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Purificação de Frutooligossacarídeos obtidos através de Síntese Enzimática utilizando Colunas de Leito Fixo e Membranas de Nanofiltração

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Engenharia de Alimentos.

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"Para realizar grandes conquistas, devemos

não apenas agir, mas também sonhar; não

apenas planejar, mas também acreditar"

Anatole France

Aos meus pais, Tânia e Erno

À minha irmã, Kátia

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RESUMO GERAL

Os frutooligossacarídeos (FOS) têm recebido considerável atenção, devido aos benefícios associados ao seu consumo, na proliferação de bifidobactérias no cólon humano, diminuição de níveis sanguíneos de colesterol, triglicerídeos e fosfolipídios, não cariogênicos, sendo classificados como prebióticos. Estão presentes em pequenas concentrações em plantas e vegetais, como banana, aspargo, alho, trigo, entre outros. Industrialmente são produzidos pela degradação da inulina e também enzimaticamente pela frutosiltransferases (FTase) ou β-frutofuranosidade de espécies de fungos e bactérias. Os FOS sintetizados enzimaticamente consistem de misturas compostas por glicose, frutose e resíduos de sacarose, o que pode vir a ser inconveniente para certas aplicações. Portanto, a minimização, ou mesmo eliminação, desses açúcares é desejável, aumentado seu caráter funcional e valor comercial, possibilitando aplicação em uma maior gama de produtos alimentícios. Em vista disso, o objetivo principal deste trabalho foi a definição de uma técnica simples, de baixo custo e robusta, para a purificação de frutooligossacarídeos presentes em uma mistura de açúcares composta por frutooligossacarídeos, glicose, frutose e sacarose. Nesse sentido foram desenvolvidas técnicas com o uso de colunas de leito fixo e membranas de nanofiltração. Os frutooligossacarídeos foram sintetizados por enzima produzida pelo microrganismo, *Rhodotorula* sp., e foram obtidos a partir de solução de sacarose 50% (v/v) como substrato. Os adsorventes carvão ativo, celite e zeólita X trocada com os cátions Na^{+}, Ca^{+2}, Ba^{+2}, Sr^{+2}, K^{+} e Mg^{+2} foram investigados em reatores de mistura contendo glicose, frutose e sacarose. Através dos resultados obtidos experimentalmente e pela modelagem matemática foi selecionada a zeólita NaX, a qual apresentou a maior taxa de adsorção e menor resistência a transferência de massa dos adsorventes testados.

A eficiência de separação (ES) foi uma das respostas utilizadas como critério para caracterizar a separação nos ensaios em colunas de leito fixo. Na separação em coluna de leito fixo com zeólitas NaX foram estudados efeitos de temperatura, vazão de alimentação, quantidade de amostra injetada e concentração de etanol no eluente através de um planejamento experimental fracionário (2^{4-1}). A partir dos resultados no planejamento fracionário foi definido um delineamento composto central rotacional (DCCR (2^{2})) sendo estudada a influência da temperatura e da concentração de etanol no eluente sobre a separação dos açúcares. Pureza de 90% dos frutooligossacarídeos e

uma eficiência de separação de 6,86 entre frutooligossacarídeos e glicose foram obtidas utilizando etanol 60% (v/v) no eluente a 45^{0}C.

Para colunas de leito fixo de carvão ativo um DCCR (2^2) foi desenvolvido para investigar o efeito das variáveis, temperatura e concentração de etanol do eluente. Os resultados mostram que a melhor eficiência de separação foi de 3,99 ± 0,07 entre frutooligossacarídeos e glicose, sendo conseguida uma pureza de 80% dos frutooligossacarídeos e recuperação de 97,8% do açúcar injetado na coluna utilizando etanol 15% (v/v) a 50^{0}C.

Seis membranas: NP010, NP030, NF270, Desal-5 DK, Desal-5 DL, Desal-5 HL foram investigadas, sendo que a membrana NP030 foi a que apresentou maior diferença entre a retenção dos frutooligossacarídeos e da glicose, portanto foi selecionada para a purificação dos frutooligossacarídeos. Esta membrana foi utilizada em dois processos de nanofiltração: uma diafiltração (com volume constante) e uma etapa de concentração. Na etapa de concentração foi realizada uma nanofiltração do permeado da diafiltração até uma concentração similar à solução inicial. O processo permitiu uma pureza de frutooligossacarídeos acima de 90% com rendimentos em torno de 80%.

Os resultados obtidos neste trabalho possibilitaram a definição de duas técnicas para purificação de frutooligossacarídeos de uma mistura de açúcares composta também de glicose, frutose e sacarose: a purificação através de colunas de leito fixo com zeólita NaX ou carvão ativo, e a nanofiltração utilizando membrana NP030, através de diafiltração, com purezas acima de 90% dos frutooligossacarídeos.

Neste trabalho, portanto, dois processos para a purificação de frutooligossacarídeos foram desenvolvidos, permitindo a aplicação industrial destes açúcares como ingredientes de produtos alimentícios, aumentando seu valor de mercado.

Como resultado final, duas patentes foram requeridas, cujos números são PI 0904119-2 e PI 0904143-5, e os artigos estão apresentados nesta tese.

ABSTRACT

Fructooligosaccharides (FOS) have received considerable attention due to the benefits associated with to its consumption, in the proliferating of bifidobacteria in the human colon, decreasing levels of cholesterol, triglycerides and phospholipids in the serum, besides being noncariogenic and are classified as prebiotics. FOS are found in small concentrations, in several kinds of plants and vegetables such as banana, asparagus roots, onion, wheat, and others. They are industrially produced by inulin degradation and enzymatically through the fructosyltransferase (FTase) or β-fructofuranosidase from bacterial and fungal species. The FOS synthesized enzymatically consists of mixtures containing glucose, fructose and sucrose residues, which may be inappropriate to some applications. Therefore, the minimization, or even the elimination, of these sugars from the mixture is desirable, what will increase its functionality and commercial value, as wel as enabling it for applications in a broader range of food products. Then, the aim of this work was the purification of fructooligosaccharides from mixture of sugars composed by fructooligosaccharides, glucose, fructose and sucrose. Therefore, with this purpose, two techniques were developed, which are fixed bed columns and nanofiltration membranes. The fructooligosaccharides were synthesized by enzyme isolated from *Rhodotorula sp.,* and were obtained from sucrose 50% (v/v) as substrate. The adsorbents, which are activated charcoal, celite and X zeolites exchanged with cations Na^{+}, Ca^{+2}, Ba^{+2}, Sr^{+2}, K^{+} e Mg^{+2} were investigated in stirred tank reactor, with glucose, fructose and sucrose. Through the experimental results as well as mathematical modeling the NaX zeolites was selected, since it presented the highest adsorption rates and the lowest mass transfer resistance, amongst all the materials evaluated.

The separation efficiency (ES) was one of the responses used to characterize the separation in the fixed bed columns experiments. In the fixed bed packed with NaX zeolites were studied the effects of temperature, flow rate, amount of sample injected, and ethanol concentration in the eluent through a fractional factorial design (2^{4-1}). Analysing these results in the fractional factorial design a central composite rotatable design (2^2 CCRD) was studied the influence of temperature and ethanol concentration in the eluent in the separation efficiency of sugars. Fructooligosaccharides at 90% purity and a separation efficiency of 6.86 was obtained between fructooligosaccharides and glucose, using 60 % ethanol (v/v) as eluent at 45^0C.

In the fixed bed column with activated charcoal as adsorbent, a 2^2 CCRD was performed to investigate the effects of temperature and ethanol concentration in the eluent. The results showed that the better separation efficiency was 3.99 ± 0.07 between fructooligosaccharides and glucose, and the final FOS purification degree and recovery were about 80% and 97.8%, respectively using 15% (v/v) ethanol eluent, at 50^0C.

Six membranes: NP030, NP010, NF270, Desal-5 DK, Desal-5 DL and Desal-5 HL were investigated, and the NP030 membrane showed higher differences in the observed retention of FOS and glucose, and so was the membrane selected for purification of FOS. The NP030 membrane was used to perform two nanofiltration stages: a diafiltration process (at constant volume) and a concentration process. In the concentration process was performed a nanofiltration of the permeate of the diafiltration to obtain a concentrate similar in its characteristics to the initial solution. The process allows getting purities over 90% in fructooligosaccharides with yields around 80%.

The results of this work defined two techniques for purification of fructooligosaccharides from a mixture of sugars containing also glucose, fructose and sucrose, which were the fixed bed columns with NaX zeolites or activated charcoal and the nanofiltration using the NP030 membrane, through diafiltration, leading to fructooligosaccharide solutions with purities higher than 90%.

In this work, therefore, two processes for the purification of fructooligosaccharides solutions were developed, allowing its industrial application as ingredients of food products and increasing your market value.

As final result, there was the application of two patents, which numbers are PI 0904119-2 and PI 0904143-5, and the articles are found in the body of this thesis.

Capítulo 1

Introdução Geral

1. Introdução e Justificativa

Atualmente existe grande preocupação em todo o mundo com a qualidade de vida e a saúde, originando um maior interesse dos consumidores por alimentos mais saudáveis, dentre os quais estão os alimentos funcionais. Estes alimentos proporcionam um efeito positivo sobre a microbiota intestinal, além de possuírem caráter nutricional, como os probióticos, e mais recentemente, os prebióticos. Prebióticos são definidos como ingredientes de alimentos não digeríveis que afetam beneficamente o homem pela estimulação da seletividade e/ou atividade de espécies de bactérias (bifidobactérias). Oligossacarídeos não digeríveis, como os frutooligossacarídeos (FOS) são prebióticos devido aos efeitos benéficos na proliferação das bifidobactérias no cólon humano (Shin et al., 2004; Antosová & Polakovic, 2001).

Destes oligossacarídeos, podem-se destacar os frutooligossacarídeos que, além de seu poder adoçante, possuem algumas propriedades funcionais. Os FOS são classificados como ingredientes e não aditivos alimentares, sendo considerados fibras dietéticas, e nos Estados Unidos possuem o status GRAS (Generally Recognized As Safe). Estes açúcares têm recebido destaque pelos benefícios associados com a saúde que vêm promovendo, conduzindo a um aumento da sua popularidade como ingredientes de alimentos e também como adoçantes alternativos nas formulações de alimentos para diabéticos. Nos Estados Unidos e na Europa o consumo diário de FOS é bastante disseminado, sendo a média diária de ingestão de 1-4 g e 3-15 g, respectivamente (Sangeetha et al., 2005).

Os frutooligossacarídeos estão presentes naturalmente em alguns alimentos em pequenas quantidades, como: trigo, banana, tomate, mel, além da ocorrência natural em alimentos podem ser produzidos enzimaticamente através de invertases (β-frutofuranosidase) ou transferases (β-D-frutosiltransferase) (Monsan & Paul, 1995); em alguns estudos vem sendo reportada a produção de FOS por espécies de fungos como *Aspergillus niger* (L'Hocine et al., 2000) e *Aspergillus japonicus* (Chen & Liu, 1996). A estrutura e as ligações dos FOS produzidos dependem da origem microbiana da frutosiltransferase utilizada na produção. Eles podem ser classificados como FOS do tipo inulina, que possuem ligações β (2-1), classificados

como kestose, nistose e frutofuranosil nistose, e os neo-FOS, que possuem ligações β (2-6), como a neokestose (Mabel et al., 2008). Os FOS são obtidos comercialmente da hidrólise enzimática da inulina através da inulinase, consistindo de ligações lineares de unidades frutosil que são comercialmente conhecidas como "Raftilose", produzido pela Beneo-Orafti (Bélgica) ou como "Frutafit", produzido pela Sensus (Holanda).

Quando são produzidos frutooligossacarídeos enzimaticamente, outros açúcares estão presentes na mistura como a glicose, produto da hidrólise da sacarose, resíduos de sacarose e frutose, portanto uma alternativa interessante para aumentar seu valor comercial seria através de técnicas de purificação ou concentração destes frutooligossacarídeos, possibilitando uma aplicação industrial em alimentos. Uma separação dos açúcares de baixa massa molecular como a glicose, frutose e a sacarose da mistura de açúcares é desejada, já que estes açúcares não contribuem com os benefícios dos frutooligossacarídeos. Os FOS mais puros podem ser aplicados como edulcorantes em produtos para diabéticos e também diminuem em 34% as calorias de um produto comparado com a sacarose (Sangeetha et al. 2005). Dentre as técnicas empregadas atualmente para separação de açúcares, podem-se destacar os métodos cromatográficos, como as colunas de leito fixo com a utilização de zeólitas (Lorenço, 2004; Burkert, 2003), com carvão ativo (Kawazoe et al., 2008; Morales et al., 2006) e também as colunas com resinas de troca iônica (Lin & Lee, 1998; Vanková et al., 2010).

A cromatografia por eluição em colunas é um método que permite uma efetiva separação e purificação de vários componentes de uma mistura com diferentes afinidades por um determinado adsorvente cromatográfico. Este método apresenta algumas desvantagens como a baixa produtividade e o longo tempo de separação, mas, apesar disto, vêm sendo bastante estudado e aplicado em larga escala nas últimas duas décadas (Guiochon et al., 1994). Para a otimização deste processo de separação o mais importante é a escolha do adsorvente adequado.

Como uma das alternativas, surgem as zeólitas, que são aluminosilicatos cristalinos, podendo ser classificadas como naturais ou sintéticas, tendo a capacidade de troca iônica, seletividade e especificidade. Têm sido aplicadas em diferentes campos, desde a remoção de amônia (Sirkecioglu & Erdem-Senatalar,

1995) a tratamento de efluentes (Engin et al., 2008), principalmente na indústria química. Mas atualmente, já vem sendo utilizadas na indústria de alimentos na estabilização da cerveja, pois as zeólitas NaA e LiX adsorvem as proteínas que são responsáveis por uma futura degradação do produto, eliminando, adicionalmente, os ácidos graxos de óleo comestível, como ocorre com o uso da zeólita X (Auerbach et al., 2003).

O carvão ativo é outro adsorvente que tem sido utilizado em várias aplicações como na purificação do ar, água potável e tratamento de águas residuárias, e também na descolorização do açúcar (Pendyal et al., 1999). Tem sido aplicado em colunas cromatográficas para remoção de impurezas de soluções de glicose (Montgomery & Weakley, 1951) e na purificação de solução de dextrose de oligossacarídeos (Urbanic, 1982). É utilizado também na separação de açúcares, sendo que nos estudos de Kawazoe et al. (2008); Morales et al. (2006) e Weston & Brocklebank (1999) foi estudada na separação de oligossacarídeos através de colunas de leito fixo de carvão e celite. Gulewicz et al. (2000) purificaram oligossacarídeos de baixa massa molecular com terra diatomácea, carvão ativo e cromatografia de troca catiônica. Mesmo sendo aplicado em vários setores industriais, o carvão ativo apresenta uma desvantagem que é a dificuldade de se conseguir uma reprodutibilidade absoluta em diferentes bateladas e a existência da distribuição de tamanho de poros, mesmo que restrita, significando que a seletividade por tamanho da molécula, usando carvão, não é tão favorável quanto à distribuição por tamanho de poro proporcionada por zeólitas (Ruthven, 1984).

Outro método bastante utilizado atualmente para purificação de oligossacarídeos são as membranas de ultra e nanofiltração (Li et al. 2005; Goulas et al. 2002; 2003). Apresentam algumas vantagens em relação aos demais métodos de recuperação já utilizados, dentre elas a recuperação de compostos de baixa massa molecular (Aydogan et al., 1998). Também constituem uma alternativa para os métodos cromatográficos mais dispendiosos e vem sendo utilizadas na separação de açúcares devido a faixa de separação destas membranas, que compreende a faixa do tamanho de molécula dos açúcares.

Nos últimos anos, no Laboratório de Engenharia de Bioprocessos (DEA-FEA-UNICAMP) têm sido pesquisados micro-organismos isolados de flores e frutas da

Mata Atlântica Brasileira (Maugeri & Hernalteens, 2007), com grande potencial de transfrutosilação na produção de frutooligossacarídeos. Como resultado desses estudos, algumas patentes já foram registradas relacionadas à produção dos frutooligossacarídeos (Maugeri et al., 2007), relacionada ao processo de imobilização da enzima em nióbio (Maugeri & Aguiar-Oliveira, 2007) e relacionadas à purificação dos frutooligossacarídeos (Kuhn & Maugeri, 2009a; 2009b). Todos estes estudos têm sido motivados pela diversidade de aplicação dos FOS em indústrias de alimentos devido à sua funcionalidade. Portanto, este trabalho tem como interesse ampliar o conhecimento adquirido nos últimos anos nesta linha de pesquisa, dando ênfase principalmente à purificação e separação destes frutooligossacarídeos obtidos enzimaticamente.

1.1. Objetivos

1.1.1. Objetivo Geral

Desenvolver uma metodologia para a purificação de frutooligossacarídeos obtidos através de síntese enzimática, utilizando colunas de leito fixo e membranas de nanofiltração.

1.1.2. Objetivos Específicos

- ✓ Avaliar os adsorventes zeólita X, carvão ativo e celite, através de modelos de isotermas e taxas de adsorção, verificando sua potencialidade na separação dos açúcares através de colunas de leito fixo;

- ✓ Determinar parâmetros de adsorção através de um modelo matemático utilizando reatores de mistura na adsorção de glicose, frutose e sacarose;

- ✓ Purificar os frutooligossacarídeos obtidos de síntese enzimática através de coluna de leito fixo com zeólita NaX;

✓ Purificar os frutooligossacarídeos obtidos de síntese enzimática através de coluna de leito fixo com carvão ativo;

✓ Selecionar membranas de nanofiltração com potencial para a purificação de frutooligossacarídeos;

✓ Determinar uma metodologia de purificação da mistura de açúcar obtida de síntese enzimática através de membranas de nanofiltração.

Desta forma a apresentação deste trabalho foi dividido em 8 capítulos, descritos a seguir.

1.2. Descrição dos capítulos

Capítulo 1. Introdução Geral e Justificativa

Capítulo 2. Revisão Bibliográfica

Este capítulo aborda uma revisão bibliográfica relatando a literatura recente e mais relevante sobre o tema proposto neste trabalho.

Capítulo 3. Selection of adsorbents and determination of parameters for the separation of glucose, fructose, sucrose and fructooligosaccharides.

Neste capítulo foi realizado um estudo da adsorção de glicose, frutose, sacarose e frutooligossacarídeos, utilizando diferentes adsorventes, dentre os quais zeólita, celite e carvão ativo, através de isotermas. O principal objetivo foi determinar parâmetros importantes na separação e verificar o adsorvente com maior potencial para o estudo posterior em colunas de leito fixo.

Capítulo 4. Mathematical modeling as a tool to investigate the separation of mono- and disaccharides from enzymatic synthesis of fructooligosaccharides by adsorption in zeolites

Este capítulo relata o estudo da adsorção de glicose, frutose e sacarose utilizando zeólita X trocada com diferentes cátions (Na^{+}, Ca^{+2}, Ba^{+2}, Sr^{+2}, K^{+} e Mg^{+2}). Foi feita uma seleção do adsorvente mais adequado através dos parâmetros do modelo em relação às taxas de adsorção e resistência à transferência de massa.

Capítulo 5. Separação e purificação de frutooligossacarídeos em coluna de leito fixo de zeólitas

Este capítulo relata o estudo de parâmetros importantes na purificação dos frutooligossacarídeos através de colunas de leito fixo com zeólitas NaX. Foram definidos parâmetros importantes na separação, através da aplicação de metodologia do planejamento experimental. O primeiro planejamento experimental realizado foi do tipo fracionário (2^{4-1}), onde foram estudados os efeitos de temperatura, concentração de etanol no eluente, quantidade de amostra injetada na coluna e vazão de alimentação. Após a análise deste planejamento, foi realizado um DCCR onde as variáveis temperatura e concentração de etanol no eluente foram estudadas.

Capítulo 6. Purification of fructooligosaccharides in an activated charcoal fixed bed column

Neste capítulo estão apresentados os resultados referentes ao estudo da separação de frutooligossacarídeos através de coluna de carvão ativo, onde foram estudadas variáveis importantes na separação como concentração de etanol no eluente e temperatura através de um delineamento central composto rotacional 2^{2}.

Capítulo 7. Selection of membranes for purification of fructooligosaccharides

Este capítulo apresenta o estudo da seleção de membranas de nanofiltração para a purificação de frutooligossacarídeos. Foram estudadas 6 membranas: NP010, NP030, NF270, Desal-5 DK, Desal-5 DL e Desal-5 HL. Através dos resultados, selecionou-se a membrana considerada mais promissora na purificação e separação de frutooligossacarídeos.

Capítulo 8. Mass transfer and transport during purification of fructooligosaccharides by nanofiltration

Neste capítulo foi projetado um processo de purificação de frutooligossacarídeos presentes em uma mistura de açúcares obtidos através de síntese enzimática. O processo de purificação compreendeu duas etapas, na primeira foi realizada uma diafiltração até a concentração desejada de frutooligossacarídeos, na segunda etapa foi realizada uma nanofiltração do permeado obtido na primeira etapa, sendo conseguida uma concentração similar a solução inicial.

Capítulo 9. Conclusões Gerais

Neste capítulo as principais conclusões sobre os resultados obtidos são relatadas e discutidas.

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Capítulo 2

Revisão Bibliográfica

2. Revisão Bibliográfica

2.1. Frutooligossacarídeos: Conceito e produção

Frutooligossacarídeos (FOS), como a kestose, nistose e frutofuranosil nistose, são oligômeros de frutose que possuem unidades de frutosil, ligadas a posição β 2,1 da sacarose. Não são açúcares calóricos, carcinogênicos e quando ingeridos causam um efeito benéfico para a saúde (Vanková et al., 2010).

Os FOS podem ser divididos em dois grupos do ponto de vista comercial. O primeiro grupo é preparado a partir da hidrólise enzimática da inulina pela enzima inulinase através da sacarose e consiste de unidades lineares de frutosil, com ou sem uma unidade final de glicose. Este produto é comercializado como "Raftilose", produzido pela BENEO-Orafti (Bélgica) ou como "Frutafit", produzido pela Sensus (Holanda). Este processo ocorre amplamente na natureza, e esses oligossacarídeos podem também ser encontrados em uma grande variedade de plantas (Roberfroid, 1993), principalmente em tubérculos, yacon, alcachofras, aspargos, beterraba, chicória, banana, alho, cebola, trigo e tomate (Spiegel, 1994; Yun, 1996), mas em pequenas quantidades. O segundo grupo é preparado por reação enzimática de transfrutosilação em resíduos de sacarose, e consiste tanto de cadeias lineares quanto de cadeias ramificadas de oligossacarídeos. Este produto é produzido pela Meiji Seika Ltd (Japão), e comercializado como "Neosugar", "Profeed", "Meioligo" e "Nutraflora"; o "Actilight" é produzido e comercializado pela Béghin Meiji Industries (França).

As enzimas que catalisam a produção de frutooligossacarídeos são classificadas como frutosiltransferases (FTase, E.C. 2.4.1.9) ou β–frutofuranosidase (FFase, E.C. 3.2.1.26). Diversos microrganismos têm sido reportados como produtores da enzima frutosiltransferase responsável pela produção dos frutooligossacarídeos, dentre eles, o *Aspergillus oryzae* CFR 202 (Sangeetha et al., 2002), *Aspergillus japonicus* (Chien et al., 2001), *Aspergillus niger* AS 0023 (L'Hocine et al., 2000), *Aureobasidium pullulans* CFR 77 (Sangeetha et al., 2004), *Bacillus macerans* EG-6 (Park et al., 2001), *Zimomonas mobilis* (Beker et al., 2002), *Penicillium citrinum* (Hayashi et al., 2000).

Os rendimentos de produção de frutooligossacarídeos variam de 24 a 61%, sendo que rendimentos de 61% foram encontrados utilizando *Aspergillus japonicus* com altas concentrações de sacarose (40%) como substrato (Sangeetha et al., 2005). Rendimentos maiores, em torno de 90-98% foram obtidos quando duas enzimas foram utilizadas (β-frutofuranosidase e glicose oxidase) na conversão dos frutooligossacarídeos (Sheu et al., 2001).

Diversos são os trabalhos encontrados onde se utilizam bactérias e fungos para a produção de frutooligossacarídeos dentre eles, destaca-se o *Bacillus macerans,* que produz frutooligossacarídeos com rendimento de 33%, utilizando sacarose 50% como substrato (Park et al., 2001). A bactéria *Zimomonas mobilis* também tem sido reportada como produtora de frutooligossacarídeos, através da enzima levanasacarase, produzindo uma mistura de frutooligossacarídeos (1-kestose, 6-kestose, neokestose e nistose) com um rendimento de 24-32% (Beker et al., 2002). Já entre as espécies fúngicas, destaca-se o *Aspergillus niger* AS 0023 com rendimentos de 54% utilizando sacarose 50% como substrato (L´Hocine et al., 2000)

2.2. Propriedades

Os FOS apresentam várias características que os tornam interessantes para aplicação em alimentos, como a solubilidade que é maior que a da sacarose, não são degradados em processos de aquecimento, não são calóricos, podem ser utilizados na formulação de alimentos destinados a diabéticos. Devido a essas características, podem ser utilizados em muitos alimentos dentre eles: nas formulações de sorvete e sobremesas lácteas, iogurtes, biscoitos, produtos de panificação, barras de cereais e sucos, dentre outros.

Estes açúcares são solúveis em água, levemente doces, possuem de 0,4 a 0,6 vezes o poder de doçura da sacarose, possuindo baixo valor calórico, sendo esta última propriedade benéfica em dietas com restrição de açúcares. A doçura dos frutooligossacarídeos depende da estrutura química e massa molecular do oligossacarídeo presente e dos níveis de mono e dissacarídeos na mistura (Yun, 1996).

Os FOS apresentam outras características interessantes, como não cariogenicidade, e em alguns estudos foram observadas diminuições nos níveis de colesterol e triacilgliceróis após a sua ingestão (Williams & Jackson, 2002).

Os frutooligossacarídeos são altamente higroscópicos, sua viscosidade e estabilidade térmica são relativamente maiores que a da sacarose nas mesmas concentrações. Mesmo não existindo muitos estudos comparando as propriedades físico-químicas dos frutooligossacarídeos à sacarose, acredita-se que estes açúcares são semelhantes em muitas propriedades como solubilidade, temperatura de congelamento e ponto de ebulição (Antosová & Polakovic, 2001; Yun, 1996).

O maior interesse na utilização dos oligossacarídeos ocorre devido aos benefícios das suas propriedades fisiológicas. Muitos oligossacarídeos não são digeridos pelo organismo, não sendo utilizados como energia para o corpo humano. E esta propriedade confere a possibilidade de sua utilização também em alimentos com menor teor de açúcares, produtos *diet*, podendo ser consumidos por diabéticos (Yun, 1996).

2.3. Purificação de FOS

Na produção enzimática de FOS a partir de sacarose, os FOS formados possuem de duas a quatro ligações β (1-2) frutosil ligadas à glicose, sendo eles a kestose, nistose e a frutofuranosil nistose, respectivamente. Glicose e pequenas porções de frutose são formadas como produtos da reação de transfrutosilação, sendo que estes açúcares podem ser removidos da mistura através de processos de separação de açúcares (cromatografia e separação através de membranas) para a produção de frutooligossacarídeos de elevada pureza (Crittenden & Playne, 1996). Geralmente os xaropes de frutooligossacarídeos obtidos apresentam rendimento de frutooligossacarídeos entre 55-60%, portanto, torna-se necessário um processo de separação para a purificação destes açúcares (Yung & Song, 1993) devido aos benefícios gerados através de seu consumo.

2.3.1. Nanofiltração

A micro e a ultrafiltração são processos de separação bem conhecidos em biotecnologia e na indústria de fermentação, e já vem sendo utilizados como meios para purificação de oligossacarídeos de alta massa molecular. A nanofiltração (NF) aparece como um método em escala industrial para a concentração e purificação de misturas de oligossacarídeos (Goulas et al., 2002), apresentando algumas vantagens em relação aos demais métodos de recuperação já utilizados, dentre elas a recuperação de compostos de baixa massa molecular (Aydogan et al., 1998). A NF vem sendo cada vez mais utilizada em processos industriais, objetivando o *design* de novos processos de nanofiltração e a otimização da aplicação das membranas já existentes, com o desenvolvimento de boas ferramentas para purificação de compostos (Cavaco Mourão et al., 2008). As membranas de NF separam moléculas na faixa de 200-1000 daltons, sendo muito interessante para aplicação em açúcares.

A transferência de massa na separação por nanofiltração é baseada em dois mecanismos: peneiramento e efeitos de cargas. O peneiramento é mais importante na separação de moléculas neutras, onde moléculas maiores que os tamanhos dos poros da membrana permanecerão retidas, e as moléculas que são menores que os tamanhos dos poros da membrana passarão através dos poros. Na separação de açúcares, o transporte de massa através das membranas de nanofiltração é controlado pela convecção e difusão já que os açúcares são considerados moléculas neutras em soluções aquosas (Goulas et al., 2003).

2.3.1.1. Fatores que afetam a nanofiltração

Dentre os fatores que afetam a separação por nanofiltração, podemos citar a pressão transmembrana, velocidade de fluxo, concentração de alimentação do soluto, temperatura, força iônica e pH (Suwattana, 2007).

A pressão é um fator importante na separação por nanofiltração porque fornece a força motriz necessária para a separação. Em geral, um aumento na pressão melhora a transferência de massa. Entretanto, isto também pode causar

um aumento na formação da concentração de polarização na superfície da membrana. Sempre que a pressão é aumentada é observado um aumento na retenção de mono, di e trissacarídeos (Goulas et al., 2002), ocorre a redução da espessura da membrana e o tamanho de poro devido ao aumento do fluxo. Portanto, a rejeição de solutos neutros, que dependem principalmente do efeito de peneiramento, aumenta. O aumento da pressão faz diminuir a diferença de rejeição entre mono, di e trissacarídeos. Em outras palavras, a separação destes três açúcares é menos efetiva com o aumento da pressão (Goulas et al., 2002). Sjöman et al. (2007) observaram que a retenção de monossacarídeos dependia fortemente da pressão de filtração, essa retenção aumentava com o aumento do fluxo de permeado ou pressão até atingir um limite de pressão, após este limite, a pressão não apresentava influencia sobre a retenção dos açúcares.

O aumento da concentração do açúcar é outro fator que pode aumentar a polarização da concentração na superfície da membrana. Goulas et al. (2002) e Vellenga & Tragardh (1998) observaram que a rejeição de mono, di e trissacarídeos diminuía com o aumento da concentração da solução de açúcar.

Um aumento no fluxo do permeado geralmente ocorre com o aumento da temperatura, que diminui a viscosidade da solução e aumenta a difusividade das moléculas (Garem & Jeantet, 1995). No entanto, a temperatura não deve ultrapassar a faixa de temperatura da membrana, pois pode danificá-las de forma permanente e, portanto diminuir o seu desempenho (Mänttäri et al., 2002).

2.3.1.2. Separação de açúcares por nanofiltração

Diversos estudos têm sido realizados utilizando membranas de nanofiltração para separação de misturas de oligossacarídeos. Goulas et al. (2003) demonstraram a remoção de monossacarídeos de misturas comerciais de oligossacarídeos com uma menor perda de oligossacarídeos após quatro processos de diafiltração. Entretanto, este estudo avaliou o desempenho das membranas somente com baixas concentrações de açúcares entre 10 e 20 g/L.

Goulas et al. (2002) estudaram os efeitos de pressão, concentração da alimentação e temperatura de filtração, sendo que fatores de rejeição dos açúcares

na solução aumentaram com o aumento da pressão causando aumento do fluxo e compactação da membrana. Este efeito foi maior para soluções com açúcares de menor massa molecular. O aumento da concentração do açúcar na alimentação causou um decréscimo na rejeição do açúcar. Já a temperatura de filtração quando aumentada causou um decréscimo na rejeição observada para açúcares de menor massa molecular.

Aydogan et al. (1998) observaram que o aumento da vazão na alimentação aumentou o fluxo do permeado e a rejeição pela minimização dos efeitos da polarização da concentração. Também observaram que a rejeição do açúcar não foi influenciada pelo aumento da pressão.

Pontalier et al. (1997) estudaram os mecanismos que envolvem a retenção de glicose e lactose em membranas de nanofiltração, observando que a transferência de massa da glicose ocorre por difusão e por convecção no momento em que sua molécula penetra nos poros da membrana, e a lactose, que possui uma molécula maior não penetra nos poros da membrana e a sua transferência de massa ocorre somente por difusão.

Li et al. (2005) determinaram parâmetros de transporte para glicose e sacarose através de dados experimentais. Com estes resultados foi proposto um modelo de poro para definir parâmetros de transporte para frutooligossacarídeos.

González-Muñoz & Parajó (2010) estudaram a diafiltração de solução de açúcares contendo oligossacarídeos, sendo a diafiltração avaliada através de um modelo matemático baseado na diferença de pressão e também nos efeitos da polarização da concentração e na adsorção dos açúcares na superfície interna e nos poros da membrana. Foi observado que a diafiltração foi eficiente na remoção dos monossacarídeos devido à baixa retenção destes compostos (18,4% glicose, 7,9% xilose e 10,9% arabinose) pela membrana, enquanto que os oligossacarídeos apresentaram uma maior retenção, entre 72-80%.

2.3.2. Zeólitas

Uma alternativa muito interessante na separação de açúcares é o uso de zeólitas, sólidos microporosos e cristalinos, de natureza inorgânica, contendo alumínio, silício e oxigênio arranjados em uma estrutura altamente regular. Podem ser de origem natural ou sintética e apresentam microporosos uniformes, seletividade de adsorção pelo tamanho molecular, propriedades de troca iônica, habilidade de desenvolver acidez interna, estabilidade térmica e facilidade de ser regenerada (Burkert, 2003). Sendo assim, podem ser utilizadas como "peneira molecular" nos processos de separação. As zeólitas X são membros da família faujasita, que apresentam uma estrutura de cristal estável, grande volume de poros e possibilidade de troca iônica (Hammoudi et al., 2008).

O termo zeólita foi inicialmente utilizado para designar uma família de minerais naturais, que apresentam como propriedades particulares à troca de íons e a dessorção reversível de água. O termo engloba um grande número de minerais naturais e sintéticos que apresentam características comuns. Apresenta um esqueleto cristalino formado pela combinação tridimensional de tetraedros TO_4 (T = Si, Al, B, Ga, Ge, Fe, P, Co) unidos entre si através de átomos de oxigênio comuns (Giannetto, 1990). Na Figura 1 está representada a estrutura da zeólita.

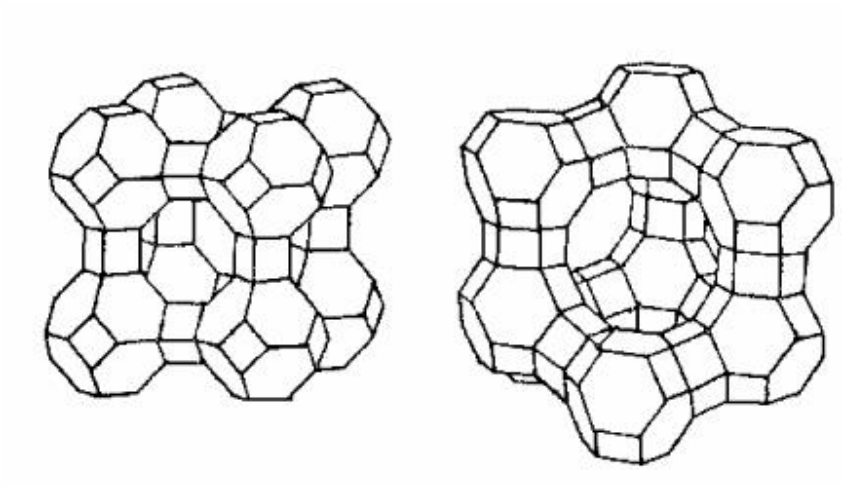

Figura 1. Estrutura das zeólitas

A zeólita X é um aluminosilicato de material cristalino e um adsorvente hidrofílico. Sua composição química é expressa pela fórmula:

$$Na_{86}[(AlO_2)_{86}(SiO_2)_{106}].264H_2O$$

Consiste de arranjos tridimensionais de tetraedros de SiO_4 e AlO_4 ligados por átomos de O_2 (Chang et al., 2006). É positivamente carregada com íons sódio na estrutura dos poros, podendo ser trocada por outros cátions (Yu et al., 1998). A proporção de alumínio é uma propriedade extremamente importante, afetando a distribuição da densidade de carga negativa na rede, a capacidade de troca iônica, a estabilidade térmica, a hidrofobicidade/hidrofilicidade das superfícies interna e externa e as dimensões de cela unitária (Braga & Morgon, 2007).

Aplicações importantes das zeólitas estão em processos de secagem, separação e purificação, destacando-se em processos de troca iônica (Hammoudi et al., 2008), purificação de diferentes compostos, dentre eles proteínas (Yu et al., 1998), tratamento de efluentes (Engin et al., 2008), remoção de chumbo de águas (Turan et al., 2005). Muitos pesquisadores estudaram a purificação de açúcares com zeólitas, dentre estes podemos citar Holtkamp et al. (2009); Berensmeier & Buchholz (2004); Cheng & Lee (1992) e Ho et al. (1987).

As zeólitas geralmente são utilizadas como adsorvente seletivo de carboidratos em solução aquosa, sendo o processo de adsorção reversível baseado no tamanho molecular e seletividade. Esta propriedade permite que a zeólita seja utilizada muitas vezes, entre os passos de adsorção e dessorção do processo, enfatizando o considerável valor econômico em aplicações de adsorção. As zeólitas são caracterizadas não só pela alta seletividade, mas também pela habilidade de separar substâncias com base na diferença do tamanho molecular e forma (Berensmeier & Buchholz, 2004).

2.3.2.1. Zeólitas X e Y

As zeólitas podem ser classificadas em zeólitas sintéticas e naturais. Mesmo existindo várias zeólitas naturais, a indústria possui interesse na produção de zeólitas sintéticas, pois as zeólitas naturais apresentam em sua grande maioria

impurezas indesejáveis, cuja composição química pode variar significativamente de um depósito para outro, inclusive no mesmo meio não há como obter zeólitas de mesma composição, enquanto a indústria consegue desenvolver zeólitas otimizadas e específicas aos seus interesses (Braga & Morgon, 2007).

De acordo com Kärger & Ruthven (1991), as zeólitas X e Y formam o maior volume de poro entre as zeólitas e a diferença entre elas está na proporção Si/Al e na ordenação do silício e do alumínio nos tetraedros. O número de íons de alumínio na célula unitária da zeólita X varia de 96 a 77 e na zeólita Y de 76 a 48, resultando em uma relação de Si/Al de 1 a 1,5 para a zeólita X e de 1,5 a 3 para a zeólita Y (Breck, 1974).

A área superficial e o diâmetro efetivo do poro das zeólitas X e Y são aproximadamente 800m^2/g e 7-8 Å, respectivamente, que são duas vezes superior ao valor da zeólita A (Chang et al., 2006).

2.3.2.2. Separação de açúcares utilizando zeólitas

Nos últimos anos diversos trabalhos utilizaram a zeólita como adsorvente na separação e purificação de açúcares, como uma alternativa eficiente e mais econômica. Dentre os trabalhos citados na literatura, Holtkamp et al. (2009) estudaram a remoção de isomaltose através de zeólitas do produto de reação de formação de isomaltose por síntese com glicosiltransferase, verificando a influência do eluente e da temperatura no processo e dessorção.

Berensmeier & Buchholz (2004) estudaram a adsorção de glicose, frutose e isomaltose em reatores de mistura, onde foi observado que a isomaltose apresentou maior afinidade pela zeólita que os demais açúcares estudados. Dentre os estudos com leito fixo de zeólitas, destaca-se o trabalho de Lorenço (2004) que estudou a melhor condição para a separação de glicose e frutose obtidos do leito de hidrólise, sendo que a condição em que se obteve a melhor separação foi através da utilização de três colunas de zeólitas Y em série utilizando etanol 15% (v/v) como eluente. Foi observado que quanto maior a concentração de etanol e tamanho do leito, melhor a separação obtida. Frações de frutose de alto grau de pureza (63,5% da frutose injetada) foram obtidas na saída das colunas cromatográficas. Burkert (2003)

estudou a separação de glicose, frutose e dextranas utilizando colunas de leito fixo com zeólitas. Foi verificado que através da troca iônica da zeólita com bário se obteve uma separação mais eficiente dos açúcares. No estudo da separação de glicose e frutose, verificou-se que a temperatura mostrou efeito pronunciado sobre a eficiência de separação. Já na separação de dextrana e frutose, temperatura e volume injetado, apresentaram maior influência na separação.

Cheng & Lee (1992) estudaram a eficiência de separação de glicose e frutose através de zeólita Y, definindo como parâmetros para o cálculo da eficiência de separação (ES), a temperatura, volume injetado, a vazão de alimentação e troca iônica. A melhor separação foi obtida com a zeólita Ba-Y, sendo que, em ordem de eficiência de separação, tem-se: $Ba^{2+} > Ca^{2+} > K^{+} > Na^{+}$, com diâmetro de partícula de 20-40 mesh. Água deionizada foi utilizada como eluente, sendo que os ensaios foram realizados numa vazão de 2, 1,5, 1 e 0,5 mL/min. Em vazões mais baixas (0,5 e 1 mL/min) foram obtidas as melhores separações. Três níveis de temperatura foram utilizados (25, 40 e 60^{o}C) sendo que os melhores resultados foram encontrados a 40^{o}C. O volume injetado na coluna variou de 1 a 8 mL, onde as melhores separações foram obtidas com menores volumes de injeção.

Ho *et al.* (1987) estudaram a adsorção de glicose e frutose através de duas resinas (Ca^{2+} Zerolit 225 SRC14 e Ca^{2+} Duolite C-204) e duas zeólitas (Ca-Y e Ca-X). A zeólita Ca-X não apresentou seletividade entre glicose e frutose, já a zeólita Ca-Y apresentou seletividade e capacidade semelhante às resinas. A zeólita Ca-Y apresentou a vantagem de uma menor resistência à transferência de massa que as resinas.

2.3.3. Carvão ativo

O carvão ativo é obtido da decomposição química de materiais carbonáceos seguido da ativação com vapor ou dióxido de carbono a elevadas temperaturas (700-1100^{0}C). A superfície do carvão é essencialmente não polar, entretanto uma ligeira polaridade pode surgir da oxidação da superfície, tornando o carvão adsorvente levemente hidrofóbico (Ruthven, 1984).

No processo de ativação do carvão é possível preparar um carvão ativo com uma estreita distribuição do tamanho do microporo, com um diâmetro em torno de 4 a 9 Å. Para uma determinada separação pode-se fazer uma modificação do tamanho de poro, pelo ajuste das condições do processo de manufatura, que é relativamente fácil. Entretanto a dificuldade de se atingir uma reprodutibilidade absoluta em diferentes bateladas e a existência da distribuição de tamanho de poros, mesmo que restrita, significa que a seletividade por tamanho da molécula usando carvão não é tão favorável quanto a distribuição por tamanho de poro proporcionada por zeólitas (Ruthven, 1984).

O processo de adsorção no carvão ativo é dependente, dentre outras coisas, de sua área superficial, envolvendo uma interface sólido-líquido, no caso de tratamento de soluções ou água, e sólido-gás, no caso de tratamento de gases. A adsorção pode ocorrer de duas formas, pela fisissorção, onde as moléculas do líquido são adsorvidas no sólido através de forças de van der Waals, resultando em uma adsorção em multicamadas, e pela quimissorção, quando acontece uma reação química entre o adsorbato e o adsorvente.

Os produtos comerciais possuem freqüentemente uma área superficial que varia de 500 a 1500 m^2/g, sendo que o carvão ativo também é caracterizado pelo volume do poro, cujos diâmetros podem ser maiores que 50 nm (macroporos), entre 2 - 50 nm (mesoporos) e menores do que 2 nm (microporos). A capacidade de adsorção do carvão ativo é determinada não só pela sua área superficial, mas também pela estrutura dos poros internos e pela presença de grupos funcionais na superfície do poro (Ahmedna et al., 2000).

O carvão ativo é classificado em pó ou granulado, sendo este considerado mais versátil devido a sua maior facilidade de regeneração. Este tipo de carvão é mais encontrado no mercado, sendo principalmente utilizado na descoloração do açúcar (Ahmedna et al., 2000).

2.3.3.1. Aplicações

O carvão ativo tem sido utilizado na purificação do ar, água potável e tratamento de águas residuárias e também na descoloração do açúcar (Pendyal et

al., 1999). Também vem sendo aplicado em colunas cromatográficas para remoção de impurezas de soluções de glicose (Montgomery & Weakley, 1951) na purificação de solução de dextrose de oligossacarídeos (Urbanic, 1982) e em processos de separação de açúcares.

2.3.3.2. Separação de açúcares utilizando carvão ativo

Na literatura a utilização de carvão ativo em processos industriais é bastante conhecida, mas ultimamente o carvão também vem sendo utilizado na separação e purificação de açúcares. Kawazoe et al. (2008) estudaram a separação de sacarídeos através de colunas de leito fixo de carvão e celite. A coluna foi eluída com água, etanol (5%) e etanol (30%), os monossacarídeos foram eluídos com água e os oligossacarídeos foram eluídos com solução de etanol (30%). Morales et al. (2006), estudaram a separação de oligossacarídeos através de colunas de carvão ativo e celite (1:1), onde os ensaios com os melhores resultados foram com eluição dos monossacarídeos com solução de água/etanol (90/10) (v/v) e oligossacarídeos sendo eluídos com solução de água/etanol (50/50) (v/v).

Boon et al. (2000) estudaram a remoção de oligossacarídeos por adsorção em carvão ativo durante a reação catalisada pela enzima β-galactosidase de *Bacillus circulans*. Para determinação dos parâmetros de Langmuir foi utilizado 1,6 g de carvão ativo com 8 mL de tampão citrato-fosfato 0,02 M (pH 5,0) contendo açúcares em diferentes concentrações (6 a 108 g/L). A afinidade por trissacarídeos foi maior do que para mono e dissacarídeos. As curvas de ruptura foram obtidas para a mistura contendo lactose (5,9 g/L), galactose (5,8 g/L) e maltotriose (2,9 g/L), utilizando colunas de leito fixo pré-tratadas com tampão citrato-fosfato 0,02 M (pH 5,0) e lactose (5,9 g/L). Os resultados demonstram que a coluna pré-tratada com tampão mostrou maior afinidade por trissacarídeos de acordo com os resultados encontrados para as isotermas de Langmuir.

Gulewicz et al. (2000) isolaram, com 90% de pureza, oligossacarídeos de baixa massa molecular. Estes oligossacarídeos, da família rafinose (RFOs), foram precipitados com etanol 100%; purificados com terra diatomácea e carvão ativo; e cromatografia de troca catiônica. Observou-se que após a precipitação com etanol

100%, foram conseguidas soluções com 60% de oligossacarídeos e após filtração com terra diatomácea e carvão ativado soluções com cerca de 75% de oligossacarídeos e 90% de pureza após eluição em coluna de troca catiônica.

Kaplan & Hutkins (2000) estudaram a purificação de frutooligossacarídeos para a aplicação em meio MRS para identificação de bactérias ácido lácticas e bifidobactérias capazes de fermentar frutooligossacarídeos. Foi utilizada uma mistura comercial de frutooligossacarídeos composta de GF_2 (32%), GF_3 (53,6%), GF_4 (9,8%), glicose e frutose (2,3%) e sacarose (2,3%), sendo preparada uma solução 40% de frutooligossacarídeos, que foi adicionada a uma coluna (30 x 5 cm), contendo carvão ativo. Glicose e frutose foram eluídas com água, sacarose foi eluída com etanol (5%) e frutooligossacarídeos com etanol (15%).

No trabalho de Weston & Brocklebank (1999) foi relatada a separação de frações de oligossacarídeos dos monossacarídeos. Amostras de mel contendo os oligossacarídeos foram diluídas em 20 mL de água e 4 g de carvão ativo foi adicionado. A mistura foi adicionada a uma coluna empacotada com carvão ativo e celite. Os monossacarídeos foram eluídos da coluna com solução de etanol em água (1:999, 1 L) e os oligossacarídeos com etanol/água (1:1, 500 mL).

Swallow & Low (1990) também trabalharam com amostras de mel na separação de vinte estruturas similares de carboidratos do mel. Na coluna de leito fixo contendo carvão e celite aproximadamente 99% dos monossacarídeos foram removidos com eluição da coluna com solução de etanol (0,1%) (v/v) a uma vazão de 10 mL/min, os oligossacarídeos foram eluídos da coluna a 60ºC com 500 mL de solução etanol 50% (v/v) na mesma vazão.

Hidaka et al. (1988) sintetizaram oligossacarídeos através da enzima de *Aspergillus niger* ATCC 20611, onde após 72 h de síntese obtiveram rendimentos de glicose (33,4%), traços de frutose, sacarose (9%), GF_2 (17,3%), GF_3 (32%) e GF_4 (7,2%). A mistura de açúcares foi adicionada numa coluna de carvão ativo, após eluição com água destilada para remoção dos monossacarídeos e sacarose, a coluna foi sucessivamente eluída com 5, 10 e 20% de etanol para a separação dos FOS.

2.4. Fenômeno da adsorção

A teoria da adsorção baseia-se na separação de componentes de uma mistura tendo a transferência de massa como fenômeno físico. Nesta mistura têm-se duas fases, o componente que está diluído na fase líquida, denominado adsorbato e um sólido denominado adsorvente. Assim quando estas duas fases entram em contato, o composto que está diluído se difunde indo do seio da fase fluida para a superfície do adsorvente. A força motriz desta difusão é a diferença de concentração entre o seio da solução e a superfície do material sólido. Dependendo do processo empregado na remoção, existe também um componente de difusão forçada originada pelo fluxo da fase fluida.

Diferentes tempos de adsorção sugerem a existência de diferentes graus de interação entre os compostos e o sólido adsorvente. Isso acontece porque existem dois tipos de interação que podem ocorrer neste processo: adsorção química e adsorção física. A adsorção física ou fisissorção é resultado de forças de interação entre moléculas do adsorvente e do adsorbato maiores do que forças atrativas entre as moléculas do próprio fluido. Este processo envolve forças de van der Waals e devido à magnitude destas forças é um processo reversível, podendo o adsorvente ser usado outras vezes e o adsorbato ser reciclado com uma concentração superior a do efluente antes do tratamento. Na adsorção química ou quimissorção ocorre ligação química entre o sólido adsorvente e o adsorbato, isto é, há troca ou compartilhamento de elétrons entre eles. O adsorbato é fixado mais fortemente à superfície do adsorvente. As moléculas não são atraídas para todos os pontos da superfície e dirigem-se para os centros ativos. A adsorção química ocorre em uma única camada e é um processo praticamente irreversível devido à alteração da natureza química do adsorbato (Ciola, 1981).

2.4.1. Isotermas de adsorção

A isoterma de adsorção demonstra o equilíbrio atingido relacionando a concentração de um determinado composto na fase fluida e a concentração no adsorvente a uma dada temperatura. Geralmente são representadas por equações

matemáticas que relacionam as concentrações de equilíbrio entre as fases numa dada temperatura.

Os modelos citados na literatura geralmente são: Linear, Langmuir e Freundlich (Nobre et al., 2009; Serpen et al., 2007).

2.4.1.1. Isoterma Linear

Na Equação 1 está representado o modelo para a isoterma Linear.

$$q = K.c \tag{1}$$

onde:
q = quantidade de adsorbato adsorvida no equilíbrio;
K = coeficiente de partição
c = concentração de adsorbato em solução no equilíbrio.

2.4.1.2. Isoterma de Langmuir

O modelo de Langmuir foi originalmente desenvolvido para representar a sorção química, sem sítios distintos de adsorção, de gases e vapores em sólidos. Uma das características da isoterma de Langmuir é presumir a formação de uma monocamada e assumir uma aproximação da quantidade limite de adsorção. Esta teoria assume as seguintes hipóteses:

- O sólido adsorvente possui um número definido de sítios disponíveis para a adsorção de determinadas espécies;
- Todos os sítios possuem o mesmo nível de atividade;
- A adsorção em um sítio não influencia os sítios vizinhos;
- Cada sítio pode ser ocupado por somente uma molécula da espécie a ser adsorvida, a adsorção é limitada a uma monocamada.

Para casos em que a adsorção ocorre em fase líquida, a equação que representa a isoterma de Langmuir é dada pela Equação 2 (Ruthven, 1984).

$$q = \frac{q_m.C}{k_D+C} \tag{2}$$

onde:
q_m = capacidade máxima de adsorção;
k_D = constante de dissociação.

2.4.1.3. Isoterma de Freundlich

A isoterma de Freundlich (Eq. 3) é utilizada para energias superficiais heterogêneas. Só é valida para soluções diluídas e não prediz a linearidade quando a concentração tende a zero. É um dos modelos mais usados, devido a sua simplicidade (Claudino, 2003).

$$q = k_F.(c)^{n_F} \quad (3)$$

onde:
k_F = constante do modelo Freundlich;
n_F = índice do modelo Freundlich.

2.4.2. Adsorção em leito fixo

O estudo de adsorção em sistemas dinâmicos reflete melhor o comportamento real do processo, pois envolvem fluxo líquido e transferência de massa complexa. O comportamento dinâmico envolve a saturação ao longo da coluna em relação ao tempo, espaço e comprimento da coluna de adsorção, simultaneamente (Volesky, 2001), enquanto que, nos experimentos em batelada variam somente com o tempo (Sánchez et al., 1999).

O projeto de colunas de adsorção ou troca iônica (apesar de serem duas ocorrências distintas) seguem basicamente os mesmos procedimentos. O comportamento de troca iônica ou adsorção pode ser evidenciado de maneira dinâmica, nestes casos longe do equilíbrio, a troca ocorre de forma mais semelhante aos processos industriais. Os sistemas contínuos mais comumente utilizados são os de leito fixo. Trata-se de uma técnica de separação altamente seletiva que pode remover até mesmo traços de componentes iônicos de grandes volumes de soluções diluídas (Barros et al., 2001).

O leito fixo é constituído por uma coluna com o sólido adsorvente que irá remover a substância desejada diluída em um fluido. O leito é considerado como fixo porque a vazão de operação é suficientemente baixa para não permitir que as partículas sólidas se movimentem dentro da coluna, ou seja, a força da gravidade sobre o sólido é maior do que a força de arraste do fluido sobre as partículas, não ocorrendo a fluidização.

Em um leito fixo o fluído geralmente entra pela parte inferior e flui pelo leito até a parte superior, por onde deixa o sistema. Em alguns sistemas, principalmente quando o adsorvente é mais frágil utiliza-se o fluxo de cima para baixo porque o fluxo ascendente a altas velocidades poderia fluidizar as partículas, causar atrito destas com a parede do leito e arrastar o adsorvente.

O processo de adsorção em colunas de leito fixo é geralmente o mais empregado e tem como vantagens: pequeno espaço, simples operação, tratamento de grandes volumes de efluentes de forma contínua, rendimento considerável e a fácil ampliação da escala de laboratório para a escala industrial (Costa, 1998).

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Chapter 3

Selection of adsorbents and determination of parameters for the separation of glucose, fructose, sucrose and fructooligosaccharides

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Abstract

This work aimed to describe the adsorption of sugars (glucose, fructose, sucrose and fructooligosaccharides) employing different adsorbents (celite, active carbon and NaX zeolite) by means of isothermal models. At the adsorbent selection step, the active carbon exhibited a higher affinity for the adsorption of fructooligosaccharides. On the other hand, the adsorbents NaX zeolite and celite presented the highest adsorption stability with glucose, giving values of 1.496 g glucose/g zeolite and 1.35 g glucose/g celite. Therefore, zeolite and celite were the adsorbents selected for the determination of isothermal kinetics and it was shown that the linear model ($q^* = k.C^*$) provided the best fit for the experimental data. The temperatures studied were (30, 40 and 50^0C) and the 50^0C resulted in the highest partition coefficient (k) for glucose on celite, indicating that higher temperatures benefit sugar adsorption.

KEYWORDS: Adsorption, glucose, zeolite, celite, active carbon, fructooligosaccharides.

3.1. Introduction

Fructooligosaccharides (FOS) are regarded as functional food ingredients that may be employed in product formulation. FOS synthesis from sucrose, although being highly competitive, also produces mono and disaccharides, which are undesirable, and should be removed to improve product quality. In addition, the commercial value of oligosaccharides increases with purity, boosting the interest in recovering oligosaccharides by techniques such as chromatography, thus obtaining high purity products. However, these products are currently found on the market mainly as mixtures containing the mono and disaccharides, requiring purification by means of inexpensive techniques. Therefore, over the last few years, research has been carried out in the Bioprocess Engineering Laboratory (UNICAMP) in order to obtain compounds containing substantially purified amounts of prebiotic fructooligosaccharides.

Previous studies have shown that zeolites adsorb fructose from mixtures of glucose and fructose, with a result similar to that of using an ion-exchange resin, with the advantage of presenting lower costs (Buttersack et al., 1993; Cheng & Lee, 1992; Ching et al., 1987; Ching & Rutheven, 1988; Ho et al., 1987; Schöllner et al., 1993). Due to their adsorptive properties and to the fact that their structure and composition are known, the zeolites may be employed in sugar recovery (Silva, 1998). Zeolites have attracted considerable attention due to their potential applications in many fields, such as gas storage, catalysis and ion-exchange (Breck, 1974 and Dyer, 1988). Type X zeolites, an aluminum-rich member of the faujasite family, are extensively used in adsorption, separation processes and ion-exchange, due to their stable crystal structure, large pore volume and high cation content (Hammoudi et al., 2008).

Active carbon has shown outstanding performance in purification processes, detoxification, deodorization, filtration, discoloration, removal or alteration of flavor, and concentration of various materials as well as liquid and gaseous substances (Ahmedna et al., 2000a). All these applications have rendered active carbon a product of great interest in many economic sectors in diverse fields of action, such as the food, pharmaceutical, chemical and petroleum industries, and it may be employed in the granular or powdered form (Ahmedna et al., 2000b).

Some studies have reported the purification of sugars, specially those carried out by Hidaka et al. (1988), using an active carbon column and preparative column, obtaining good yields and purification of the fructooligosaccharides. Boon et al. (2000) using an active carbon column for the removal of oligosaccharides from a reaction mixture for synthesis, showed that the best performance of the process occurred when the removal and synthesis were performed in a batch process.

With respect to the use of zeolites in the separation of oligosaccharides, Burkert (2003) indicated the zeolites as a new perspective for sugar separations, achieving good separation of dextran, fructose and glucose in a zeolite fixed bed column. Weston & Brocklebank (1999), Morales et al. (2006) and Kawazoe et al. (2008) studied the separation of monosaccharide and oligosaccharide fractions by passing through a column packed with active carbon and celite.

Therefore, this study aims to contribute knowledge about alternative adsorbents that have affinity for sugars, by characterizing these adsorbents and defining their equilibrium parameters. Thus studies on new purification techniques for these sugars are of great interest in order to provide knowledge on separation processes for such molecules and possibly make their industrial application feasible.

The main objective of this study was to obtain adsorption data for sugars (fructose, glucose, sucrose and fructooligosaccharides), employing alternative adsorbents, and attempt to describe the adsorption behavior by the use of isothermal models, carrying out a selective stage to determine the most adequate adsorbent with a view to studying purification on a fixed bed column.

3.2. Material and Methods

3.2.1. Sugars

Samples of glucose, fructose and sucrose from Sigma and fructooligosaccharides from Wako Pure Chemical Industries (Osaka, Japan), all of analytical grade, were tested using the stirred tank reactor, and only glucose was used in the determination of the isotherms.

3.2.2. Determination of sugars by ion-exchange chromatography

The reducing sugars were determined using the spectrophotometric method of Nelson (1944) and Somogyi (1945). Identification and quantification of the sugars was carried out by ion exchange chromatography with pulsed amperometric detection (HPLC-PAD). Chromatography was performed on a Carbopac PA100 (4x250mm) column with a PA100 (4x50 mm) guard column at 22-24°C, using a GP50 gradient pump, ED40 electrochemical detector and the software PEAKNET, all from Dionex (U.S.A.). The sugars were eluted in 50 mM sodium hydroxide with a linear gradient of sodium acetate (0-500 mM) at a flow rate of 1.0 mL/min. The standards were kestose (GF_2), nystose (GF_3) and fructofuranosyl nystose (GF_4) from Wako Pure Chemical Industries (Osaka, Japan) and sucrose, glucose and fructose from Sigma, all of analytical grade.

3.2.3. Adsorbents

Samples of active carbon, celite and NaX zeolite (UOP/U.S.A), were employed as adsorbents, the zeolites being exchanged with the cations Ca^{+2}, Ba^{+2}, Sr^{2+}, K^{+} and Mg^{2+}. A selection of the adsorbents for use in the stirred tank reactor was carried out with all the adsorbents. Only those selected in the adsorption tests in the stirred tank reactor, were used for the adsorption isotherms and image analysis.

3.2.4. Composition and density

The composition of the zeolite was ascertained by means of Fluorescent X-Ray Spectroscopy, employing a Philips PW 2404 Spectroscope (Vendemiatto & Enzweiler, 2001).

3.2.5. Surface area and porous structure

The isotherm for nitrogen adsorption was obtained at a temperature of 77 K using the Quantachrome Nova 1200 equipment, within the interval of $0.05 < P/P0 < 1.00$, in the Thermal Analysis Laboratory (DEQ/UFSCar/SP/Brazil). The specific surface area (S_{BET}), total pore volume (V_{pores}), micro pore volume (V_{micro}) and the

average pore diameter (D_{pores}), were calculated by fitting the data to the linear BET equation (Equation 1) within the interval 0.05 < P/P0 < 0.29, as well as the pore size distribution (BJH method), with the assistance of the Autosorb software for Windows® v. 1.19.

$$\frac{1}{V\left[\left(\frac{P_0}{P}\right)-1\right]} = \frac{1}{V_m.C_{BET}} + \left(\frac{C_{BET}-1}{V_m.C_{BET}}\right)\frac{P}{P_0} \quad (1)$$

3.2.6. Image analysis

Microphotographs of the support particles of the adsorbents selected in the stirred tank reactor step were taken at different magnitudes, using a JSM5900 LV scanning electron microscope (SEM) at the Brazilian Synchrotron Light Laboratory (LNLS-Brazil). The images were generated by the secondary electrons technique (SEI). The particles received no kind of pre-treatment.

3.2.7. Ion exchange of the zeolites

Were evaluated as potential adsorbers the synthetic zeolites from the group faujasite at six different ionic forms as Na^{+}, Ca^{+2}, Ba^{+2}, Sr^{2+}, K^{+} and Mg^{2+} obtained from the ion exchange with the corresponding salt.

The moisture content of the zeolite was first established in a muffle furnace at 300ºC. The amount of ions to be exchanged for the equivalent amount of grams of Na_2O present in the zeolite was then calculated (10.57% in the original zeolite). The amounts of zeolite, water and saline were also calculated in order to obtain a final concentration of 15% solids, which is actually equivalent to the dry zeolite present within the ionic exchange reactor. The amount of zeolite calculated was suspended in water and the pH calibrated to between 5 and 6 with 10% hydrochloric acid. A 35% solution of the counter cation compound was then added in conformity with the stoichiometry required for exchange. The final suspension was kept under constant mild agitation (100 rpm) for 24 h. The temperature for the exchange processes was 75ºC. After 24 h, the suspension was filtered and washed twice. It was first washed with a 35% solution of the counter cation, using the same amount

used in the exchange. The second wash was carried out with deionized water, using twice volume as employed in the exchange.

3.2.8. Adsorption kinetics in the stirred tank reactor

A solid mass (adsorbent), in the proportion of 1 to 20 of suspension volume (water), was added to the reactor connected to a thermostatically controlled water bath, and left for approximately 12 h with stirring (150 rpm). A 150 g/L solution of the respective sugars (glucose, fructose, sucrose and fructooligosaccharides) was then added, maintaining the 1:20 proportion of solid mass/suspension volume. Samples were removed approximately every 4 h to determine the sugar concentration.

3.2.9. Tukey´s test

Tukey's test was applied in the case of the NaX zeolite at a significance level of 95% (α = 0.05), in order to check whether there were any significant differences between the exchanges accomplished.

3.2.10. Adsorption isotherms

The adsorption isotherms were obtained at temperatures of 30, 40 and 50^{0}C within stirred jacketed reactors containing a solution of pure glucose/zeolite and pure glucose/celite in a (1:20) proportion of solid mass/suspension volume, with mechanical stirring (150 rpm) in a thermostatically controlled water bath for the temperature control. After the equilibrium time (t*), which was defined based on previous studies on adsorption kinetics carried out in this project, the final concentrations were analyzed and the equilibrium isotherms obtained. The amount of glucose adsorbed at equilibrium was calculated according to Equation 2.

$$q^* = \frac{(C_0 - C^*)V_{sol}}{m_a} \quad (2)$$

Where: q* (amount of sugar adsorbed at equilibrium); C_0 (initial concentration of the sugar in solution); C*(sugar concentration in solution at equilibrium); V_{sol} (volume of solution) and m_a (mass adsorbent).

The fitting of the data and estimation of the parameters were accomplished using the Nonlinear Estimation tool of the software Statistica (StatSoft, Inc., USA), according to the linear model, which best described the process.

3.3. Results and Discussion

3.3.1. Adsorption kinetics

According to the results obtained in the stirred reactors at 40^0C, a more significant drop in concentration was observed after 120 min, showing small oscillations probably due to water adsorption/desorption. Therefore an equilibrium time of (t* = 120 min) was adopted, since this was the best time observed for the different adsorbents and saccharides.

On examining the results of the equilibrium time (t*=120 min) (Table 1), it can be seen that with respect to the different cations exchanged, the NaX zeolite present significant differences from the BaX and MgX zeolites. Therefore the NaX zeolite was chosen to continue the study, since this appeared to be the most promising from the isotherm results, where the equilibrium adsorption capacity was 1.496 g glucose/g zeolite. This represents a high capacity for sugar adsorption, mainly for glucose, and did not require the ion exchange stage, since Na^+ is the major ion of NaX zeolite.

Table 1– Adsorption equilibrium (t*=120 min) of glucose, fructose and sucrose on X zeolite exchanged with different cations.

Cation	C/C_o(glucose)	C/C_o(fructose)	C/C_o(sucrose)
Na^+	0.72 ± 0.13^{A}	0.67 ± 0.13^{A}	0.99 ± 0.05^{BC}
K^+	0.83 ± 0.094^{AB}	0.90 ± 0.15^{AD}	0.97 ± 0.03^{BC}
Sr^{+2}	0.84 ± 0.00^{AC}	0.99 ± 0.04^{BCD}	0.97 ± 0.08^{BC}
Ca^{+2}	0.91 ± 0.08^{AD}	0.81 ± 0.10^{AB}	0.78 ± 0.06^{A}
Ba^{+2}	0.95 ± 0.03^{BCD}	0.85 ± 0.08^{AC}	0.90 ± 0.02^{AC}
Mg^{+2}	1.05 ± 0.06^{CD}	0.98 ± 0.11^{BCD}	0.86 ± 0.06^{AB}

The same letters indicate no statistical difference amongst them, according to Tukey's test (α=0.05).

C = adsorbate concentration in the solution at equilibrium.

C_0 = initial concentration of the adsorbate in the solution.

Figures 1, 2 and 3 show the adsorption kinetics for glucose, fructose and sucrose on zeolite, exchanged with the three ions that presented the highest adsorption rates.

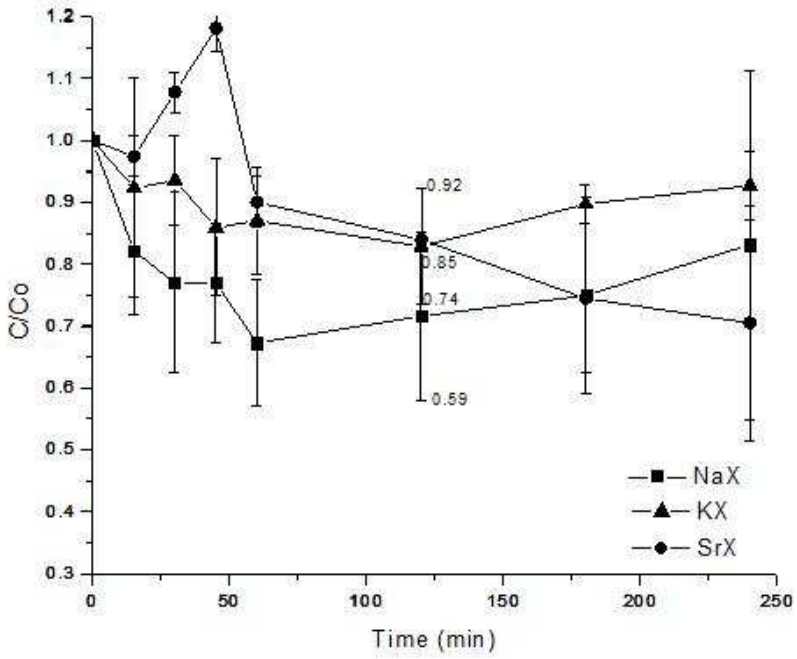

Figure 1 – Adsorption kinetics of glucose on zeolite.

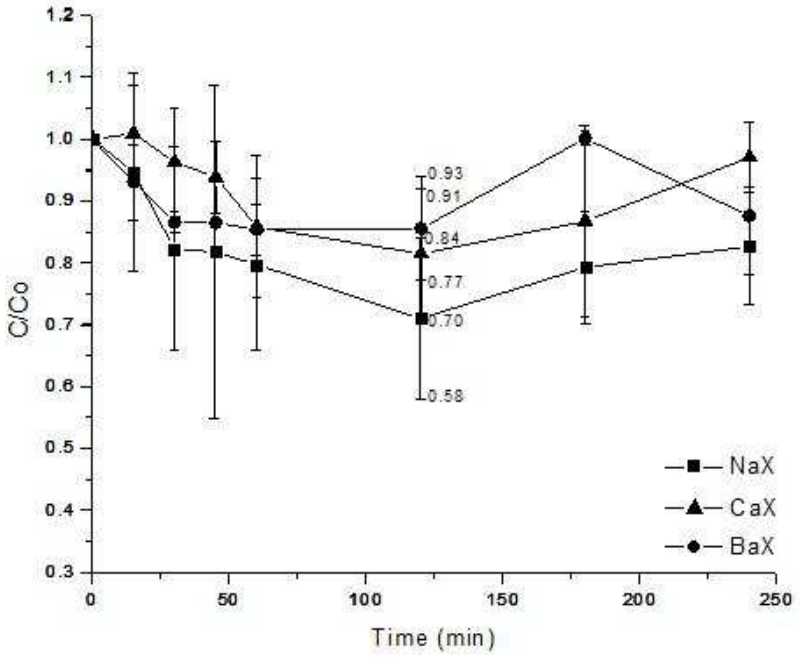

Figure 2 – Adsorption kinetics of fructose on zeolite.

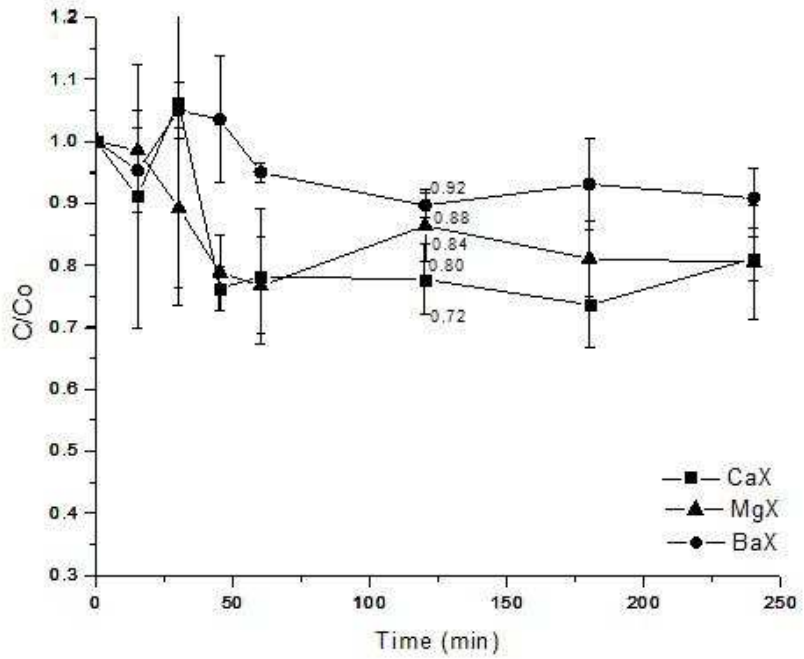

Figure 3- Adsorption kinetics of sucrose on zeolite.

In the case of ion Ba^{2+} there is interactions with three oxygen-containing groups of monosaccharides independent of the Si:Al ratio, because the ionic radii of Ba^{2+} is higher than that for Ca^{2+} and the number of coordinated water molecules is

three or more for these divalent cations in their positions in X and Y zeolites. Therefore the dependence of the separation factor on the Si:Al ratio can be neglected (Schöllner et al., 1993).

Heper et al. (2007) studied the separation of glucose and fructose using Y zeolites exchanged with various ions (Na^+, NH_4^+, Ca^{2+} and Mg^{2+}), the results indicating that only the NH_4^+ and Ca^{2+} forms were potentially selective adsorbents for the separation of glucose and fructose. In this study the zeolite exchanged with Ca^{2+} presented a significant adsorption of fructose (C/C_o = 0.81±0.10) and sucrose (C/C_o = 0.78±0.06).

An examination of the results from stirred tank reactors with active carbon and celite as adsorbents (Table 2), showed that the adsorption of glucose on celite was highly pronounced (C/C_o= 0.42±0.03) (Figure 4). Considering the maximum adsorption capacity at equilibrium of 1.35 g glucose/g celite, the latter was selected as one of the adsorbents for the isothermal determination.

Table 2 – Adsorption equilibria for glucose, fructose, sucrose and FOS on active carbon and celite

C/C_0	**Carbon**	**Celite**	**Carbon/Celite**
Glucose	0.92±0.01	0.42±0.03	0.92±0.01
Fructose	0.96±0.12	0.70±0.01	0.94±0.01
FOS	0.57±0.07	1.01±0.07	-----
Sucrose	0.90±0.02	0.77±0.05	0.95±0.06

C = adsorbate concentration in the solution at equilibrium.
C_0 = initial concentration of the adsorbate in the solution.
(----)Not determined

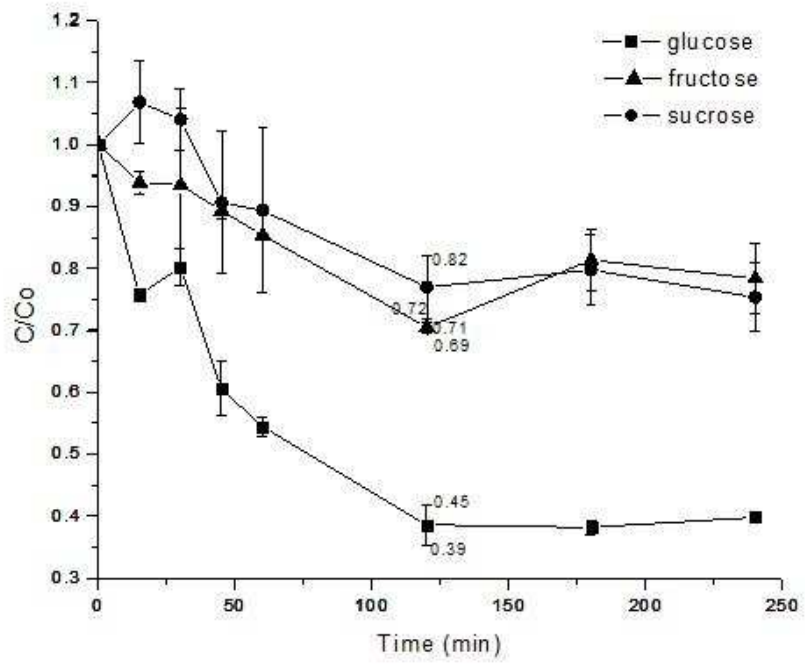

Figure 4 - Adsorption kinetics of saccharides on celite.

Active carbon satisfactorily adsorbed the fructooligosaccharides (FOS) (C/C_o=0.57±0.07) (Figure 5), but it showed little affinity for the other sugars (glucose, fructose and sucrose) (Figure 6). Thus this adsorbent was only employed in a later stage of this work, in a study with fixed bed columns for the separation of fructooligosaccharides, glucose, fructose and sucrose.

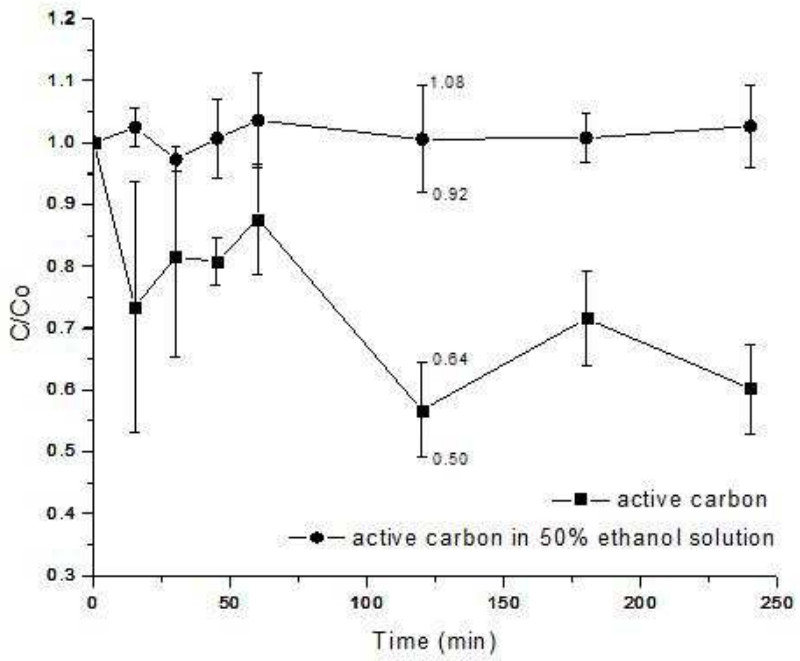

Figure 5 - Adsorption kinetics of fructooligosaccharides on active carbon.

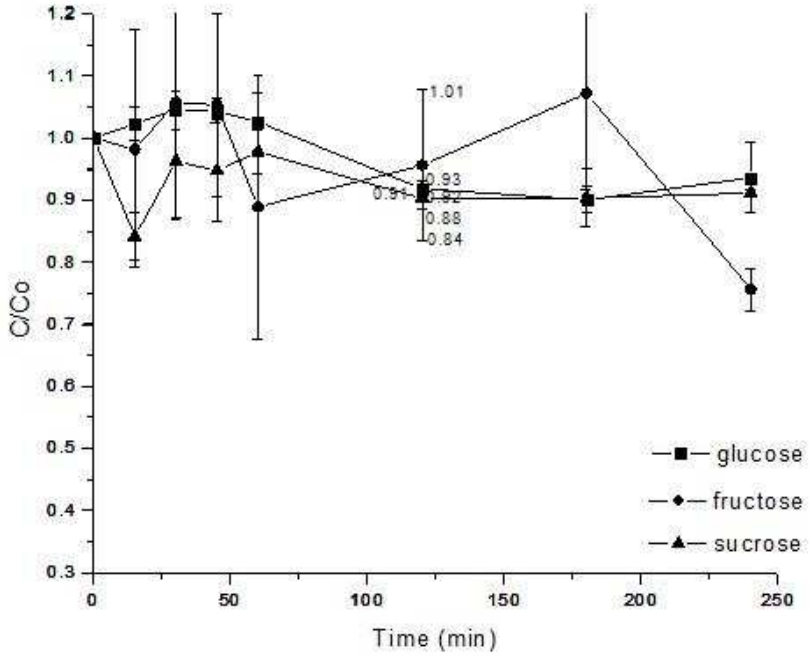

Figure 6 - Adsorption kinetics of saccharides on active carbon.

Some assays employing a mixture of celite/active carbon (1:1) as the adsorbent were carried out, and at the adsorption equilibrium, similar results to those obtained with pure active carbon were obtained for glucose, fructose and

sucrose. The assay with active carbon and celite was performed because the active carbon didn't show affinity by monosaccharides and the celite has affinity for these saccharides, and then a mixture of these adsorbents was tested to observe the adsorption.

The influence of adding an ethanol/water solution (50/50) (v/v) was studied in order to verify the possibility of improving adsorption (Figure 5). The results showed that a high ethanol concentration in the suspension did not increase the adsorption of fructooligosaccharides (C/C_o= 1.00±0.08) at the adsorption equilibrium. The study carried out by Morales et al. (2006) showed good recovery of fructooligosaccharides using ethanol/water (50/50) % (v/v). The ethanol in higher concentration has influence on the recuperation of saccharides, and in small concentration has influence in the adsorption/desorption.

The adsorption isotherms were only determined for glucose since, along with the fructooligosaccharides, this sugar was found to have one of the highest concentrations in the sugar mixture. The sugar mixture consisted of approximately 270 g/L FOS, 176 g/L glucose, 44 g/L sucrose and 10 g/L fructose, obtained by enzymatic synthesis. Therefore the equilibrium parameters are of extreme importance in the FOS purification process.

According to results found for the stirred reactor with respect to the adsorption kinetics and equilibrium time, celite and NaX zeolite were selected for the determination of the adsorption isotherms with glucose.

These adsorbents can be useful in adsorption processes in fixed bed columns, since they are inexpensive and show high regeneration capacity. Ho et al. (1987) worked with the CaY zeolite and proved it to be similar, in terms of selectivity, to ion exchange resins, with the advantage of presenting less resistance to mass transfer, showing the benefit of using it in sugar adsorption processes, since zeolite has lower operational costs.

In general, zeolite has excellent mechanical properties which can withstand extremely unfavorable chemical conditions and can be sterilized by conventional methods (Yu et al., 1998). Zeolites are porous crystalline materials and have effective pore openings of 8-9 Å. They are also known as molecular sieves,

compounds larger in structure than the pore diameter being excluded by the zeolite, and compounds smaller in structure being adsorbed by the micropores. In addition, they have electrostatic force fields inside the cages and channels. Consequently they are capable of both physical sieving and selectivity with respect to the incoming molecules. This influence is enhanced by the stripping function of the desorbent, hence they are very effective in column separation process (Lu & Lee, 1987).

3.3.2. Characterization of the selected zeolite

NaX zeolite is composed of approximately 42% SiO_2, 24.70% Al_2O_3 and 13.6% Na_2O. Such data represent the characteristic composition of zeolites of the NaX type, since the major oxide was Na_2O in a Si/Al rate of 1.5, and therefore the zeolite is an X type one (Breck, 1974).

Table 3 shows the properties of the NaX zeolite, the results being similar to those found in the literature. Yu et al. (1998) worked with an X zeolite with a specific area of 400 m^2/g, and Burkert (2003) worked with a Y zeolite with a specific area of 448 m^2/g.

Table 3 – Specific surface area (S_{BET}), micropore volume (V_{micro}), total pore volume (V_{pores}) and average pore diameter (D_{pores}) of NaX zeolite.

Property	Value
S_{BET}	444.86 m^2/g
V_{micro}	0.2028 cm^3/g
V_{pores}	0.308 cm^3/g
D_{pores}	27.7 Å

3.3.3. Image analysis

Images of the morphology and topography of the microcrystalline structure of the NaX zeolite were obtained by scanning electron microscopy (SEM). Figure 7 shows a micro-photograph of a NaX zeolite particle enlarged 230 and 2000 times, showing the zeolite microstructure. As reported by Moscou (1991), it can be seen that the zeolites display a micro-porous structure and uniform pore dimensions.

From the images, it can be seen that the zeolite crystal had dimensions between 2-3 µm. Yu et al. (1998) observed zeolite crystals of between 3-4 µm using an electron microscope. As reported by Serralha et al. (1998), type NaX, NaY and NaA zeolites are constituted of 2 µm, 0.5 – 1 µm and 1 - 2 µm crystallites, respectively.

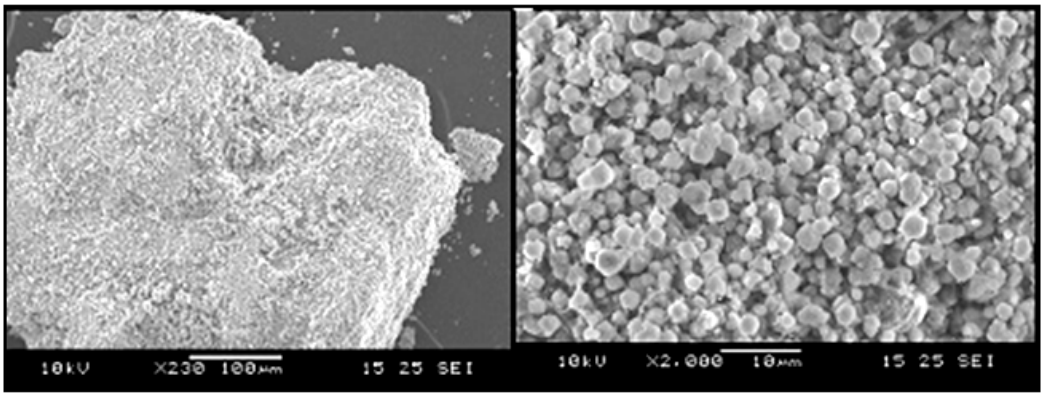

Figure 7- SEM of NaX zeolite, enlarged x 230 and x 2000.

Figure 8 shows images of celite, enlarged 270 and 2200 times, showing the microstructure and confirming that the pore dimensions of this adsorbent are not uniform, making it difficult to standardize the pores of this adsorbent.

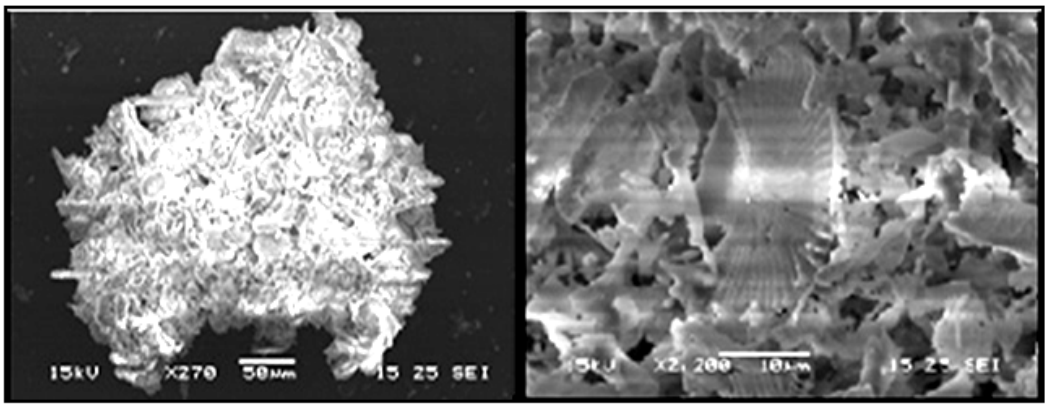

Figure 8- SEM of celite, enlarged x 270 and x 2200.

When a comparison was made between the pore structure of the two adsorbents it could be seen that the celite presented an irregular pore structure, although it showed good adsorption of the sugars. However, as a natural adsorbent, it must be remembered that the samples come from different origins, and the adsorption properties can change greatly. This adsorption process involves weak

forces, such as Van der Walls and ion exchange forces, in accordance with the adsorption affinity of the sugar. Zeolite is a synthetic adsorbent and has a homogeneous pore structure, presenting an interesting sugar adsorption property. It is a thus a promising adsorbent in sugar adsorption processes.

3.3.4. Adsorption isotherms

The isotherms were determined at temperatures of 30, 40 and 50ºC, since higher temperatures result in greater sugar adsorption. Table 4 shows the experimental data fitted using Equation 3 for celite as the adsorbent. According to the sugar, temperature and concentration conditions defined, the linear model was shown to be the one that best fitted the data collected. These results are in accordance with the work of Nobre et al. (2009) that reported the adsorption of glucose, fructose and sucrose in ion-exchange resins were appropriately fitted by the linear regression models.

$$q^* = k.C^* \quad (3)$$

where q* (amount of adsorbate adsorbed at equilibrium), k (partition coefficient) and C* (concentration of adsorbate in the solution at equilibrium).

Table 4 - Parameters of the linear model for the adsorption of glucose on celite.

Model	T(ºC)	k 10^3(L/g)	r^2
$q^* = k.C^*$	30	4.09±0.27^A	0.88
	40	5.06±0.36^B	0.94
	50	6.55±0.23^C	0.98

The same letters indicate no statistical difference amongst them, according to Tukey's test (α=0.05).

In order to achieve greater reliability with respect to the data of the effect of temperature on the adsorption of glucose on celite, Tukey's test (α=0.05) was used. An analysis of the results showed that for the temperatures studied, significant differences were found between them, with p=0.05. Thus there was higher glucose adsorption at 50ºC, since this temperature was statistically different from all the

others. In the present work, the highest temperature studied was 50°C, because higher temperatures may darken the sugar solution due to the effect of caramelization (Cheng & Lee, 1992).

Figure 9 shows the adsorption isotherm of glucose on celite, the dots representing the experimental data and the lines the data obtained by linear regression.

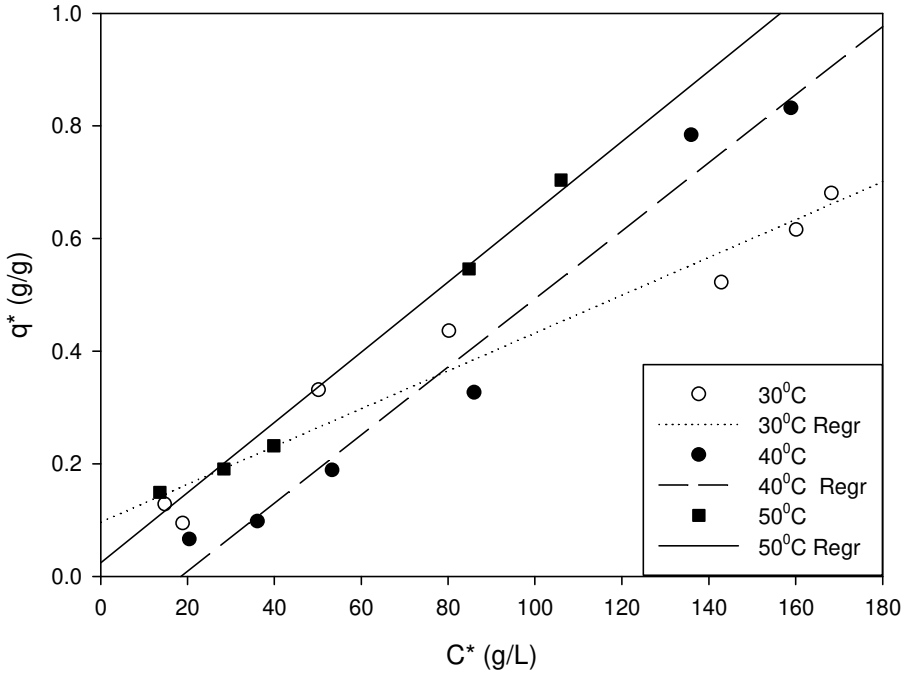

Figure 9- Linear regressions of the experimental data for the adsorption isotherms of glucose on celite.

The NaX zeolite partition coefficient (*k*) was higher at the temperature of 50°C (Table 5), where the behavior was similar to that of celite, showing that higher temperatures effectively facilitated sugar adsorption. According to Tukey's test there was no significant difference between the adsorption coefficients obtained at the temperatures of 30 and 40°C, but that obtained at 50°C was statistically different, and therefore 50°C is the most adequate temperature if high sugar adsorption is required.

Table 5 – Parameters of the linear model for the adsorption of glucose on NaX zeolite.

Model	T(^{0}C)	k 10^3(L/g)	r^2
$q^* = k.C^*$	30	3.75±0.34^A	0.88
	40	4.06±0.67^A	0.98
	50	5.79±0.37^B	0.90

The same letters indicate no statistical difference amongst them, according to Tukey's test (α=0.05).

Figure 10 shows the linear regressions obtained at the three temperatures (30, 40 and 50^{0}C), the dots standing for the experimental data and the lines standing for the data estimated by linear regression.

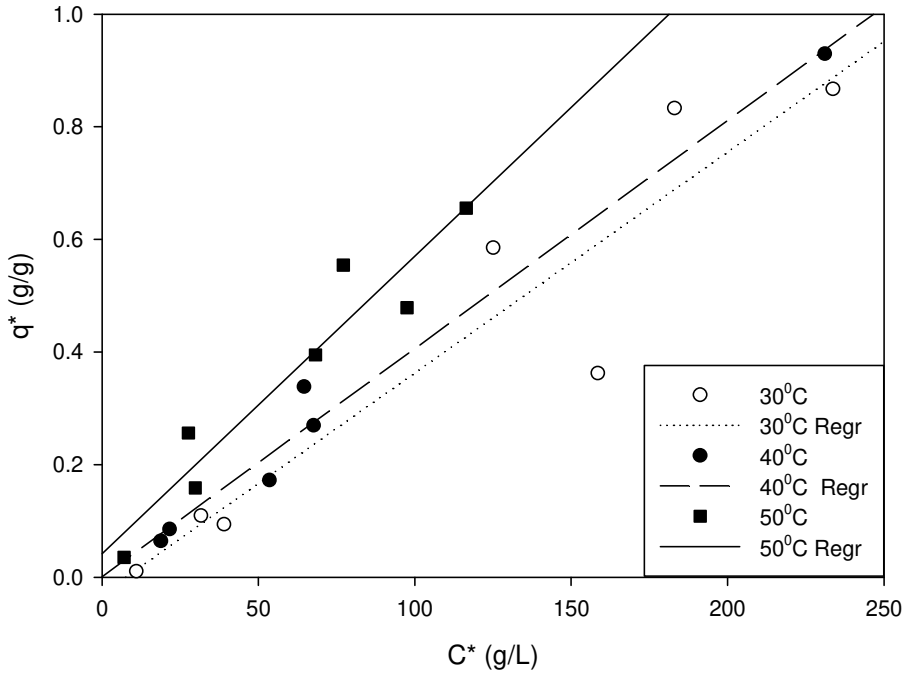

Figure 10 – Linear regressions of the experimental data for the adsorption isotherms of glucose on NaX zeolite.

3.4. Conclusions

According to the results obtained for the glucose adsorption kinetics in stirred reactors, celite and NaX zeolite were shown to have the highest affinity for glucose, where the maximum adsorption at equilibrium was 1.496 g glucose/g zeolite and 1.35 g glucose/g celite, at 40^{0}C.

The highest partition coefficient (*k*) was obtained at a temperature of 50^{0}C for both celite and NaX zeolite, thus indicating that the greatest adsorption of glucose occurs at this temperature and defining this temperature as the most favorable for sugar adsorption. In addition, this conclusion was confirmed by Tukey´s test, which showed a significant difference (α= 0.05) with respect to the other temperatures studied.

Active carbon presented a greater affinity for the adsorption of fructooligosaccharides, showing that this adsorbent could be employed in subsequent work to separate these from the other sugars, possibly using fixed bed columns.

Image analyses showed that the NaX zeolite presented a regular structure of uniform pores with micro porous characteristics, and a zeolite crystal size ranging between 2-3 μm. Celite did not present the same uniformity, but may, nevertheless, be of interest in the adsorption of sugars, mainly if glucose is the target product.

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Chapter 4

Mathematical modeling as a tool to investigate the separation of mono- and disaccharides from enzymatic synthesis of fructooligosaccharides by adsorption in zeolites

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Bioprocess and Biosystems Engineering (Submitted)

Mathematical modeling as a tool to investigate the separation of mono- and disaccharides from enzymatic synthesis of fructooligosaccharides by adsorption in zeolites

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Abstract

This work investigates the batch adsorption of glucose, fructose, and sucrose from the enzymatic synthesis of fructooligosaccharides using six cationic forms of the X zeolite (Na^{+}, Ca^{+2}, Ba^{+2}, Sr^{2+}, K^{+} and Mg^{2+}). A mathematical model was proposed taking into account the kinetic and mass transfer phenomena, where the screening was based on the estimation and analysis of the model parameters in terms of adsorption rates and mass transfer resistances involved in the process. From the screening step, the Na^{+} form zeolite was the most promising, since it presented the highest adsorption rates and the lowest mass transfer resistance amongst the materials evaluated. The strategy adopted to select the most appropriated material to separate the saccharides showed to be interesting and applicable on the choice of other material for the purification of any bioproduct, since it is based on fundaments that taking into account the adsorption rates and mass transfer phenomena.

Key-words: Adsorption; mass transfer resistance; adsorption rate; fructooligosaccharides.

Nomenclature

Bi – Biot number

C – Concentration of saccharides in the liquid phase (gL^{-1})

C_0 – Initial concentration of saccharides in the liquid phase (gL^{-1})

C_b – Mean concentration of saccharides in the liquid phase (gL^{-1})

C_s – Concentration of saccharides in the particle surface (gL^{-1})

c_1, c_2 – Search parameters for the PSO Algorithm

D_{ef} – Effective diffusivity (m^2min^{-1})

k_1 – Intrinsic constant rate ($Lg^{-1}min^{-1}$)

k_2 – Intrinsic constant rate (min^{-1})

k_D – Dissociation constant (gL^{-1})

k_s – Film coefficient ($mmin^{-1}$)

q_m – Maximum adsorption capacity of the zeolite ($g_s g_{res}^{-1}$)

r – Radial position

R – Gas constant ($Jmol^{-1}K^{-1}$)

v – Velocity (or pseudo-velocity) of the particle

v_{obs} – Observed reaction rate ($gL^{-1}s^{-1}$)

V_l – Liquid volume (L)

V_s – Solid volume (L)

w – Search parameters for the PSO Algorithm

x – Position of the particle

φ – Thiele modulus

φ^{ap} – Apparent Thiele modulus

ε_p – Resin porosity

4.1. Introduction

Fructooligosaccharides (FOS) are a group of oligomers containing one glucose unit and 2 to 10 fructose units attached by a β-(2-1) bond. The most common are the three smallest oligomers: kestose, nystose, and fructofuranosylnystose [1]. FOS successfully entered the international functional food market as ingredients, after their FDA approval in 2000. They are produced industrially either by chemical hydrolysis of inulin from chicory or Jerusalem artichoke or by enzymatic transfructosylation of concentrated sucrose solutions [2]. In the latter case, one glucose molecule is release per transferred fructose molecule. The reactor outlet product contains, besides FOS, a large amount of glucose, un-reacted sucrose, and a small portion of fructose, which is produced by the hydrolytic side reaction. These mono- and disaccharides should be separated from FOS in order to maintain their functional properties [3].

The design of an industrial-scale separation process, including the selection of suitable adsorbent materials, needs the knowledge of adsorption equilibrium of individual compounds contained in the feed stream and the mass-transfer effects on the overall kinetics, taking into account mass-transfer and kinetic limitation [3,4]. Recent technologies on adsorption processes use resins or zeolites. Many researchers have studied zeolites, focusing mostly on zeolites X and Y, using chromatographic methods. NaX was reported to be glucose selective, KX and certain cationic forms of Y (Ca-, K-, and Sr-) were found to be fructose selective [5,6].

Regarding the use of zeolites to recovery mono- and disaccharides from enzymatic synthesis of FOS it is interesting to determine the dynamic and thermodynamic properties of the system in terms of equilibrium data, film mass transfer coefficient, intraparticle diffusivity, which are important to help the selection and optimization of a purification process. They determine the range within of which the sorption step can be carried out to ensure a good performance of the equipment, and give support to the process scale-up. The usefulness and confidence of the kinetic and mass transfer parameters are dependent of the quality of the estimation. The use of empirical correlations to estimate mass transport parameters impose restrictions to the model, since its predictions are validated for a narrow range of independent variables, implicating in a bad estimation of model parameters. Regarding the equilibrium parameters, it is preferable to estimate their values from process data instead of equilibrium data because some imprecision

during the estimation procedure probably will jeopardize the overall model performance.

In the present work, adsorption experiments were carried out in stirred tank reactor to verify the kinetics and mass-transfer effects on the purification of mono- and disaccharides from enzymatic synthesis of FOS. A mathematical model was proposed for the adsorption of glucose, fructose, sucrose and FOS in a stirred tank reactor using several kinds of zeolites as adsorbers. The model was used as a tool for the screening of the most appropriated material, where the choice was based on the mass transfer phenomena, by the analyzing the estimated parameters for each kind of material.

4.2. Materials and methods

4.2.1. Adsorbents

Were evaluated as potential adsorbers the synthetic zeolites from the group faujasite at six different ionic forms as Na^{+}, Ca^{+2}, Ba^{+2}, Sr^{2+}, K^{+} and Mg^{2+} obtained from the ion exchange with the corresponding salt.

The moisture content of the zeolite was first established in a muffle furnace at 300ºC. The amount of ions to be exchanged for the equivalent amount of grams of Na_2O present in the zeolite was then calculated (10.57% in the original zeolite). The amounts of zeolite, water and saline were also calculated in order to obtain a final concentration of 15% solids, which is actually equivalent to the dry zeolite present within the ionic exchange reactor. The amount of zeolite calculated was suspended in water and the pH calibrated to between 5 and 6 with 10% hydrochloric acid. A 35% solution of the counter cation compound was then added in conformity with the stoichiometry required for exchange. The final suspension was kept under constant mild agitation (100 rpm) for 24 h. The temperature for the exchange processes was 75ºC. After 24 h, the suspension was filtered and washed twice. It was first washed with a 35% solution of the counter cation, using the same amount used in the exchange. The second wash was carried out with deionized water, using twice volume as employed in the exchange.

4.2.2. Adsorption kinetics in the stirred tank reactor

A solid mass (adsorbent), in the proportion of 1:20 (w/v) of suspension volume (water), was added to the reactor connected to a thermostatically controlled

water bath, and left for approximately 12 h with stirring (150 rpm) at 40°C. A 150 g/L solution of the respective sugars (glucose, fructose, sucrose or fructooligosaccharides) was then added, keeping the 1:20 proportion of solid mass/suspension volume. Samples were removed approximately every 2 h to determine sugar concentration.

4.2.3. Determination of sugars by ion-exchange chromatography

Identification and quantification of the sugars was carried out by ion exchange chromatography with pulsed amperometric detection (HPLC-PAD). The chromatography was performed on a Carbopac PA100 (4x250mm) column with a PA100 (4x50 mm) guard column at 22-24ºC, using a GP50 gradient pump, ED40 electrochemical detector and the software PEAKNET, all from Dionex (U.S.A.). The sugars were eluted in 50 mM sodium hydroxide with a linear gradient of sodium acetate (0-500 mM) at a flow rate of 1.0 mL/min. The standards were kestose (GF_2), nystose (GF_3) and fructofuranosylnystose (GF_4) from Wako Pure Chemical Industries (Osaka, Japan) and the sucrose, glucose and fructose from Sigma were all of analytical grade.

4.2.4. Mathematical modeling for the adsorption step in a stirred tank reactor

It was assumed that V_s is the volume of adsorber immersed in a volume V_l of liquid with sugars dissolved at an initial concentration C_0, contained in a perfectly stirred reactor. In formulating the model, it is assumed that the resin particles are spherical; sugars diffusion in the solid particles follows Fick's law; diffusion occurs in the *r* direction only; and adsorption takes place under isothermal conditions. The adsorbed sugars are assumed to be in equilibrium with that in the pore fluid at each radial position within the particle. The following conservation equations and boundary conditions are used to describe the sugars uptake kinetics for spherical particles of radius R_p in a closed batch system. Based on the above assumptions, the adsorption process in a constant volume stirring tank can be described by Eq. (1)

$$\frac{dC_b}{dt} = -\frac{3}{R_p}\frac{V_s}{V_l}k_s\left(C_b - C_s\right) \qquad (1)$$

where C_b is the bulk and C_s the surface of solid concentration of sugars. The initial condition for Eq. (1) is:

$$t = 0 \rightarrow C_b = C_0 \quad (2)$$

The differential material balance inside the solid particles, where adsorption takes place on the porous surface is expressed by Eq. (3).

$$\frac{\partial C_i}{\partial t} = D_{eff}\left(\frac{\partial^2 C_i}{\partial r^2} + \frac{2}{r}\frac{\partial C_i}{\partial r}\right) - \frac{(1-\varepsilon_p)}{\varepsilon_p}\frac{\partial q_i}{\partial t} \quad (3)$$

If one considers that equilibrium occurs at the surface and assuming Eq. (4), Eq. (3) can be reduced to Eq. (5).

$$\frac{\partial q_i}{\partial t} = \frac{\partial C_i}{\partial t}\frac{\partial q_i}{\partial C_i} \quad (4)$$

$$\left[\varepsilon_p + (1-\varepsilon_p)\frac{\partial q_i}{\partial C_i}\right]\frac{\partial C_i}{\partial t} = D_{eff}\left(\frac{\partial^2 C_i}{\partial r^2} + \frac{2}{r}\frac{\partial C_i}{\partial r}\right) \quad (5)$$

The initial and boundary conditions associated with the diffusion process inside the solid particles are, respectively:

$$t = 0 \rightarrow C_i = q_i = 0 \quad (6)$$

$$r = R \rightarrow \frac{\partial C_i}{\partial r} = \frac{k_s}{\varepsilon_p \cdot D_{eff}}(C_b - C_s) \quad (7)$$

$$r = 0 \rightarrow \frac{\partial C_i}{\partial r} = 0 \quad (8)$$

To solve the partial differential equation (Eq. 5), the spatial derivatives were approximated by finite differences using equal size elements. This approximation results in a system of ordinary differential equations composed of n equations inside the solid particle plus the differential mass equation in liquid-phase (Eq. 1), which was solved using the LIMEX routine [7]. The model parameters, namely q_m, k_1, k_2, D_{ef} and k_f were estimated using a heuristic method, such as Particle Swarm Optimization (PSO).

The estimation of the parameters consisted of minimizing the sum of the least squares (SSR) as described in Eq. 9:

$$SSR = \sum_{i=1}^{n=NPE}\left(y_i - y_i^{calc}\right)^2 \quad (9)$$

where NPE is the number of experimental points used in the estimation, y is the vector of the experimental data points and $\mathbf{y}^{calc}$ is the vector calculated by the model.

The parameters were estimated for each form of the zeolite and, after this procedure, they were used to evaluate the mass transfer resistance in the process, the dissociation constant and the maximum adsorption capacity of the zeolites. The mass transfer resistances were analyzed by estimating the Biot number (Eq. 10), also known as dimensionless concentration gradient in liquid film surrounding the solid particles, which is related to the external mass transfer resistance, and by the estimation of the apparent Thiele modulus (Eq. 11), that is related to the internal mass transfer resistance.

$$Bi = \frac{k_f R}{D_{ef}} \tag{10}$$

$$\varphi^{ap} = \frac{R^2}{9} \frac{v_{obs}}{D_{ef} C_0} \tag{11}$$

where

$$v_{obs} = \frac{\Delta C}{\Delta t} \tag{12}$$

The criteria used to select the most appropriated material to separate saccharides from a liquid solution were based on the following statements: internal and external resistances as low as possible, highest binding capacity of the saccharides into the zeolite and the lowest value for the dissociation constant.

4.2.5. Particle swarm optimization

The PSO version used in this study was based on the work of Schwaab et al. [8] which presents a detailed description of the algorithm. The PSO technique was originally proposed by Kennedy and Eberhart [9] based on the social behavior of collection of animals. Each individual of the swarm, called particle, remembers the best solution found by itself and by the whole swarm along the search trajectory. The particles move along the search space and exchange information with others particles, in accordance with the following equations:

$$v_{p,d}^{k+1} = w \cdot v_{p,d}^{k} + c_1 \cdot r_1 \left(x_{p,d}^{ind} - x_{p,d}^{k} \right) + c_2 \cdot r_2 \left(x_{d}^{glo} - x_{p,d}^{k} \right) \tag{13}$$

$$x_{p,d}^{k+1} = x_{p,d}^{k} + v_{p,d}^{k+1} \tag{14}$$

In the Eqs. (13)-(14), p denotes the particle, d is the search direction, k represents the interation number, v is the velocity (or pseudo-velocity) of the particle

and x is the position of particle, x^{ind} and x^{glob} represent the regions of the search space where the objective function attains low (optimum) values, where x^{ind} is the best position found by the particle itself, while x^{glob} is the best position found by whole swarm. In addition, r_1 and r_2 are two random numbers with uniform distribution in the range [0,1]. The parameters w, c_1 and c_2 are search parameters, which there are called of inertial weight, the cognition and social parameters, respectively.

After a preliminary set of simulations, it was defined the best configuration of the PSO algorithm that is as following: forty particles were used for the PSO algorithm, and the inertial weight, cognition and social parameters were set at 0.7, 1.0, 1.0, respectively.

4.3. Results and Discussion

Figure 1 presents the dynamic behavior of single-component adsorption of glucose, fructose and sucrose on various cationic forms of X zeolite. It is observed that the equilibrium is almost established within 60 min in all the cases. Heper et al. [6] studied the glucose adsorption, observing that the equilibrium is almost established within 30 min in all cases. The amount adsorbed at equilibrium increases according to the sequence $NH_4^+ < Mg^{2+} < Ca^{2+} < Na^+$ forms of the zeolite. In this study, when glucose adsorption is considered, the amounts adsorbed decreases according to the sequence $Na^+<Sr^{2+}<K^+<Ca^{2+}<Mg^{2+}<Ba^{2+}$. Since the amount adsorbed for the forms Ca^{2+}, Mg^{2+}, Ba^{2+} was very low, the most attractive material to separate glucose are the forms Na^+, Sr^{2+} and K^+. Considering the fructose separation, attractive results were obtained (according to the sequence) for the forms $Na^+>Ba^{2+}>Ca^{2+}$. The hydrated Ba ions cannot migrate into the sodalite unit and the hexagonal prism during ion exchange because of their large ionic radii. They occupy positions in the supercage and can interact with the adsorbents even at a low degree of exchange [10]. In the study reported by Herper et al. [6] when fructose adsorption was considered, there was an increase according to the sequence $Ca^{2+}<Na^+<NH_4^+<Mg^{2+}$. In the case of sucrose separation it was verified that all the cationic forms presented low adsorption capacity, resulting in an inefficient separation. Gramblicka and Polakovic [3] reported that the capacity of the adsorbents (Diaion, Dowex, Lewatit and Amberlite) in respect to individual

saccharides decreased in the order fructose > glucose > sucrose > kestose > nystose > fructofuranosylnystose.

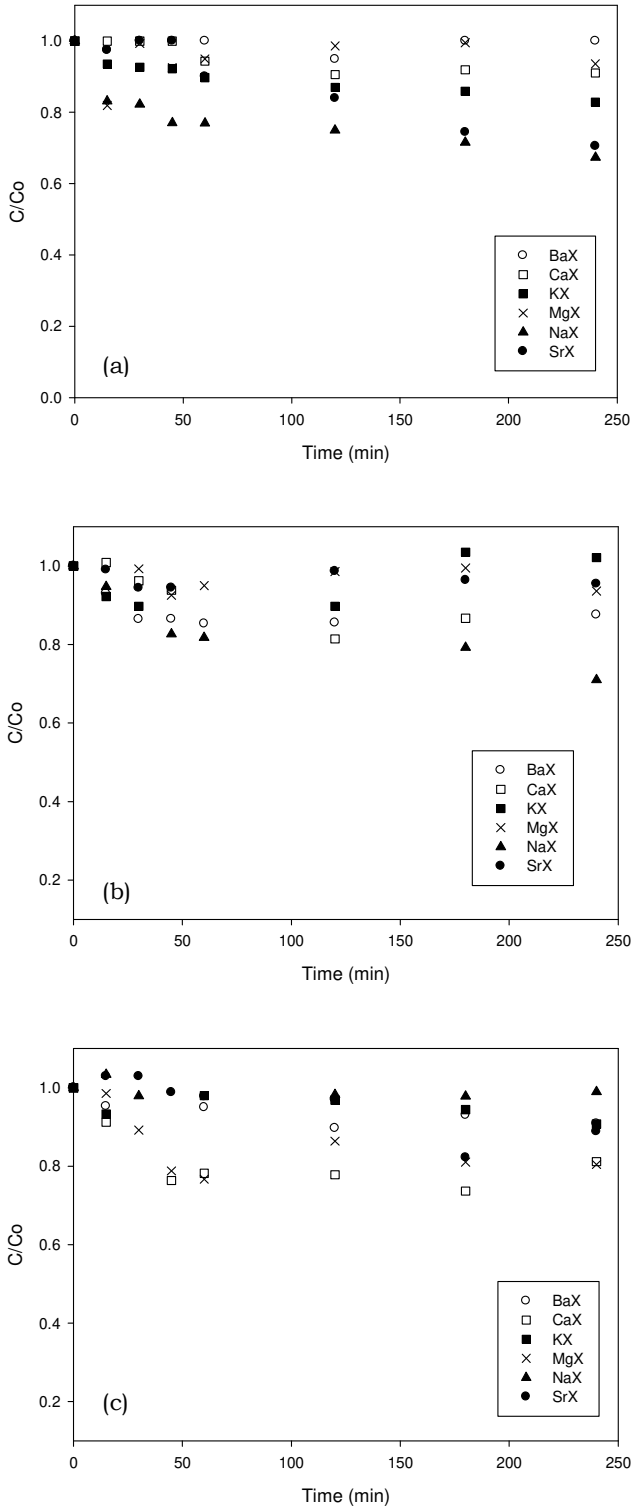

Figure 1: Adsorption kinetics for the glucose (a), fructose (b) and sucrose (c) in all the cationic forms of the X zeolite.

Based on the experimental results showed at Figure 1 it is rather difficult to select the most appropriated zeolite form to purify the FOS, since there are some forms with very similar results. To overcome this difficulty, it was been proposed a strategy based on mathematical modeling of the adsorption process in stirred tank reactor. The strategy is based on the estimation and analysis of model parameters. Table 1 presents the estimated model parameters for glucose, fructose and sucrose for all the cationic forms.

By analyzing the kinetic parameters k_1 and k_2 it is possible to verify that the adsorption process is less favorable when Na^+ form is used, since the relation of k_2/k_1 is higher than 1, indicating that the rate of desorption is higher than the adsorption ones. Nevertheless, it was verified the highest values for the maximum adsorption capacity (q_{max}) for Na^+ form, which can balance the higher desorption rate compared to the adsorption ones. The low adsorption capacity and the unfavorable adsorption rates could be affected by the high film resistance to mass transfer expressed in terms of the film coefficient k_f. For all the situations the estimated values for k_f were very low, including the Na^+ form that presented the lowest external resistance to mass transfer compared to any other cationic forms. According to this, the increase in the adsorption rate does not imply the increasing of the adsorption capacity, because the external mass transfer is a limiting step in this process.

In these heterogeneous systems, the reaction occurs inside the solid particles and there may be problems due to external and internal mass transfer resistances. The mechanisms could include film diffusion, surface or pore diffusions, capture of solute that could be by chemisorptions, phisisorption, ion exchange or complexation, amongst others [11]. An alternative to investigate these phenomena is evaluating the model parameters by mean of dimensionless number as Biot and Thiele module. The Biot number, which is related to the external mass transfer resistance, presented low values for glucose, fructose and sucrose in all the ionic forms, showing that the external mass transfer is the limiting step for the adsorption process of these sugars into the zeolites, corroborating with the above discussion regarding the low values for the film mass transfer coefficient (k_f). The exceptions were for sucrose and FOS in the Na^+ form and sucrose for Mg^{2+} form,

which presented high values for Biot number, indicating that the internal mass transfer is the limiting step.

Usually values of φ^{ap} < 0.3 indicate limitation by adsorption rate and φ^{ap} > 0.3 mass-transfer limitation due to diffusion [4]. Based on this statement, the most significant limitation step during the separation of sugars by zeolites is the adsorption, since high values for Thiele number were verified for all sugars. Based on the Biot and apparent Thiele numbers both external mass transfer and adsorption rate are significant limitations for the separation of saccharides by zeolites for all ionic forms.

Based on the experimental results, on the estimated kinetic and mass transfer parameters the most appropriated zeolite for separation of glucose, fructose and sucrose was the Na^+ form, since high observed adsorption rates and, mainly, low mass transfer resistance were observed in comparison with any other cationic forms. Adsorption kinetics of FOS was carried out using the Na^+ form zeolite. It was verified a low adsorption capacity and a higher mass transfer resistance, resulting in an inefficient separation. This is desirable because in a mixture containing sucrose, fructose, glucose and FOS it is possible to separate FOS in the solution, since the others sugars will be bond to the zeolite.

Table 1: Estimated model parameters for each sugar at all the cationic forms of the X zeolite

	$q_{máx}$ g_s/g_{res}	k_1 L/g.min	k_2 min^{-1}	k_s 10^3 m/min	D_{ef} 10^8 m^2/min	Bi	K_D g/L	φ^{ap}	$v_{obs\ X}$ 10^3 g/L.min
NaX									
Glucose	0.0518	0.02	0.0481	0.011	0.0449	0.129	2.405	2.979	0.520
Fructose	0.0272	0.0147	0.0582	0.004	0.0341	0.612	3.959	4.376	0.592
Sucrose	0.0553	0.0003	0.0721	0.473	0.285	8.796	240.3	0.036	0.027
FOS	0.0028	0.0201	0.018	30.8	48.9	3.338	0.895	0.119	0.028
BaX									
Glucose	0.0201	0.018	0.0308	0.0005	0.145	0.0178	1.71	0.080	0.036
Fructose	0.0511	0.0234	0.0831	0.0038	0.092	0.218	3.551	0.763	0.385
Sucrose	0.0221	0.0232	0.0587	0.0011	0.158	0.0365	2.53	0.321	0.111
CaX									
Glucose	0.0099	0.0202	0.0227	0.001	0.156	0.0332	1.124	0.299	0.121
Fructose	0.0397	0.0538	0.0627	0.0021	0.043	0.255	1.165	2.147	0.561
Sucrose	0.0166	0.0215	0.0652	0.0044	0.035	0.669	3.032	3.147	0.268
KX									
Glucose	0.0557	0.0292	0.0857	0.0017	0.031	0.299	2.934	2.635	0.237
Fructose	0.0277	0.0195	0.0576	0.0063	0.077	0.438	2.954	0.722	0.285
Sucrose	0.0144	0.0424	0.0456	0.0005	0.81	0.0033	1.075	0.019	0.033
MgX									
Fructose	0.0351	0.0245	0.0398	0.0003	0.789	0.0018	1.624	0.012	0.050
Sucrose	0.0096	0.0196	0.0606	0.004	0.0027	7.979	3.091	24.988	0.122
SrX									
Glucose	0.0129	0.0136	0.042	0.0015	0.057	0.143	3.088	1.383	0.214
Fructose	0.0425	0.0309	0.055	0.0012	0.684	0.0089	1.779	0.011	0.027
Sucrose	0.0116	0.0285	0.028	0.0004	0.785	0.0027	0.982	0.021	0.038

The validation of model parameters for the zeolite Na^+ is showed at Figure 2, where experimental data are plotting against predicted data. As can be seen, there is a satisfactory fitting for all saccharide experimental data, indicating that the model

parameters represent confidently the adsorption. It is obvious that the estimated parameters representing the behavior of a unique experimental condition and a more robust structure for parameter estimation require more experiments to compute the objective function. However, it is important emphasize that the model parameters were used as a criterion for the choice of the best material for the separation of the glucose, fructose, sucrose and FOS.

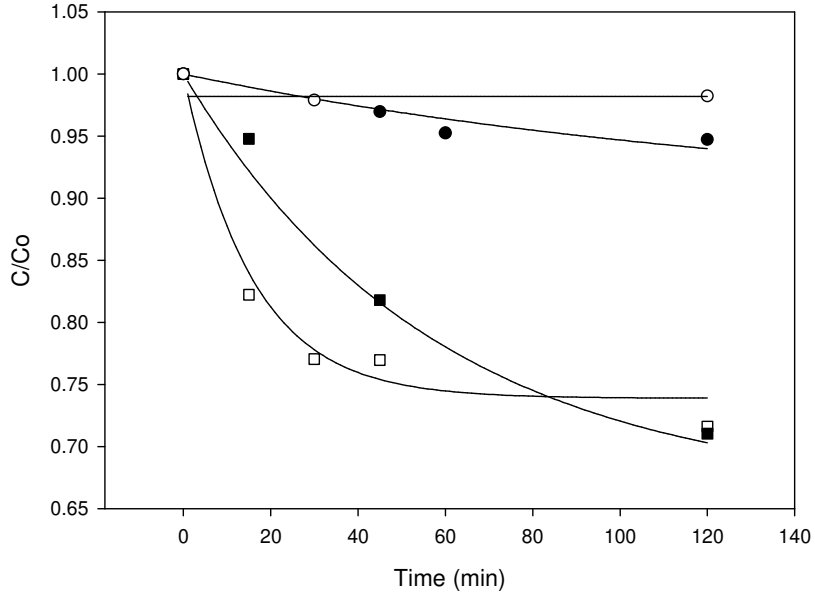

Figure 2: Experimental (points) and predicted (lines) kinetics for glucose (□), fructose (■), sucrose (o) and FOS (●) for the NaX zeolite.

In addition, it is important to emphasize that the Na^+ form presented very low adsorption capacity for the FOS molecules, what is interesting under the point of view of the application of this kind of zeolite to separate FOS from sucrose, glucose, and fructose, which are sugars produced in the enzymatic synthesis of FOS using sucrose as substrate.

4.4. Conclusions

In this work, a strategy of selection of the most appropriated cationic form of the X zeolite for the separation of glucose, fructose, and sucrose from the enzymatic synthesis of FOS was proposed. The strategy was based on the estimation and

analysis of the model parameters in terms of adsorption rates and mass transfer resistances involved in the process. Six cationic forms of the X zeolite were evaluated and the most promising was the Na^+ form, since it presented the highest adsorption rates and the lowest mass transfer resistance. In fact, the external mass transfer resistance was the major factor for all the cationic forms, but the best results were obtained for the Na^+ form. The strategy adopted to select the most appropriated material to separate the saccharides was shown to be interesting and applicable in the choice of other materials for the purification of any bioproduct of interest, since the criterion is based in the adsorption rates and mass transfer phenomena.

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4.5. References

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Capítulo 5

Separação e purificação de frutooligossacarídeos em coluna de leito fixo de zeólitas

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Separação e purificação de frutooligossacarídeos em coluna de leito fixo de zeólitas

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Resumo

O principal objetivo deste trabalho foi desenvolver uma técnica de purificação de frutooligossacarídeos obtidos através de síntese enzimática de uma mistura contendo mono e dissacarídeos através de coluna de leito fixo de zeólitas. Para purificar esta mistura de açúcares, foram estudadas a influência das variáveis temperatura, vazão de alimentação, volume de amostra injetado na coluna e concentração de etanol no eluente, através da aplicação de metodologia do planejamento experimental. O primeiro planejamento experimental realizado foi do tipo fracionário (2^{4-1}), onde foram estudados os efeitos de temperatura (40, 50 e 60^0C), vazão de alimentação (0,10; 0,12 e 0,14 mL/min), quantidade de amostra injetada na coluna (2,9; 3,8 e 5,1 % do volume do leito) e concentração de etanol no eluente (40, 50 e 60%) (v/v). Após a análise deste planejamento, foi realizado um DCCR onde as variáveis temperatura e concentração de etanol no eluente foram estudadas. Mostrou-se que as colunas de leito fixo são uma boa alternativa na purificação de frutooligossacarídeos, tendo sido obtido neste trabalho frutooligossacarídeos com 90% de pureza e uma eficiência de separação de 6,86 entre frutooligossacarídeos e glicose utilizando etanol 60% (v/v) no eluente a 45^0C.

Palavras-chave: Purificação, frutooligossacarídeos, eficiência de separação, colunas de leito fixo, zeólitas

Nomenclatura

E (%): Concentração de etanol (v/v)

ν: Vazão de alimentação ($mL.min^{-1}$)

V: Volume injetado na coluna (mL)

T: Temperatura (^0C)

μ: primeiro momento do leito

Δt: intervalo de tempo

ES: eficiência de separação

σ^2: segundo momento do leito

C_i: concentração do componente na fração

t_i: tempo

5.1. Introdução

Os frutooligossacarídeos (FOS) têm recebido considerável atenção nos últimos anos devido aos benefícios promovidos à saúde humana, dentre eles: estimulação da absorção de cálcio e magnésio, diminuição do colesterol total, fosfolipídios e triglicerídeos do organismo (Yun, 1996). São classificados como prebióticos, porque além de não serem hidrolisados e nem absorvidos na parte superior do trato gastrointestinal, eles promovem de forma seletiva o crescimento e estimulam a atividade metabólica de bactérias benéficas à saúde.

Estes FOS podem ser obtidos em pequenas quantidades através de alimentos como banana, trigo, alho entre outros, ou podem ser obtidos de reação enzimática através de sacarose pela enzima frutosiltransferase. O produto dessa reação contém glicose, frutose e pequenas porções de sacarose não transformada na etapa de síntese (Vanková et al. 2010). O seu valor comercial pode ser aumentado através da sua purificação, sendo então possível a sua adição a alimentos como barra de cereais, sucos, biscoitos, conferindo um caráter funcional ao alimento e aumentando seu valor de mercado.

Na literatura são encontrados trabalhos com purificação de frutooligossacarídeos em colunas de leito fixo, dentre eles: Antosová et al. (1999) estudaram a separação utilizando coluna de troca iônica (Ag^{+}), Hidaka et al. (1988), Bonn et al. (2000) e Kuhn e Maugeri (2010) trabalharam com coluna de carvão ativo e Lin e Lee (1998) estudaram a separação utilizando coluna de leito fixo com sílica.

As zeólitas atualmente são conhecidas pelas suas diferentes aplicações, destacando-se em processos de troca iônica (Hammoudi et al. 2008), purificação de diferentes compostos, dentre eles proteínas (Yu et al. 1998), tratamento de efluentes (Engin et al. 2008), remoção de chumbo de águas (Turan et al. 2005). Muitos pesquisadores têm estudado a purificação de açúcares, como glicose e frutose, através de resinas de troca iônica e também zeólitas (Ching e Ruthven, 1987; Ho et al., 1987; Cheng e Lee, 1992; Heper et al., 2007 e Gramblicka e Polakovic, 2007). As zeólitas são um adsorvente bastante interessante para processos de separação de açúcares, pois apresentam uma menor resistência à transferência de massa (Ho et al. 1987) comparadas com as resinas. Além disso, as zeólitas X são membros da

família faujasita, que apresenta uma estrutura de cristal estável, grande volume de poros e possibilidade de troca iônica (Hammoudi et al. 2008).

O objetivo principal deste trabalho foi definir uma técnica de purificação de frutooligossacarídeos presentes em uma mistura composta de mono e dissacarídeos, obtidos através de síntese enzimática. Utilizou-se um planejamento experimental em coluna de leito fixo contendo zeólitas, tendo como variáveis: a temperatura da coluna, volume de amostra injetado, vazão de alimentação e concentração de etanol no eluente, sendo avaliado como resposta a eficiência de separação dos açúcares.

5.2. Material e Métodos

5.2.1. Produção da enzima

A enzima foi produzida por *Rhodotorula* sp., isolada por Maugeri e Hernalteens (2007), sendo mantida em ágar inclinado composto de 2% de glicose, 1% de extrato de malte, 0,5% de extrato de levedura, 0,2% de Na_2HPO_4 e 2% de ágar a 5^0C. O meio de inóculo era composto de 2% de glicose, 2% de peptona, 1% de extrato de levedura, 0,5% Na_2HPO_4, pH 4,5 a 30°C, 150 rpm durante 24 h. Para a produção da enzima, 20 mL do inóculo foram transferidos para 200 mL de meio de fermentação composto por 65 g de melaço e 100 g de água de maceração de milho. A fermentação foi realizada a 30^0C, 250 rpm durante 18,5 h. Após a fermentação a enzima foi precipitada com etanol 70% (v/v) a 2^0C, centrifugada e o precipitado foi ressuspendido em tampão acetato 0,05M, pH 4,5.

5.2.2. Síntese dos FOS

Os FOS foram produzidos em reatores encamisados, contendo a enzima livre (6,5 U/mL) e solução de sacarose 50% (p/v) em tampão acetato 50mM (pH 4,5) a 50^oC durante 41 h. Ao final da reação foram sintetizados 54% de FOS, 35% de glicose, 8,8% de sacarose e 2% de frutose.

5.2.3. Adsorvente

Foi utilizada a zeólita NaX (UOP/EUA) na granulometria de 0,25-0,40 mm como adsorvente na coluna de leito fixo.

5.2.4. Análise cromatográfica

A análise cromatográfica foi realizada em sistema HPLC-PAD (DIONEX) com detector eletroquímico ED40, sistema de bombas GP50, detector de pulso amperométrico, eletrodo de ouro, software PEAKNET para aquisição e processamento de dados, pré-coluna (4 x 50 mm) e coluna carbopac PA100 (4 x 250 mm). A fase móvel utilizada foi composta de NaOH 50 mM e acetato de sódio 500 mM em NaOH 50 mM. Kestose (GF_2), nistose (GF_3) e frutofuranosilnistose (GF_4) (Wako Indústria Química, Osaka, Japão), sacarose, glicose e frutose (Sigma-Aldrich®) todos de grau analítico, foram utilizados como padrão.

5.2.5. Purificação dos açúcares em colunas de leito fixo

O sistema experimental de purificação em leito fixo consistiu de uma coluna de vidro (50x1) cm preenchida com o adsorvente zeólita NaX, sendo realizado o controle de temperatura através de banho acoplado. A solução de açúcar foi inserida na coluna através de bomba peristáltica Amersham® Biosciences Pump P-1, sendo controlada a vazão de alimentação. Ao final da coluna, amostras foram coletadas em coletor automático e quantificadas através de sistema HPLC-PAD. Na Figura 1 está representada a montagem experimental do sistema de purificação em coluna de leito fixo.

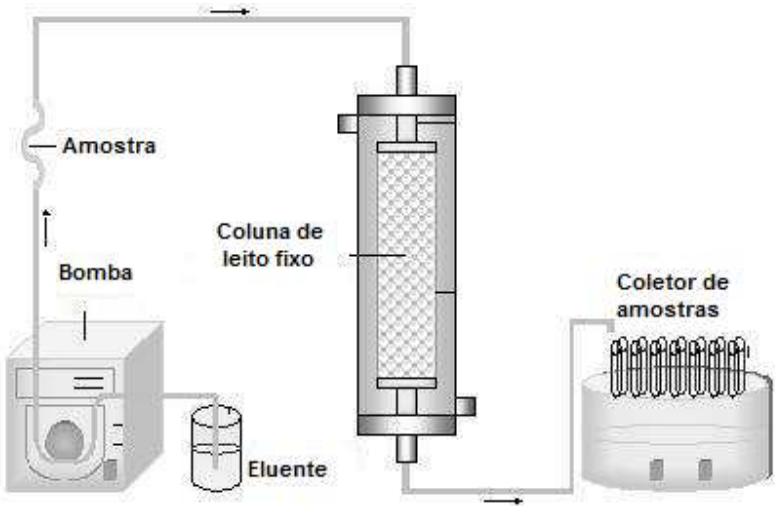

Figura 1 – Montagem experimental da purificação em coluna de leito fixo.

A análise dos resultados foi realizada utilizando metodologia definida por Lu e Lee (1987) que definem a eficiência de separação (ES) como um método para quantificar a separação de dois ou mais compostos, sendo esta definida através das Equações (1 a 5).

$$(ES)_{12} = \frac{\Delta t_{12}}{\sigma_{12}} \tag{1}$$

onde:

$$\sigma_{12} = (\sigma_1 \sigma_2)^{1/2} \tag{2}$$

$$\Delta t_{12} = |\mu_1 - \mu_2| \tag{3}$$

$$\mu_i = \frac{\sum_{i-1}^{n} .C_i t_i \Delta t_i}{\sum_{i-1}^{n} .C_i \Delta t_i} \tag{4}$$

$$\sigma^2 = \frac{\sum_{i=1}^{n} C_i (t_i - \mu_i)^2 \Delta t_i}{\sum_{i=1}^{n} C_i \Delta t_i} \tag{5}$$

A pureza dos FOS também foi considerada como resposta na análise dos resultados, sendo calculada através da Equação 6.

$$Pureza = \frac{concentração\ total\ de\ FOS}{concentração\ total\ de\ açúcares} \tag{6}$$

5.2.6. Planejamento experimental

Para interpretação dos resultados foi utilizada a metodologia de planejamento experimental com o auxílio do software STATISTICA versão 5.0. Após análise dos testes preliminares de separação em coluna de leito fixo (Tabela 1) foi definido um planejamento experimental fracionário (2^{4-1}) (Tabela 2) para verificar a influência de variáveis importantes na separação dos açúcares: temperatura, vazão de alimentação, volume de amostra injetado na coluna e concentração de etanol no eluente. Após a análise do planejamento experimental fracionário (2^{4-1}) foi delineado um planejamento experimental completo (DCCR), com as variáveis que apresentaram efeito estatístico significativo ($p<0,1$) (Tabela 3).

5.3. Resultados e Discussão

5.3.1. Ensaios preliminares de purificação dos FOS em colunas de leito fixo

Na etapa de separação dos açúcares, um maior fator $(ES)_{12}$ representa uma melhor eficiência de separação entre os compostos em estudo (Lu e Lee, 1987). A Tabela 1 mostra os resultados preliminares com o objetivo de estabelecer alguns parâmetros importantes a serem utilizados no planejamento experimental. Na definição destes níveis foi analisada somente a separação entre glicose e FOS por serem estes os açúcares de maior concentração na mistura, representando cerca de 90% da solução de síntese que será purificada. Observa-se que quanto maior a concentração de etanol no eluente, melhor a eficiência de separação, sendo conseguida uma eficiência de 2,74, quando foi utilizada uma solução com 40% (v/v) de etanol no eluente; já esta eficiência de separação foi bastante reduzida quando se utilizou etanol 10% (v/v) no eluente, caindo para 0,60. Portanto, quanto maior a concentração de etanol no eluente melhor a eficiência de separação entre os açúcares.

Tabela 1 - Eficiência de separação entre FOS e glicose utilizando diferentes eluentes.

Eluente	$ES_{FOS\text{-}glic}$
Água	0,14
Água/Etanol (90/10) (v/v)	0,60
Água/Etanol (80/20) (v/v)	1,08
Água/Etanol (60/40) (v/v)	2,74

5.3.2. Planejamento experimental

A Tabela 2 mostra o delineamento do planejamento experimental fracionário (2^{4-1}), assim como os resultados das eficiências de separação (ES) dos açúcares. Na Tabela 3 estão apresentados os resultados das eficiências de separação entre os açúcares e a pureza dos FOS através do DCCR.

Tabela 2 - Delineamento do planejamento experimental fracionário (2^{4-1}), variáveis codificadas e reais (em parênteses) e resultados de eficiências de separação entre os açúcares.

Ensaios	T(^{0}C)	ν (mL/min)	V(%)	E (%)	$ES_{FOS/glic}$	$ES_{FOS/frut}$	$ES_{FOS/sac}$
1	-1(40)	-1(0,10)	-1(2,6)	-1(40)	1,73	0,82	0,65
2	+1(60)	-1(0,10)	-1(2,6)	+1(60)	4,29	0,16	0,80
3	-1(40)	+1(0,14)	-1(2,6)	+1(60)	3,64	1,22	0,05
4	+1(60)	+1(0,14)	-1(5,1)	-1(40)	1,55	2,05	0,20
5	-1(40)	-1(0,10)	+1(5,1)	+1(60)	3,94	2,18	0,78
6	+1(60)	-1(0,10)	+1(5,1)	-1(40)	0,85	0,41	0,42
7	-1(40)	+1(0,14)	+1(5,1)	-1(40)	1,22	0,99	0,16
8	+1(60)	+1(0,14)	+1(5,1)	+1(60)	2,17	1,72	1,00
9	0(50)	0(0,12)	0(3,8)	0(50)	2,27	4,47	0,16
10	0(50)	0(0,12)	0(3,8)	0(50)	2,45	4,30	0,24
11	0(50)	0(0,12)	0(3,8)	0(50)	2,30	4,80	0,16

Tabela 3 – Delineamento do planejamento experimental completo (DCCR), variáveis codificadas e reais (em parênteses) e resultados de pureza de FOS e eficiências de separação entre os açúcares.

Ensaios	T(ºC)	E (%)	$ES_{FOS-glic}$	$ES_{FOS-frut}$	$ES_{FOS-sac}$	Pureza (%)
1	-1(35)	-1(50)	5,56	5,53	0,79	87
2	+1(45)	-1(50)	3,29	1,67	3,69	83
3	-1(35)	+1(60)	5,12	1,39	1,46	86
4	+1(45)	+1(60)	6,86	6,31	1,88	90
5	-1.41(33)	0(55)	4,95	4,95	0,92	86
6	+1.41(47)	0(55)	3,70	3,49	2,03	85
7	0(40)	-1.41(48)	3,56	4,23	0,76	85
8	0(40)	+1.41(62)	5,67	2,48	1,59	90
9	0(40)	0(55)	3,76	2,96	0,43	88
10	0(40)	0(55)	4,31	6,93	2,06	89
11	0(40)	0(55)	4,92	4,40	3,17	89

Na Figura 2 estão representados os efeitos das variáveis temperatura, vazão de alimentação, volume de amostra injetado na coluna e concentração de etanol no eluente, para a resposta eficiência de separação entre FOS e glicose, segundo o planejamento experimental fracionário ($p<0,1$). Verifica-se que a concentração de etanol do eluente, o volume de amostra injetada na coluna e a vazão de alimentação apresentaram efeito estatisticamente significativo a 90% de confiança, sendo que a concentração do etanol no eluente apresentou um efeito positivo, ou seja, ao passar do nível -1 (menor concentração) para o nível +1 (maior concentração) houve um incremento na eficiência de separação entre FOS e glicose. No entanto, o volume injetado na coluna e a vazão de alimentação apresentaram efeitos negativos, ao passar do nível -1 para o nível +1, ocorrendo diminuição da eficiência de separação entre estes açúcares. Pela análise dos efeitos concernentes às variáveis vazão de alimentação e volume de amostra injetado na coluna, conclui-se que menores vazões de alimentação e menores volumes de amostra injetados possibilitam uma melhor eficiência de separação entre FOS e glicose. A temperatura neste planejamento não apresentou efeito estatisticamente significativo dentro das faixas estudadas.

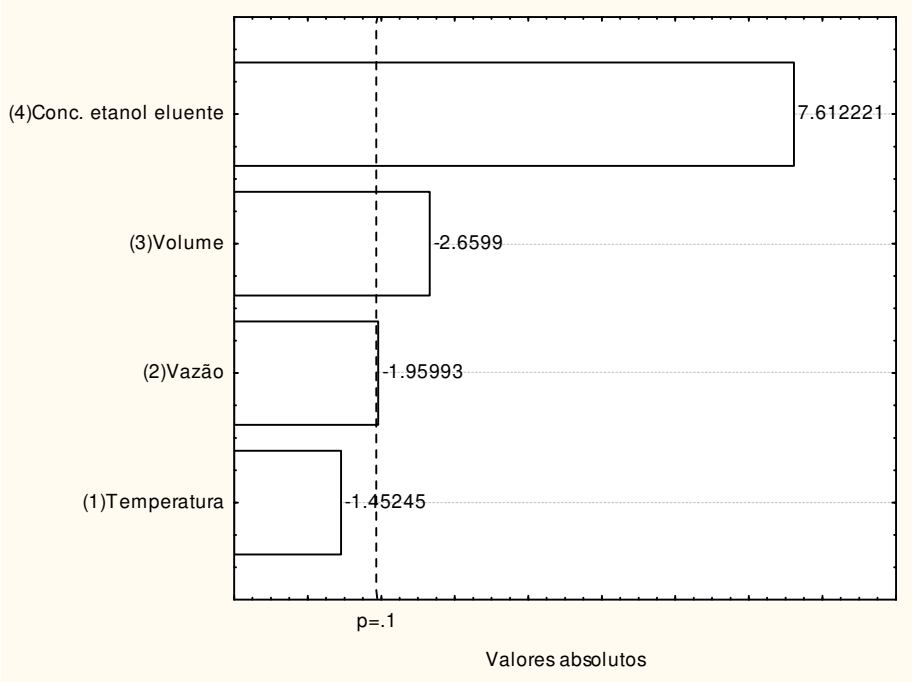

Figura 2 - Estimativa dos efeitos para a resposta eficiência de separação entre FOS e glicose no delineamento experimental fracionário (2^{4-1}).

Na análise das eficiências de separação entre FOS e frutose e entre FOS e sacarose, nenhuma das variáveis estudadas apresentou efeito significativo sobre a resposta, portanto somente a resposta eficiência de separação entre FOS e glicose foi considerada na definição dos parâmetros para o DCCR. Para este delineamento foram escolhidas as variáveis concentração de etanol no eluente e temperatura. Optou-se por trabalhar com temperaturas mais baixas que as estudadas anteriormente, visto que esta variável não apresentou efeito significativo sobre a resposta e também para redução dos custos com energia do processo. Como a concentração do etanol apresentou um efeito positivo sobre a resposta eficiência de separação entre FOS e glicose, foram definidos níveis mais elevados (48% a 62% (v/v)) desta variável no planejamento. As variáveis vazão de alimentação e volume de amostra injetado na coluna foram fixadas em 0,12 mL/min e 2,6% do volume da coluna, respectivamente.

Na Tabela 3 pode-se observar que a máxima eficiência de separação entre FOS e glicose foi obtida no ensaio 4 (Figura 4), com uma eficiência de separação de 6,86, e um grau de pureza de FOS de 90%. Nesta condição a temperatura foi de

45^{0}C e a concentração de etanol utilizada no eluente foi de 60% (v/v). Através da análise estatística dos resultados do DCCR obteve-se o modelo codificado (Equação 9) que representa a eficiência de separação entre FOS e glicose em função da temperatura e concentração de etanol no eluente, dentro das faixas estudadas. Esta equação foi validada pela análise de variância (ANOVA) (Tabela 4), onde o F calculado foi 2,4 vezes maior que o valor tabelado.

$$ES_{FOS\text{-}glic} = 4{,}327 + 0{,}765 \text{ eluente} + 1{,}00 \text{ eluente x temperatura} \quad (9)$$

Tabela 4 - Análise de variância para o DCCR.

Fonte de variação	Soma quadrática	Graus de liberdade	Média quadrática	F calculado
Regressão	8,693	2	4,3465	10,65
Resíduo	3,263	8	0,4078	
Falta de ajuste	2,59	2		
Erro puro	0,673	6		
Total	11,956	10		

R^2=72,7%; $F_{2;8;0,05}$= 4,46

Com o modelo obtido foi possível gerar a superfície de resposta (Figura 3) e analisar as melhores condições de concentração de etanol no eluente e a temperatura que proporcionaram maior eficiência de separação entre FOS e glicose. Observa-se que a eficiência de separação é maior em concentrações superiores a 60% de etanol (v/v) no eluente e em temperaturas superiores a 45^{0}C.

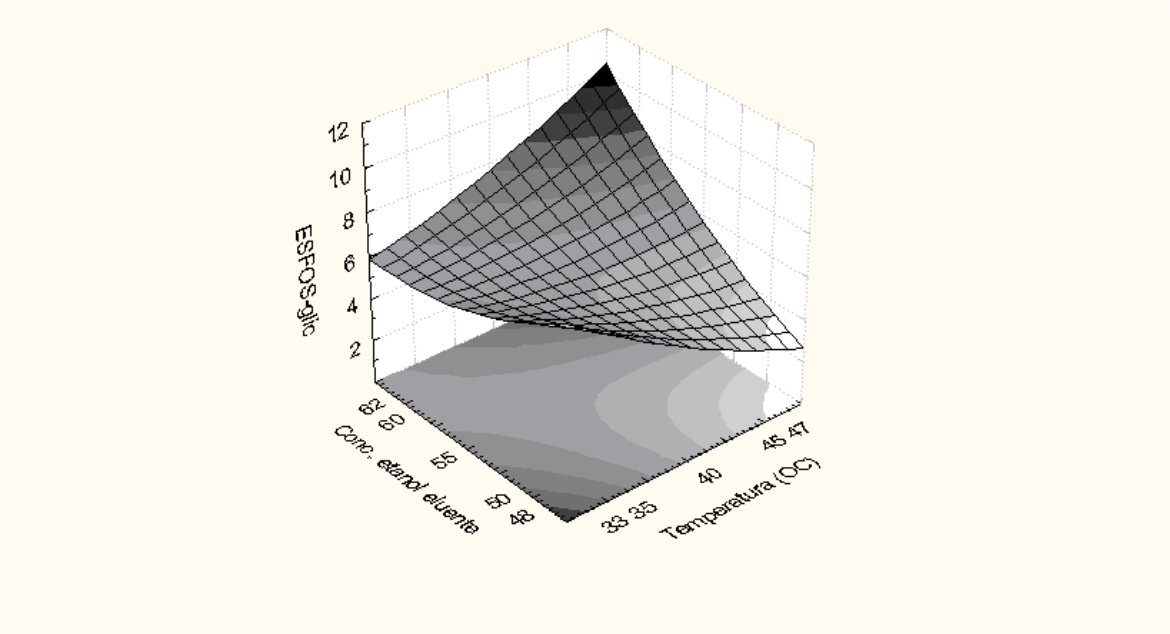

Figura 3 – Superfície de resposta obtida para a eficiência de separação entre FOS e glicose.

Analisando a separação entre os açúcares, observa-se claramente que os FOS e a glicose, que são os açúcares com maior concentração na mistura de síntese, estão totalmente separados, resultados observados pela análise das Figuras 4 e 5 que representam a concentração dos açúcares no final da coluna. Pode-se observar que os FOS não são adsorvidos praticamente pela zeólita NaX; isto ocorre pelo tamanho da molécula dos FOS que é maior que o dos poros da zeólita e pelo menos três vezes o tamanho da molécula de glicose. Por outro lado, frutose e glicose possuem uma afinidade maior pela zeólita, principalmente pelo fato de ambas moléculas possuírem tamanho menor que o diâmetro do poro da zeólita, podendo assim difundir no interior destes e, portanto, tendo tempo de residência na coluna bastante aumentado em relação aos FOS. O mesmo raciocínio pode ser aplicado à sacarose, que sai praticamente junto com os FOS, tendo em vista seu diâmetro ser maior que o diâmetro do poro; mas baixa separação observada entre estes compostos terá um efeito menor na pureza final dos FOS, pois a sacarose representa cerca de 7% da concentração total da mistura obtida da síntese enzimática. Em estudos realizados por Ho et al. (1987) também foi observado que a zeólita CaY apresentou seletividade entre glicose e frutose semelhante a resinas de troca iônica

Ca^{+2}, a zeólita ainda apresentou a vantagem frente às resinas de possuir uma menor resistência à transferência de massa.

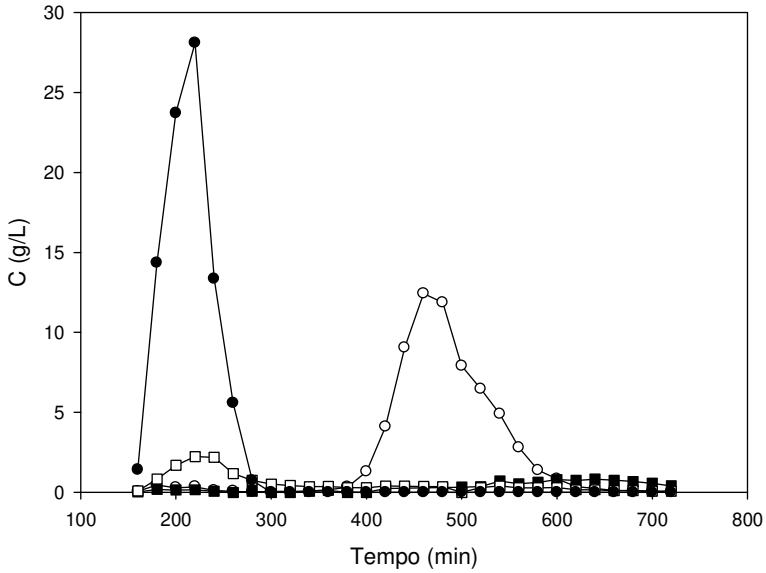

Figura 4 – Concentração de açúcares na saída da coluna de leito fixo de zeólitas (FOS (●), glicose (○), frutose (■) e sacarose (□)) a 45ºC e utilizando etanol 60% (v/v) como eluente.

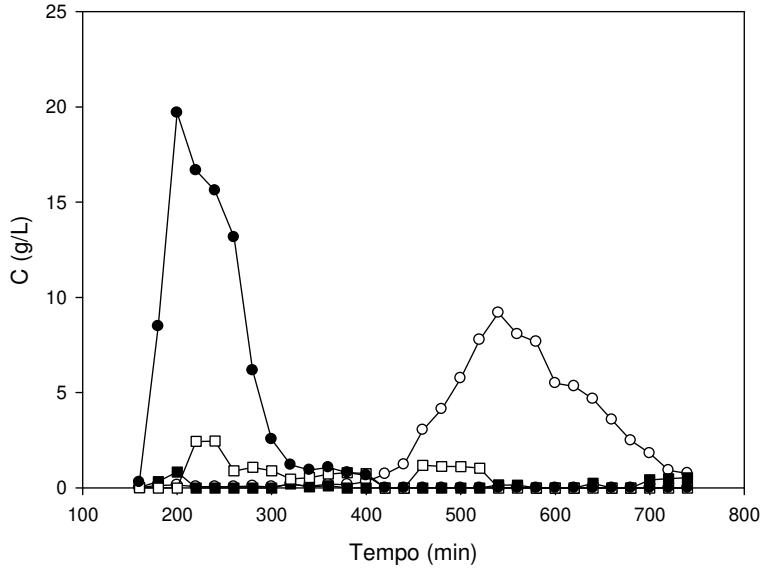

Figura 5 – Concentração de açúcares na saída da coluna de leito fixo de zeólitas (FOS (●), glicose (○), frutose (■) e sacarose (□)) a 40ºC e etanol 62% (v/v) como eluente.

Observando os melhores resultados encontrados no DCCR, pode-se verificar que a influência da temperatura é menos acentuada na purificação dos açúcares, já que os melhores resultados (ensaios 4 e 8), foram realizados a 45 e 40^{0}C, respectivamente, sendo que em ambos ensaios foram realizados com as maiores concentrações de etanol, podendo ser observado que o etanol apresenta bastante influência sobre a separação dos açúcares.

5.4. Conclusão

Através dos resultados obtidos em coluna de leito fixo com zeólitas, conclui-se que a eficiência de separação entre FOS e glicose foi aumentada em maiores concentrações de etanol. Estes resultados foram confirmados através da análise do planejamento experimental fracionário (2^{4-1}) onde a única variável que apresentou efeito significativo positivo a 90% de confiança foi a concentração de etanol no eluente. Já no DCCR foi observado que as duas melhores eficiências de separação foram conseguidas nos ensaios que utilizaram as maiores concentrações de etanol no eluente. Portanto, a concentração de etanol no eluente é determinante na eficiência de separação entre FOS e glicose. A separação entre FOS e sacarose não foi muito pronunciada em nenhum dos ensaios realizados, devido a zeólita não apresentar relevante afinidade por estes dois açúcares, principalmente por possuir o diâmetro médio de poros menor que o diâmetro destes açúcares, tendo, praticamente o mesmo tempo de residência na coluna. No entanto, este resultado não compromete significativamente a purificação dos FOS, pois a sacarose representa somente 7% da mistura de açúcares.

As colunas de leito fixo com zeólitas demonstraram ser uma boa alternativa para a separação de açúcares, principalmente FOS e glicose, visto que foram obtidos resultados bastante interessantes em termos de eficiência de separação e pureza entre estes açúcares. Sendo assim, com uma pureza final dos FOS de cerca de 90%, este produto pode ser adicionado a alimentos com caráter funcional, aumentando seu valor de mercado.

Agradecimentos

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Chapter 6

Purification of fructooligosaccharides in an activated charcoal fixed bed column

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Purification of fructooligosaccharides in an activated charcoal fixed bed column

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Abstract

Fructooligosaccharides (FOS) are mixtures of oligosaccharides containing mono and disaccharides, therefore, the separation of these sugars results in purer products for human consumption and can be added to various food products (drinks, cookies and yogurt). The aim of this work was the purification of fructooligosaccharides from a mixture of sugars, obtained by enzymatic synthesis, containing fructooligosaccharides, glucose, fructose and sucrose using activated charcoal fixed bed column. Temperature and ethanol concentration effects were analyzed using a 2^2 central composite design. Good separation conditions were obtained through central composite design. The best separation coefficient between fructooligosaccharides and glucose ($ES_{fructoolig/gluc}$) was 3.99 ± 0.07 and between fructose and fructooligosaccharides ($ES_{fructoolig/fruct}$) was 2.89 ± 0.36 using ethanol 15% (v/v) as eluent, at 50^0C. The final FOS purification degree and recovery were about 80% and 97.8%, respectively. The activated charcoal fixed bed columns were shown to be a good alternative for sugar separation, especially for rich mixtures of fructooligosaccharides.

Keywords: Fructooligosaccharides, purification, fixed bed column, activated charcoal, central composite design.

6.1. Introduction

Functional foods, which are foods that can improve health, physical performance, mental capacity, besides their nutritional value [1], have been considered with more and more interest owing to their increasing commercial importance. In the recent years, oligosaccharides have attracted the attention of researchers, because they can be used as functional ingredients, besides the traditional use as an energy source and sweetener, with a great potential to improve food quality.

The fructooligosaccharides (FOS) represent the largest class of bifidogenic oligosaccharides in terms of production volume. They can be obtained from the extraction of plants [2] and enzymatically from sucrose, using transfructosylation activity of some enzymes. The fructooligosaccharides formed in this process are formed of two to four β (1-2) fructosyl bonds, linked to glucose, named as kestose, nystose and fructofuranosyl nystose, as depicted in Figure 1. Glucose and small amounts of fructose are formed as a reaction product of transfructosylation, these sugars being removed from the mixture by chromatographic processes for the production of high purity fructooligosaccharides [3].

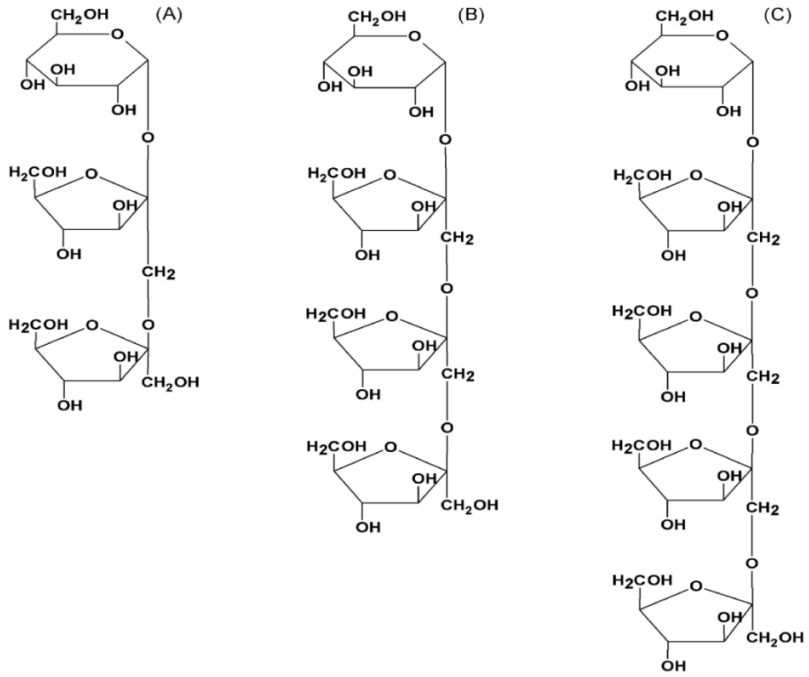

Figure 1. Chemical structure of fructooligosaccharides (A: kestose, B: nystose and C: fructofuranosyl nystose).

Fixed bed columns have been largely used in the fructooligosaccharides purification, as shown by the studies of Kuhn [4] using zeolites fixed bed columns in the separation of fructooligosaccharides, of Kawazoe *et al.* [5], Morales *et al.* [6], Weston and Brocklebank [7] using activated charcoal fixed bed columns in the sugars separation, among which are fructooligosaccharides. The adsorption capacity of activated charcoal is determined by the total surface area, the internal pores structure and the presence of functional groups in surface of pores [8]. The activated charcoal has been used in many applications such air purification, water and wastewater treatment and catalysis [9].

The aim of this work was to separate fructooligosaccharides from a mixture of sugars, which contained glucose, fructose, sucrose and fructooligosaccharides, using an activated charcoal fixed bed column and to determine the influence of the temperature and solvent composition in the separation efficiency.

6.2. Materials and methods

6.2.1. Enzyme production

The enzyme was produced by the new microorganism *Rhodotorula* sp., coded LEB-V10, from the Laboratory of Bioprocess Engineering-UNICAMP-Brazil, isolated by Maugeri and Hernalteens [10], maintained on agar slants (glucose 2%, yeast extract 0.5%, malt extract 1%, Na_2HPO_4 0.2%, agar 2%) at 5ºC. Cultivation of the strain for enzyme production was carried out in liquid culture (glucose 2%, peptone 2%, yeast extract 1% and K_2HPO_4 0.5%, pH 4.5) at 30ºC and 150 rpm for 24 h. For enzyme production, 20 mL of inoculum was transferred to a 500 mL flask containing 200 mL of main growth medium and cultured at 30ºC for 18.5 h at 250 rpm. The main medium consisting (per litre): 65 g sugar cane molasses and 100 g corn steep liquor. After cultivation, the cells were separated by centrifugation (5ºC) and the enzyme recovered as follows: ethanol was added to the supernatant solution at –20ºC up to a final concentration of 70% (v/v). The process was performed in a stirred reactor at 2^0C, under mild agitation. The precipitate was centrifuged, recovered and re-dissolved in sodium acetate buffer (0.05 M, pH 4.5) [11].

6.2.2. Enzyme assay

The reaction media used to determine enzyme activity consisted of 50% (w/v) sucrose (in 50 mM sodium acetate buffer, pH 4.5) and 10% (v/v) of adequately diluted enzyme suspension at 50^{0}C. Samples were collected at constant time intervals for 30 min of reaction time and used to quantify glucose with commercial glucose-oxidase kits (Laborlab, Brazil) and reducing sugars by the Somogyi-Nelson method [12,13].

During the cultivation, 5 mL of the culture broth was centrifuged (Sorvall RC 26 Plus/U.S.A.) at 7500 rpm, for 10 min, at 15ºC, and the supernatant was collected for the determination of extracellular enzyme activity. The hydrolytic and transfructosylating activities were measured according to the amounts of glucose and reducing sugars released into the reaction medium [14]. The Eqs (1) and (2) allow for the determination of the activities by measuring glucose (G) and reducing sugars (R) in the reaction media (F= fructose, F'= transferred fructose):

$$R = G + F \leftrightarrow F = R - G \quad (1)$$

$$F' = G - F \leftrightarrow F' = 2G - R \quad (2)$$

One unit of the fructofuranosydase activity (FA) is defined as the amount of enzyme required to hydrolyze 1 μmole of sucrose per minute at 50ºC and pH 4.5. One unit of the transfructosylating activity (FTA) is defined as the amount of enzyme required to transfer 1 μmole of fructose (F') per minute at 50ºC and pH 4.5.

6.2.3. Chemicals

Kestose (GF_2), nystose (GF_3) and fructofuranosyl nystose (GF_4) were purchased from Wako Pure Chemical Industries (Osaka, Japan), and sucrose, glucose and fructose from Sigma. All chemicals were of analytical grade.

6.2.4. Analysis of sugars

Identification and quantification of the sugars (sucrose, glucose, fructose, and FOS) was achieved by ion exchange chromatography with a pulsed amperometric detector (HPLC-PAD). Chromatography was performed on a Carbopac PA100 (4 x 250mm) column, a PA100 (4x50 mm) guard column at 22-24ºC, using a GP50

gradient pump, ED40 electrochemical detector and the software PEAKNET, all from Dionex (U.S.A.). The sugars were eluted in 50 mM sodium hydroxide with a linear gradient of sodium acetate (0-500 mM), at a flow rate of 1.0 mL/min. Before injection, the samples were filtered through 0.22 μm filters and diluted with water when needed.

6.2.5. Fructooligosaccharides synthesis

The fructooligosaccharides were produced in stirred reactors containing sucrose 50% (w/v) and enzyme (6.5 UI/mL) in 50mM sodium acetate buffer (pH 4.5), at 50^{0}C for 41h. Samples were taken and the enzyme inactivated by heating in boiling water for 5 min.

6.2.6. Adsorbent

Activated charcoal from Carvorite (Paraná, Brazil), with particles diameters between 0.25 and 0.40 mm, was used as adsorbent.

6.2.7. Adsorption kinetics in stirred tank reactors

The adsorption of sugars (glucose, fructose, sucrose and fructooligosaccharides) in activated charcoal was carried out in glass stirring vessel at 40^{0}C until adsorption equilibrium was attained. The vessel contained 100 mL of a sugar solution (150 g/L) and 5 g of activated charcoal. Samples were taken periodically, and the initial and final sugar concentrations were determined in HPLC-PAD. The amount of sugars adsorbed at equilibrium was calculated according to Equation 3.

$$q^* = \frac{(C_0 - C^*)V_{sol}}{m_a} \quad (3)$$

Where: q* (amount of sugar adsorbed at equilibrium); C_0 (initial concentration of the sugar in solution); C* (sugar concentration in solution at equilibrium); V_{sol} (volume of solution) and m_a (mass adsorbent).

6.2.8. Separation efficiency

The experimental set up consisted of a water jacketed column, 50 cm x 1 cm i.d., packed with activated charcoal. Superficial velocity 0.13 (cm/min) and 2.55 (%) injected volume/bed volume were controlled by metering pumps, the temperature

within the system was maintained by circulating hot water at a constant temperature. Samples were taken and sugars analyzed using the HPLC-PAD. The separation efficiency was calculated according to method proposed to Lu and Lee [15]:

$$(ES)_{12} = \frac{\Delta t_{12}}{\sigma_{12}} \tag{4}$$

where:

$$\sigma_{12} = (\sigma_1 \sigma_2)^{1/2} \tag{5}$$

$$\Delta t_{12} = |\mu_1 - \mu_2| \tag{6}$$

In the experiments, measurements were made by the pulse response method. The first and second moments of the pulse response were calculated according to Equation 7 and 8.

$$\mu_i = \frac{\sum_{i-1}^{n} .C_i t_i \Delta t_i}{\sum_{i-1}^{n} .C_i \Delta t_i} \tag{7}$$

$$\sigma^2 = \frac{\sum_{i=1}^{n} C_i (t_i - \mu_i)^2 \Delta t_i}{\sum_{i=1}^{n} C_i \Delta t_i} \tag{8}$$

where Ci is the concentration, ti is the time and Δti sample interval.

6.2.9. Experimental design

A central composite rotatable design (CCRD) of experiments, consisting of a two-factor-two-level pattern, was used to confirm the optimal temperature (^{0}C) and ethanol eluent composition (%), having separation efficiency as response. Factorial design and response surface analysis are important tools to determine the optimal process conditions. Factorial design is advantageous compared to the conventional method, which handles a single parameter per trial. The conventional method does not consider the effect of possible interactions between factors, very important in biological and biochemical process. The experimental conditions in the factorial design are shown in Table 1.

Table 1. Definitions and levels of independent variables in DCCR.

Independent variables	Symbol	Coded levels				
		-α	-1	0	+1	+α
Ethanol (%)	X_1	7.95	10	15	20	22.1
Temperature (^{0}C)	X_2	36	40	50	60	64

α = 1.41

6.3. Results and discussion

6.3.1. Enzyme production

The production was carried out in 500 mL flasks, in liquid medium, with *Rhodotorula* sp. for 18.5 h and samples were taken during fermentation to monitor enzyme activity. The highest activity was found with values of about 19 FTA units (transfructosylating activity) and 11 FA units (fructofuranosydase activity).

6.3.2. Fructooligosaccharides production

Fructooligosaccharides were synthesized in batch stirred reactor. The yield was 54.2%, consisting of 271 g/L of total FOS with a composition of 158 g/L of GF_2, 89 g/L of GF_3 and 24 g/L of GF_4. The mixture also contained 176 g/L of glucose, 44 g/L of sucrose and 10 g/L of fructose. Figure 2 shows that the total fructooligosaccharides produced accumulated throughout the synthesis reaction. The chromatogram corresponds to the 41 h reaction sample (Figure 3).

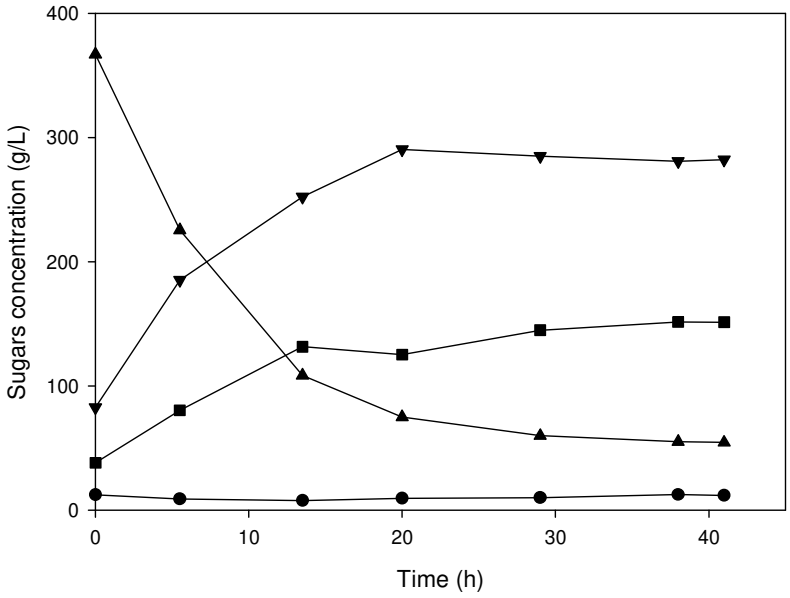

Figure 2. Batch reaction for fructooligosaccharides production from sucrose at 50^{0}C. ((▼) fructooligosaccharides, (●) fructose, (■) glucose, (▲) sucrose).

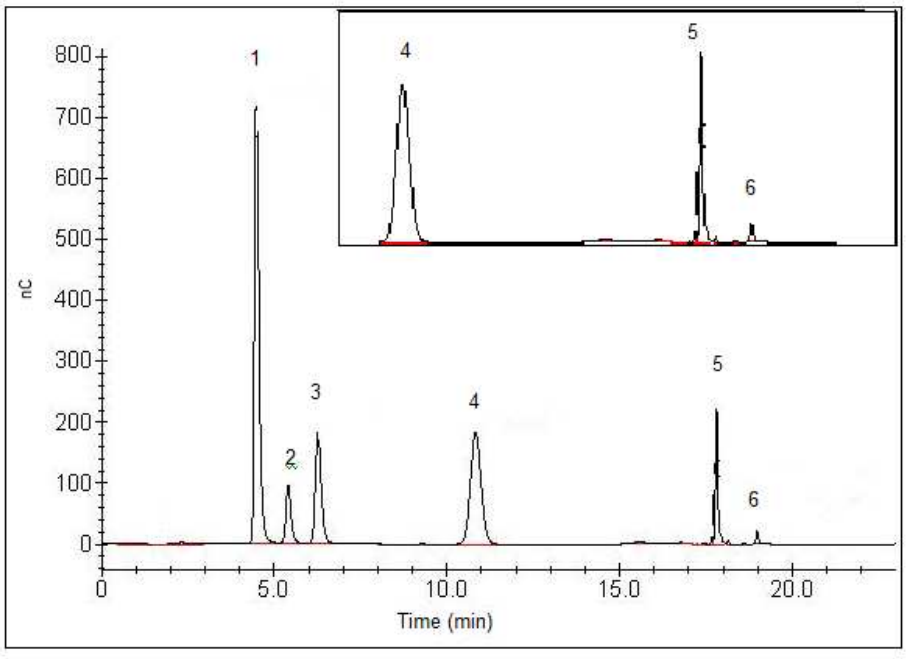

Figure 3. Chromatogram showing sugars from the 41 h reaction sample using HPLC-PAD ((1) glucose, (2) fructose, (3) sucrose, (4) kestose, (5) nystose, (6) fructofuranosyl nystose).

The fructooligosaccharides yields were satisfactory and similar to the ones found in the literature: the process with the bacteria *Zymomonas mobilis* gave yields of 24-32% fructooligosaccharides [16] and that with the extracellular fructosyltransferase from *Aureobasidium pullulans* produced yields of about 56% [17]. Fungal species have also been reported to produce FOS, with yields of about 50-60% [18-20]. Hernalteens and Maugeri [21] obtained about 240 g/L of fructooligosaccharides for 72 h of reaction with *Rhodotorula* sp. High yields of FOS production are of interest from the purification point of view, because the sugars (FOS) have a higher concentration in the final product, facilitating the separation process.

6.3.3. Adsorption kinetics

According to the results of the kinetics of adsorption (Figure 4) the activated charcoal showed good adsorption affinity for fructooligosaccharides, but less for glucose, fructose and sucrose, agreeing with the results of Kaplan and Hutkins [22]. The equilibrium was normally established after 120 min, where the ratio C/C_o was 0.57 for fructooligosaccharides, 0.92 for glucose, 0.96 for fructose and 0.90 for sucrose, using water as solvent. The maximum amount of FOS adsorbed at equilibrium was 0.92 g FOS/g activated charcoal (Eq. (3). When the adsorption kinetics were made using ethanol/water (50/50) (v/v) as solvent (Figure 5), the adsorption of fructooligosaccharides was less effective, with the ratio C/C_o at the equilibrium being about 1.00±0.08. However, in the literature we can find results of a similar procedure, such as the one of Morales *et al.* [6], who studied the recovery of maltodextrins with a solution of ethanol/water (50/50) (v/v). The experiments carried out by those authors showed good results, demonstrating that this ethanol concentration is effective in the recovery of maltodextrins.

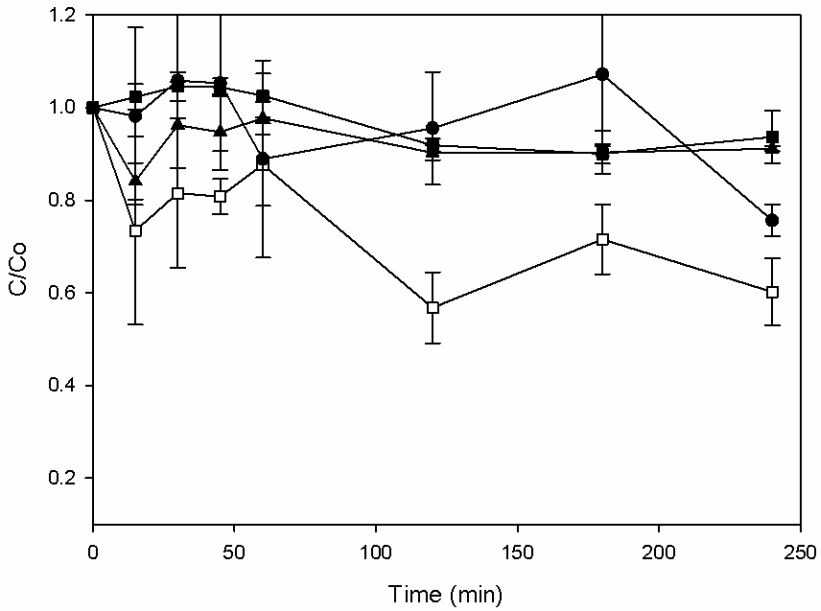

Figure 4. Adsorption kinetics of fructooligosaccharides (□), fructose (●), glucose (■) and sucrose (▲) in stirred tank reactor with activated charcoal, in water solution.

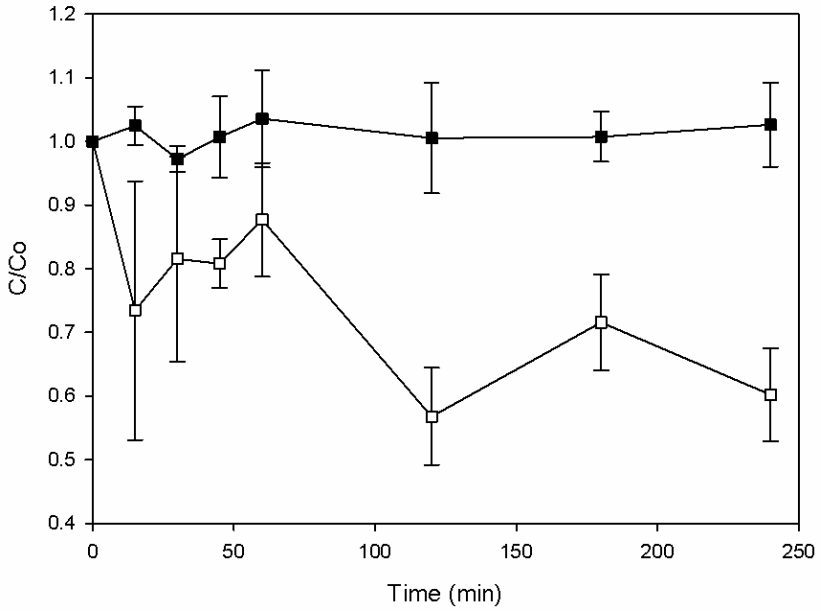

Figure 5. Adsorption kinetics of fructooligosaccharides on activated charcoal in water (□) and on activated charcoal in a 50% (v/v) ethanol solution (■).

6.3.4. Separation efficiency

In order to appraise the effects of the different parameters in the separation efficiency of the sugar mixture preliminary tests were performed. In these trials the eluent flow was 0.13 (cm/min), temperature was 40^{0}C and the amount of sample injected in the column was 2.55% of the column volume, and water, water/ethanol (90/10, 80/20 and 60/40) (v/v) were tested as eluent. The results showed that the column adsorbed a mixture of sugars, but did not desorb them when water was used as eluent. On the contrary, ethanol solutions, in different concentrations, were promising for sugars separation, since they enabled its elution. The efficiency coefficient was determined in all experiments and put together in Table 2. We can see that the efficiency for water is nil, since there is no elution from the column, and an efficiency of about 1.54 at 10% ethanol concentration, which decreases as ethanol concentration increases. Figure 6 shows the results from these experiments. Morales *et al.* [6] studied the removal of glucose and fructose in honey samples and also the recovery of maltodextrins with different concentrations of ethanol. For the mono and disaccharides removal the best condition of recovery was using a solution of water/ethanol (90/10) (v/v) as eluent, and for oligosaccharides recovery the best condition was using water/ethanol (50/50) (v/v) as eluent. In this present work, however, a solution ethanol/water (60/40) (v/v) as eluent was not effective, since there was a poor separation, as it can be seen in Figure 6. We should always have in mind, when using activated charcoal, as a natural product, that the efficiency depends very much on a variety of things, concerning the charcoal itself, where the actual distribution and the total pore volume associated with each pore size range are however sensitive to the conditions of the initial pyrolysis and activation procedures. However, by special activation procedures it is possible to prepare carbon adsorbents with a very narrow distribution of micropore size and which therefore behave as molecular sieves. The ability to modify the effective pore size by adjusting the conditions of the manufacturing process makes it relatively easy to tailor a carbon sieve to achieve a particular separation. However, it is difficult to achieve absolute reproducibility between different batches, and the existence of the distribution of pore size, even if narrow, means that the molecular sieving selectivity of a carbon sieve seldom approaches the almost perfect separation achievable under favorable circumstances with a zeolite sieve [23]. Therefore, it is quite normal that some results should not agree, and may be even opposites, as shown in this work.

Table 2. Separation efficiencies between fructooligosaccharides and glucose.

Eluent	$ES_{fructoolig/gluc}$
Water	n.d.
Water/Ethanol (90/10)	1.54±0.161
Water/Ethanol (80/20)	1.24±0.198
Water/Ethanol (60/40)	0.07±0.019

n.d.: not determined

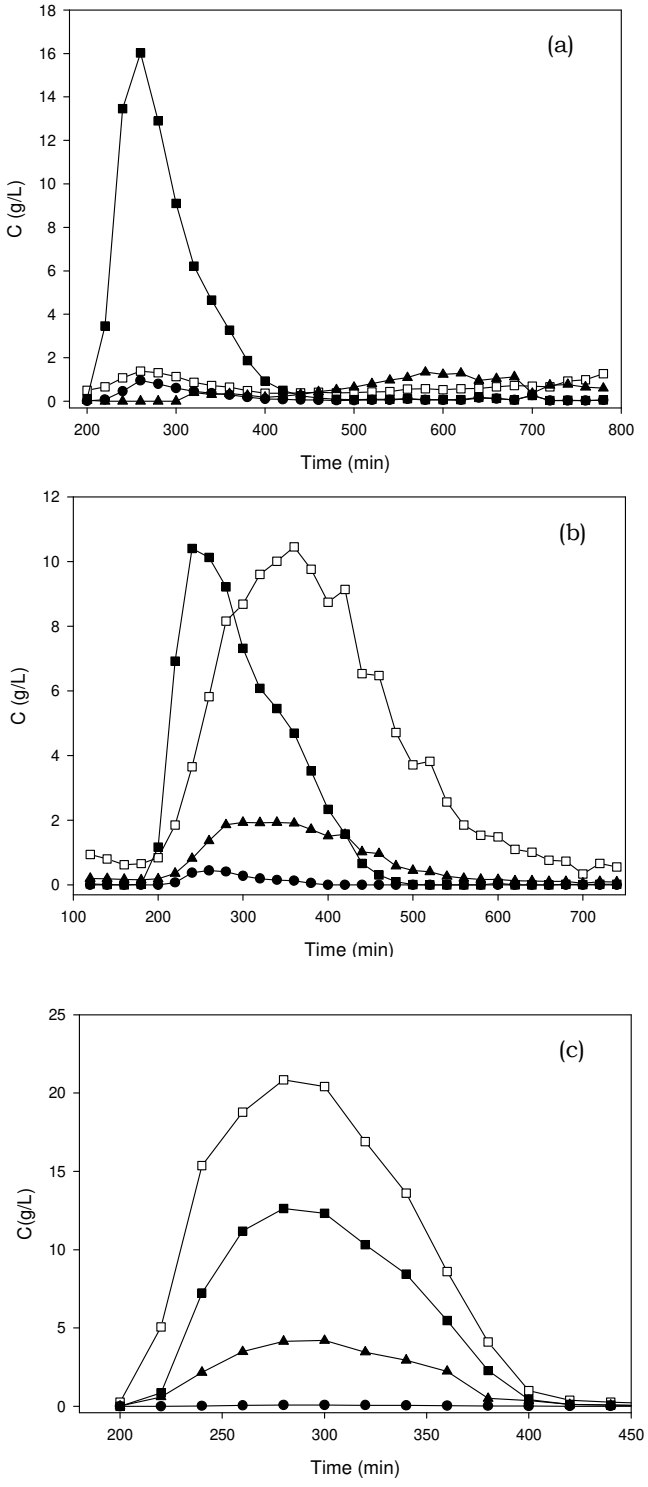

Figure 6. Time course for sugars leaving the fixed bed column, with superficial velocity = 0.13 cm/min at 40°C: (a) 10% ethanol solution, (b) 20% ethanol solution and (c) 40% ethanol solution: (□) fructooligosaccharides, (●) fructose, (■) glucose, (▲) sucrose.

In order to establish the best condition for the sugars separation, in this study the separation efficiency for fructooligosaccharides and glucose ($ES_{fructoolig/gluc}$) was taken as the main response of the experimental design described in Table 3, because these sugars together represent almost 90 % of the mixture.

6.3.5. Central composite design

Considering the above results, a factorial experimental design was planned to find the optimal conditions for the column operation. The experimental parameters and its respective values, as well as the results for the separations efficiencies between fructooligosaccharides and glucose ($ES_{fructoolig/gluc}$), fructooligosaccharides and fructose ($ES_{fructoolig/fruct}$) and fructooligosaccharides and sucrose ($ES_{fructoolig/suc}$), are shown in Table 3.

Table 3. Experimental design, coded and real levels (in parentheses) and results of the 2^2 central composite design.

Assays	Ethanol Conc. (%)	T (^{0}C)	$ES_{FOS/gluc}$	$ES_{FOS/fruct}$	$ES_{FOS/suc}$
1	-1(10.00)	-1(40.0)	1.70	0.66	0.69
2	+1(20.00)	-1(40.0)	1.12	1.52	0.19
3	-1(10.00)	+1(60.0)	0.43	0.14	1.02
4	+1(20.00)	+1(60.0)	1.00	0.70	0.18
5	-1.41(7.95)	0(50.0)	1.90	0.78	0.95
6	+1.41(22.05)	0(50.0)	1.09	0.45	0.30
7	0(15.00)	-1.41(35.9)	1.37	0.61	1.46
8	0(15.00)	+1.41(64.1)	1.23	0.51	1.30
9	0(15.00)	0(50.0)	3.94	2.49	0.86
10	0(15.00)	0(50.0)	4.08	3.21	0.96
11	0(15.00)	0(50.0)	3.97	2.97	1.01

An estimate of the main effect is obtained by evaluating the difference in process performance caused by a change from the low (-1) to the high (+1) level of the corresponding factor [24]. The statistical parameters *t* test and *p* value were used to confirm the significance of the studied factors; in this case, $p<0.05$ suggested significance at the 0.05 level or a 95% confidence level.

A model fitting was accomplished for the efficiency separation response: the independent and dependent variables were fitted to a second-order model equation, and this model was evaluated in terms of goodness of fit. The analysis of variance (ANOVA; Table 4) was used to evaluate the adequacy of the fitted model. The R^2 value provided a measure of how much the model could explain the variability in the observed response.

Table 4. ANOVA for separation efficiencies between fructooligosaccharides, glucose, fructose and sucrose.

	SS	*df*	MS	F	R^2
		$ES_{FOS/gluc}$			
Regression	16.76	2	8.38		
Residual	1.37	8	0.17	49.1[a]	92.46
Total	18.13	10			
		$ES_{FOS/fruc}$			
Regression	10.73	2	5.37		
Residual	1.37	8	0.17	31.36[a]	88.68
Total	12.11	10			
		$ES_{FOS/suc}$			
Regression	1.23	2	0.61		
Residual	0.62	8	0.07	7.97[b]	66.58
Total	1.85	10			

[a] $F_{0.05;\,2;\,8} = 4.45$

[b] $F_{0.1;\,2;\,8} = 3.11$

On the basis of the ANOVA, a second-order model was established, describing the efficiency separation as a function of eluent concentration and temperature (Eqs. 9-11), since the calculated *F* value is higher than the critical *F* value. Coded models (Eqs. 9-11) were used to generate response surface for the analysis of the variables effects on separations efficiencies between fructooligosaccharides and glucose (Figure 7a) and fructooligosaccharides and fructose (Figure 7b).

$ES_{fructoolig/gluc} = 3.99 - 1.34\ \text{Eluent concentration}^2 - 1.44\ \text{Temperature}^2$ (9)

$ES_{fructoolig/fruct} = 2.889 - 1.10\ \text{Eluent concentration}^2 - 1.13\ \text{Temperature}^2$ (10)

$ES_{fructoolig/suc} = 1.03 - 0.28\ \text{Eluent concentration} - 0.31\ \text{Eluent concentration}^2$ (11)

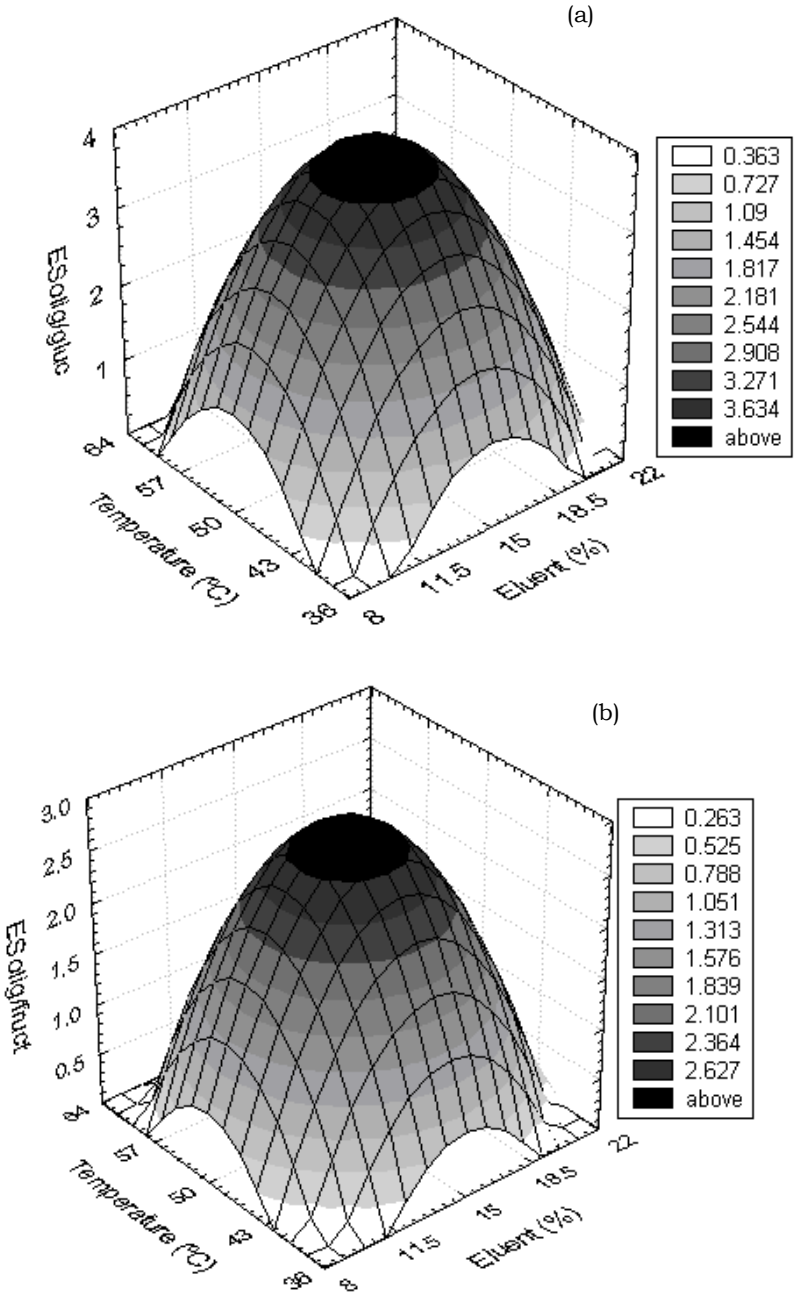

Figure 7. Response surface for separation efficiencies between (a) fructooligosaccharides and glucose and (b) fructooligosaccharides and fructose, as a function of the eluent concentration and temperature.

According to the ANOVA analysis of the separation efficiency between fructooligosaccharides and sucrose, the calculated *F* was approximately two times the critical *F* value, so Equation 11 was not used to generate a response surface for these results, since there was a lack of fit between experimental results and the ones predicted by the proposed model. However, this result concerning the poor separation between sucrose and fructooligosaccharides is of minor importance since sucrose is found in low concentrations in the sugar mixture, about 5-7%, which leads to a FOS purification degree of about 92%. On the other hand, separation of glucose and fructose from the mixture is quite efficient and can lead to a FOS purity like above, and a total recovery of 80% of FOS. Additionally, the remaining sugars can be recycled to the column in order to enhance recovery.

According to results from Figure 8 (a) and (b) the best conditions for FOS separation is using ethanol 15% (v/v) as eluent at 50^0C. In order to illustrate the results above, Figure 8 shows results for two conditions of separation: (a) using 15% ethanol solution at 35.9^0C and (b) 15% ethanol solution at 50^0C, demonstrating the above experimental design results.

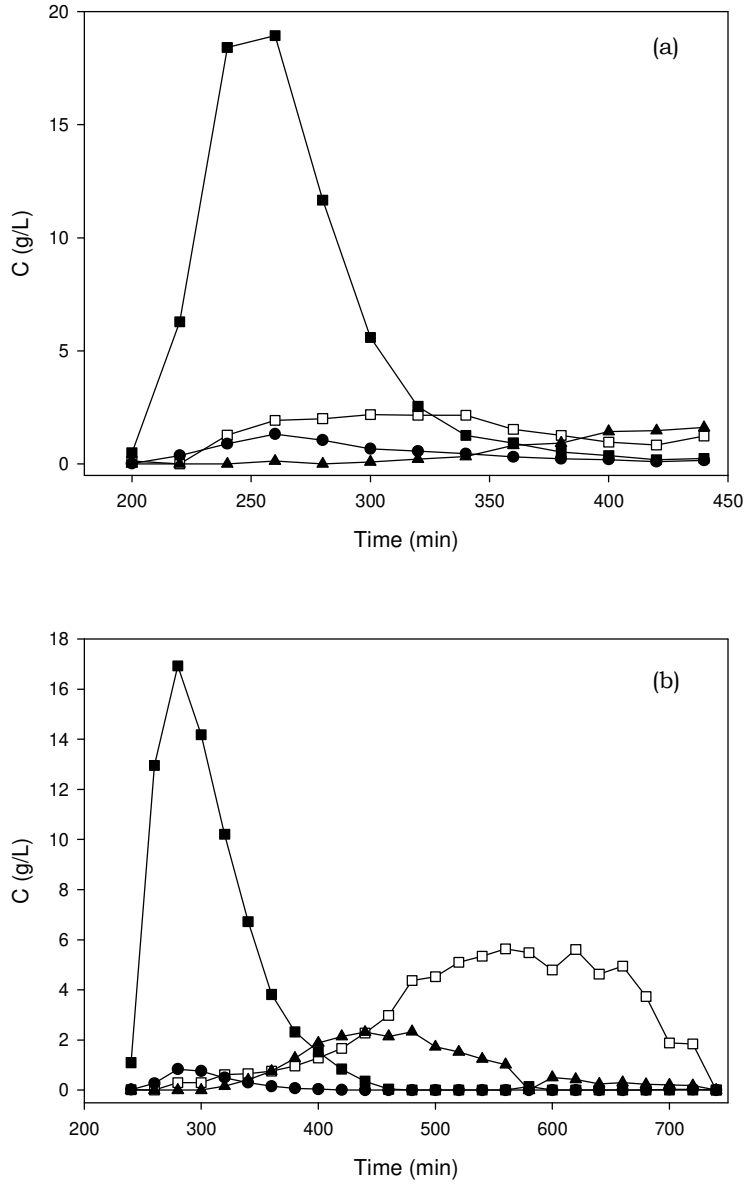

Figure 8. Time course for sugars leaving the fixed bed column, with superficial velocity = 0.13 cm/min using ethanol 15% (v/v) as eluent at (a) 35.9^{0}C and (b) 50^{0}C: (□) fructooligosaccharides, (●) fructose, (■) glucose, (▲) sucrose.

6.4. Conclusion

In this work, the development of separation technology for fructooligosaccharides was carried out. The adsorption kinetics experiments in

stirred tank reactor showed that the adsorption of fructooligosaccharides by activated charcoal follows different behavior compared to the adsorption of glucose, fructose and sucrose. Through the preliminary tests in fixed bed, it was observed that when water was the eluent there is no desorption of the sugars, especially fructooligosaccharides.

However, the activated charcoal fixed bed column was effective in separating the mixture of sugars, especially fructooligosaccharides and glucose and fructooligosaccharides and fructose, and the best separations were obtained using 15% ethanol solution (v/v) as eluent at 50^{0}C, these results were obtained after a 2^2 central composite design. A fixed bed column for the separation of sugars showed to be an interesting process, increasing the purity of the synthesized fructooligosaccharides. The final FOS purification degree and recovery were about 80% and 97.8%, respectively. The technique described in this work leads to a FOS with higher market value, and can be applied in industrial plants, since it is a low cost and robust process.

Acknowledgements: We would like to thank CNPq for the financial support.

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Chapter 7

Selection of membranes for purification of fructooligosaccharides

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Desalination and Water Treatment (in press)

Selection of membranes for purification of fructooligosaccharides

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Abstract

The aim of this study was to purify fructooligosaccharides (FOS) present in a mixture of sugars, containing also glucose, fructose and sucrose through, nanofiltration membranes. Four membranes were used: NP010 and NP030 (Microdyn Nadir, Germany), Desal-5 DL and Desal-5 HL (GE Water & Process Technologies). Experimental assays were performed in a dead-end cell and tangential cell filtration to select the most appropriate membrane. Then, with the membrane selected performed diafiltration in tangential cell filtration up to a concentration about 80% FOS. In the dead-end filtration cell experiments the NP010, NP030, HL and DL membranes were selected, since they performed the highest retentions of FOS, and lowest retention of glucose. The results showed that NP030 membrane performed the highest differences between the retention of FOS and sucrose, where the retentions of the different saccharides were: fructooligosaccharides (R_{obs} = 0.66), glucose (R_{obs} = 0.18), fructose (R_{obs} = 0.15) and sucrose (R_{obs} = 0.24). The results clearly demonstrate the potential of diafiltration using the NP030 membrane in the purification of FOS from mixtures containing mono and disaccharides.

Keywords: nanofiltration, fructooligosaccharides, separation, selection of membranes.

Nomenclature

J_w pure water flux (based on the membrane area), m/s

ΔP applied pressure drop, kN/m^2

R_{obs} observed retentions

C_p concentrated of the solute in the permeate, g/L

C concentrated of the solute in the feed, g/L

7.1. Introduction

Oligosaccharides are functional food ingredients, which have great potential to improve the quality of many foods [1]. Certain oligosaccharides are not digested or absorbed in the small intestine but are metabolized by desirable species of bacteria, mainly bifido and lactobacilli, resident in the colon. These oligosaccharides are classified as prebiotics [2,3]. Fructooligosaccharides (FOS) are oligosaccharides composed by fructose oligomers consisting mainly of kestose, nystose and 1-β-fructofuranosyl nystose, with one to three fructosyl units linked to sucrose in the β-2,1 position [4]. The FOS are present in the form of mixtures containing mono and disaccharides, so that the purification of FOS from this mixture becomes suitable, by removing the low molecular weight sugars that do not contribute to the beneficial properties of the higher molecular weight oligosaccharides. Nanofiltration (NF) appears to be a potential industrial scale method for purification and concentration of oligosaccharide mixtures because recovering low molecular weight species [5].

Several researchers have been evaluated the potential of nanofiltration to fractionated and concentration the oligosaccharides. According to Lopez Leiva and Guzman [6], the concentration and some purification of oligosaccharides mixtures are possible using NF membranes as an alternative to more expensive chromatographic techniques. In addition, Matsubara *et al.* [7] reported partial concentration of oligosaccharides from steamed soybean wastewater using NF membranes. Sarney *et al.* [8] used NF for the fractionation of human milk oligosaccharides and produced biologically active oligosaccharide mixtures with very little contaminating lactose. Kamada *et al.* [9] studied the effectiveness of combined membrane processing with ultrafiltration (UF) and nanofiltration (NF) for purifying and concentrating oligosaccharides from chicory rootstock. Goulas *et al.* [10] also studied the fractionated commercial oligosaccharides mixtures by applying diafiltration using two nanofiltration and one ultrafiltration membranes. Olano-Martin *et al.* [11] using the ultrafiltration dead-end membrane reactor to investigate the production of pectin-oligosaccharides.

The aim of this work was the selection of a NF membrane able to purify fructooligosaccharides from a mixture of sugars, which contains glucose, fructose, sucrose and fructooligosaccharides.

7.2. Material and Methods

7.2.1. Chemicals

Analytical grade purity glucose, fructose and sucrose were purchased from Panreac Quimica S.A (Spain). The commercial oligosaccharide mixture was fructooligosaccharides syrup from BENEO-Orafti (Belgium). The syrup consists of 51-53% of fructooligosaccharides, 25-28% of glucose, 10-13% of sucrose and 8-10% of fructose.

7.2.2. Membranes

Four membranes were used (Table 1), NP010 and NP030 (Microdyn Nadir, Germany), these membranes were made of polyethersulfone, Desal-5 DL and Desal-5 HL (GE Water & Process Technologies, USA), these membranes were made of aromatic polyamide.

Table 1. Permeabilities at 25 bar pressure in dead-end cell filtration.

Membranes	Permeability (m/Pa.s)	Retention (%)	MWCO (Da)[c]
NP010	1.01×10^{-12}	25-55[a]	1000
NP030	6.89×10^{-15}	80-95[a]	400
HL	6.84×10^{-14}	98[b]	150-300
DL	2.86×10^{-13}	96[b]	150-300

[a] Na_2SO_4 500mg/L at 40 bar.

[b] $MgSO_4$ 2000mg/L at 6.9 bar.

[c] MWCO of the membranes as given by the manufacturer.

7.2.3. Membrane filtration equipment

7.2.3.1. Dead-end stirred cell

The selection of membranes was performed in a dead-end stirred cell (Model Sterlitech) (Figure 1). The volume of the stirred cell is 200 mL while the effective membrane surface area is 14.6 cm^2. A magnetic stirrer was used to homogenize the feed solution and to reduce the concentration polarization. The feed of the stirred cell was pressurized using nitrogen gas from a gas cylinder.

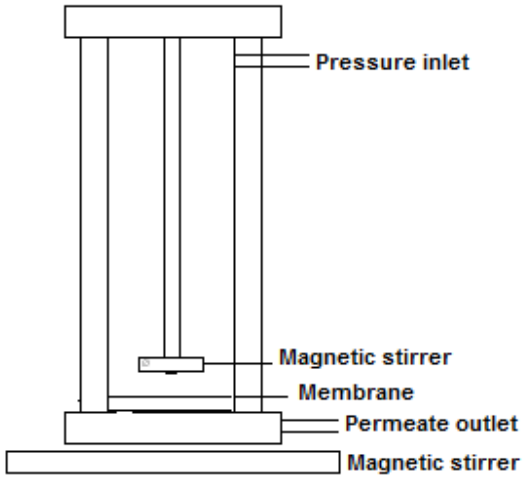

Figure 1. Dead-end filtration cell.

At the beginning of each nanofiltration experiment, distilled water was circulated and the pure water permeate flux of the membrane was measured. Pure water fluxes (Jw) were measured as a function of pressure (Δp) using ultra pure water to determine the permeability of membranes. The permeability of membranes (L_p) were calculated using the Equation 1:

$$L_p = \frac{J_W}{\Delta P} \quad (1)$$

The observed retentions for a given saccharide, based on the concentration determined from the sample analysis, were calculated from the Equation 2:

$$R_{obs} = 1 - \frac{C_p}{C} \quad (2)$$

where c_p is the permeate concentration while c is the feed concentration.

7.2.3.2. Tangential membrane cell

In order to perform the experiments in a tangential cell a system consisting of a tank for feeding the solution with temperature control (24-28ºC), a pump (Tuthill TXS2), two pressure gauges at the membrane inlet and outlet to measure the transmembrane pressure, a needle valve located after the membrane and one flowmeter to measure the retentate flow, was used (Figure 2) [12]. The effective membrane surface area was 66 cm^2. Samples of permeates and retentates were withdrawn at different times and analyzed by chromatography.

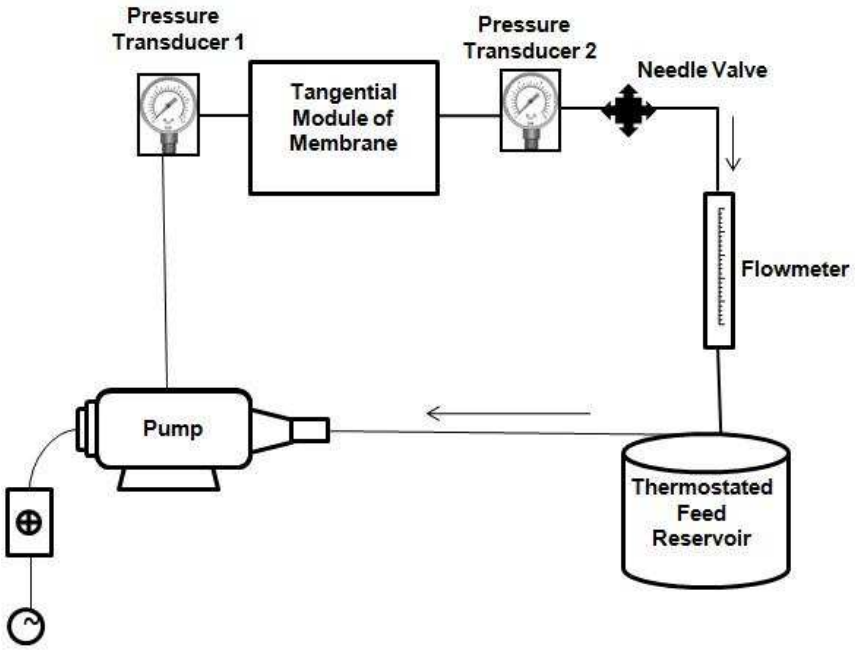

Figure 2. Experimental set-up of tangential cell filtration.

7.2.3.3. Diafiltration experiments

The diafiltration experiments were performed in tangential cell filtration consisted of a 2.3 L feed tank. The membranes were conditioned by compressing them to steady state with demineralized water as feed, at an intermediate pressure according to their pressure operation limits (40 bar). The feed concentration was about 250 g/L, and operation pressure was 3.5 MPa. The feed volume (2.3 L) was kept constant along the experiment by adding distilled water.

7.2.4. Analytical methods: analysis of sugar

Identification and quantification of saccharides (sucrose, glucose, fructose, and FOS) was achieved by HPLC Shimadzu LC-9A. Shodex KS 801 guard column and column at 22-24°C were used, using software Class-VP. The sugars were eluted in distilled water at a flow rate of 0.8 mL/min and injected volume at 20 µL. The methodology is a standard, defined by the manufacturer and clearly separated the sugars in question.

7.3. Results and Discussion

7.3.1. Dead-end stirred cell filtration

The stirring velocity used in the dead-end filtration cell experiments was 1110 rpm, was chosen the maximum stirring velocity which reduces the effects of concentration polarization [13], at 25 bar pressure, usual pressure in nanofiltration processes, room temperature and 250g/L. At low pressures, the relationship between permeate flux and rejection increases in a linear way but as soon as the pressure attains a certain level, the concentration polarization increases and the retention remains constant or decreases. It is not possible to provide a reference value for the optimal flow rate or pressure required using dead-end and membrane separation for saccharide separation, as the optimal value may vary considerably depending on the particular feedstock, i.e. solute properties (as treated above), the volume of the solution etc [13].

Table 1 shows the experimental values of permeabilities for the four membranes studied and it can be observed that the NP010 and DL membranes showed the highest permeabilities. Also the characteristics of the nanofiltration membranes provided by the manufacturer are presented in this Table.

The selection of membranes was carried out based on the highest observed retentions of FOS and lower observed retention for glucose because the glucose concentration is the highest in the mixture, after the FOS. The observed retentions depending of concentration polarization, depended largely on working conditions, how feed concentration, pressure, and stirring velocity, to decrease the effects of concentration polarization working with high stirring velocities. For the same membrane, with the same working conditions, the concentration polarization should be similar for all sugars and therefore the relative differences between the observed retentions can be considered constant. In these conditions it is possible to compare results of retentions from different membranes through observed retentions, defining the most appropriate for a particular process.

Therefore, the membrane that performed the highest retention of FOS was the DL (R_{obs} = 0.99) (Figure 3) and presenting an observed retention for glucose of R_{obs} = 0.92, the HL membrane presented the observed retention for FOS (R_{obs} = 0.97) and sucrose (R_{obs} = 0.89). The NP010 and NP030 membranes presented observed

retentions of FOS (R_{obs} = 0.81) and (R_{obs} = 0.82) and observed retentions of glucose (R_{obs} = 0.12) and (R_{obs} = 0.10), respectively. These membranes give lower observed retentions of FOS, but the observed retention of sucrose in both membranes (NP010 and NP030) are lower than for the others. In the case of DL and HL membranes, the retention of sucrose is about 90% and it would be very difficult to be separated from FOS with these membranes.

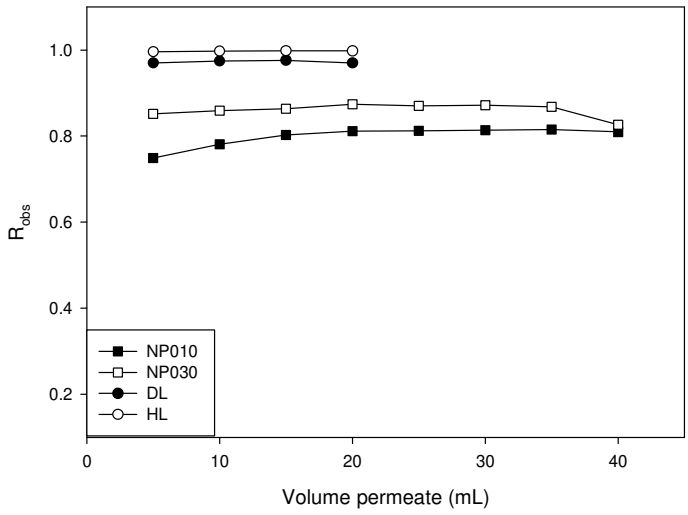

Figure 3. Observed retentions of FOS versus permeate volume using dead-end stirred cell.

7.3.2. Tangential membrane cell filtration

For a system of purification of FOS on a large scale were performed the same tests on a tangential membrane cell filtration. The Figures 4 (a-d), showed the observed retentions of the saccharides in the NP010, NP030, HL and DL membranes respectively, using tangential membrane cell filtration at 18 bar pressure, room temperature and 0.55 m/s tangential velocity and feed volume of 1 L at 250 g/L. It can be seen that the membranes NP010 and NP030 showed the lowest retention of glucose, fructose and sucrose, with values for the observed retentions at the end of the experiments of:

- glucose (R_{obs} = 0.26), fructose (R_{obs} = 0.22) and sucrose (R_{obs} = 0.47) for the NP010 membrane and

- glucose (R_{obs} = 0.18), fructose (R_{obs} = 0.15) and sucrose (R_{obs} = 0.24) for the NP030 membrane.

The DL membrane has a low retention of glucose and fructose (R_{obs} = 0.25) and (R_{obs} = 0.26), respectively, but has a similar retention of FOS (R_{obs} = 0.98) and sucrose (R_{obs} = 0.91). The HL membrane has a similar behavior even with somewhat lower values of retention for all sugars.

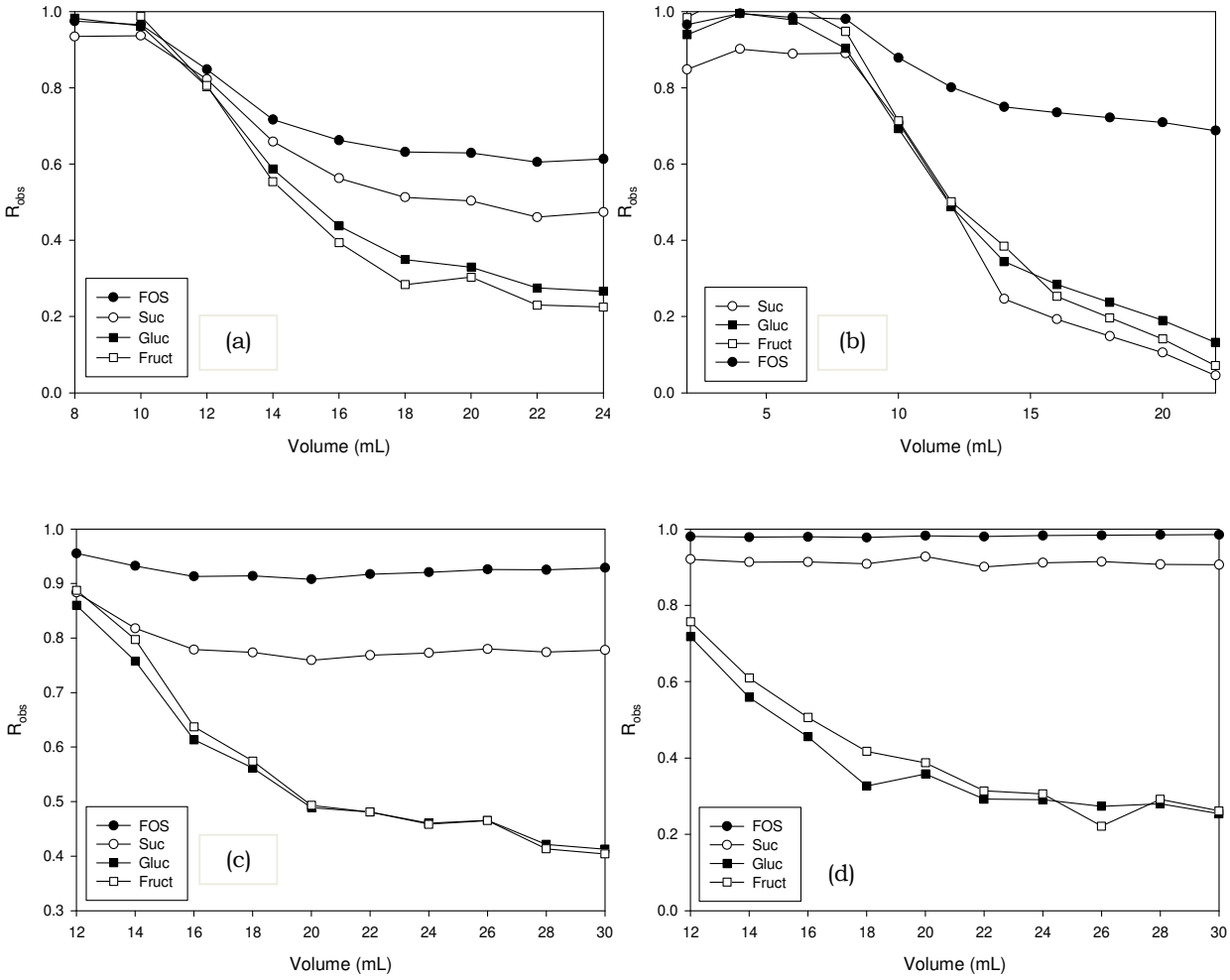

Figure 4. Observed retentions of saccharides versus permeate volume using tangential membrane cell filtration (a) NP010 (b) NP030 (c) HL and (d) DL membranes.

The NP010 and NP030 membranes were chosen to perform tangential filtration because these membranes showed greater differences in observed retention of FOS and sucrose, and they can be used to define a methodology for purification of

FOS. Although we are not considering the concentration polarization in these experiments, considering only the observed retentions, we do not believe that have relative differences between the true and observed retentions in this case. The Figure 4 shows the results for the permeate volume, in these case the permeate volume is very small and not change the feed concentration during the filtration.

The fouling is confirmed by an analysis of the flux versus time, in the Figure 5 shows a decrease of the flux of both membranes. It can observe that the NP010 membrane has a higer initial flux, then the process is faster, but the flux is decreased much faster too.

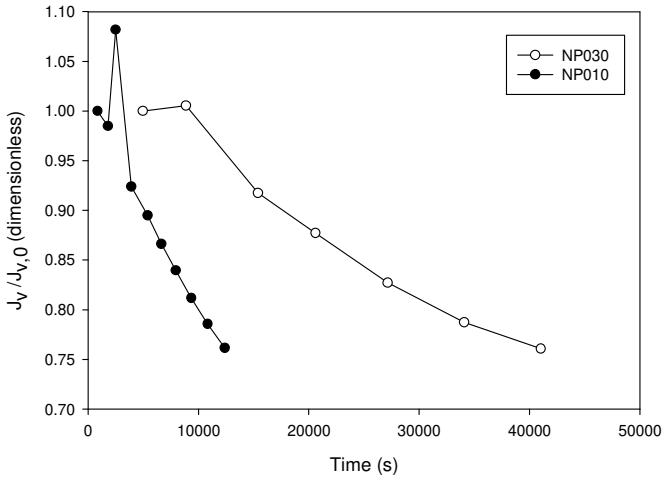

Figure 5. Relation between permeate flux versus time and the initial flux to the membranes NP010 and NP030 in the process of tangential filtration.

In the purification experiments the feed concentration can be changed, then others experiments were conducted to NP010 and NP030 membranes, at 18 bar pressure, room temperature and feed volume 2 L at 300 g/L. The first filtration was permeated half the original volume, about 1 L. The Figure 6a shows the observed retentions of FOS (R_{obs} = 0.64), sucrose (R_{obs} = 0.38), glucose (R_{obs} = 0.28) and fructose (R_{obs} = 0.31) in the NP010 membrane and the Figure 6b shows the observed retentions of FOS (R_{obs} = 0.72), sucrose (R_{obs} = 0.40), glucose (R_{obs} = 0.15) and fructose (R_{obs} = 0.27) in the NP030 membrane, it can be concluded that the NP030 membrane has the largest retention of FOS, whereas the observed retentions of sucrose and fructose are the same in both membranes, then this seems to indicate that the NP030 membrane is most suitable for the process of purification.

The Figure 6 shows the observed retentions in the NP010 and NP030 membranes, it can be observed that the membrane NP010 has an increased observed retention during filtration, since the membrane NP030 remains constant, this can be explained by fouling in the NP010 membrane.

The Figure 7 shows the observed retentions for the solution more diluted, this experiment was performed because the filtration has been slow due to high osmotic pressures generated by high concentrations of sugar, and then more dilute solutions were used to demonstrate that the relationship between retentions of sugars is the same. Then the feed volume was 2 L at 200 g/L, the volume permeate was 1 L. The observed retentions for the NP010 membrane were: FOS (R_{obs} = 0.57), sucrose (R_{obs} = 0.31), glucose (R_{obs} = 0.16) and fructose (R_{obs} = 0.31) (Figure 7a), and for the NP030 membrane the observed retentions were: FOS (R_{obs} = 0.71), sucrose (R_{obs} = 0.31), glucose (R_{obs} = 0.02) and fructose (R_{obs} = 0.001) (Figure 7b). The standard deviation between different experiments is less than 10%.

With these results we can define a methodology for the purification of FOS through diafiltration using the NP30 membrane. The diafiltration process seems more appropriate because the low concentrations the observed retention is greater and than the advancement of filtration there is a decrease of the osmotic pressure. The NP030 membrane is more suitable because this membrane shown that the biggest difference between the retentions of FOS and other sugars.

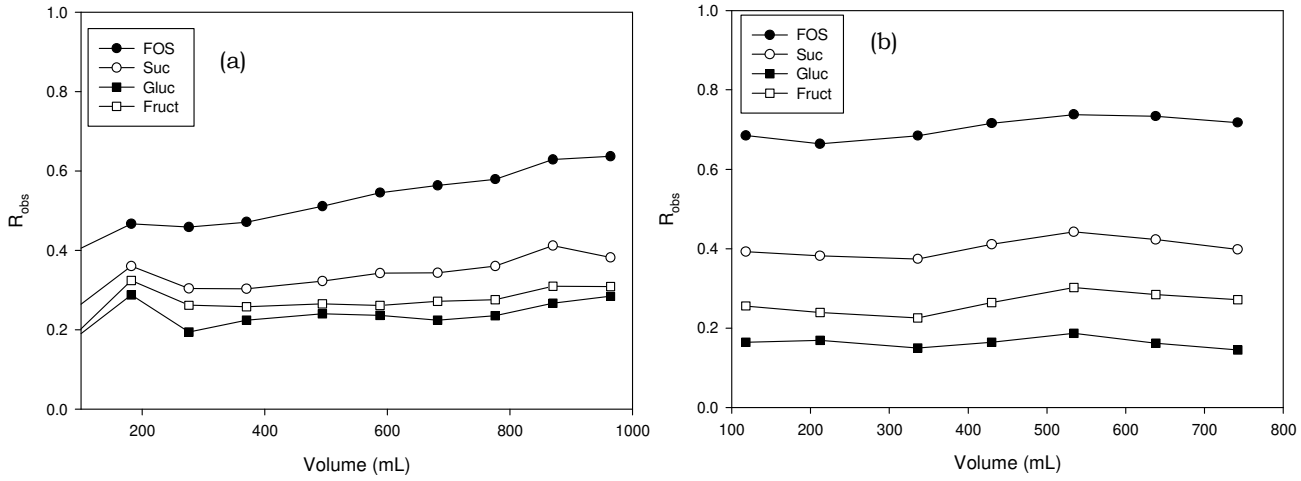

Figure 6. Observed retentions of the saccharides versus permeate volume using tangential membrane cell filtration (a) NP010 (b) NP030 membranes.

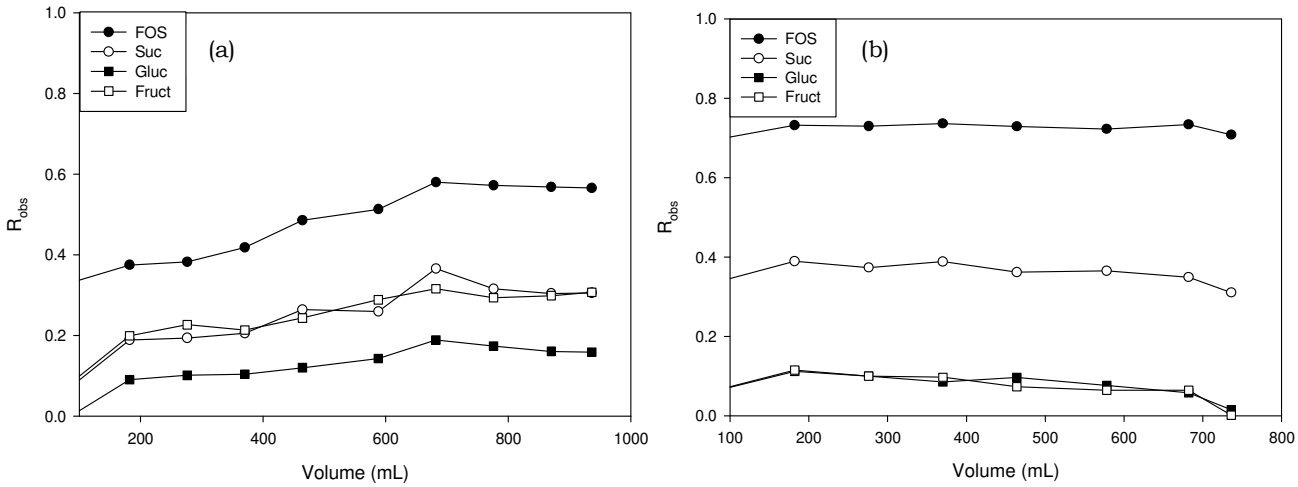

Figure 7. Observed retentions of the saccharides versus permeate volume using tangential membrane cell filtration with low feed concentration (a) NP010 (b) NP030 membranes.

Goulas et al. [10] observed that membranes composed for polyethersulfone appears to exert better separation characteristics than cellulose membranes, in this work the same was observed then the polyethersulfone membrane was the presented the better separation of mixture of sugars. This is because the sugars to be separated have a small molecular size difference that requires a more uniform pore size distribution in order for such separation to be achieved.

7.3.3. Diafiltration

The tests were carried out in the tangential cell filtration with the NP030 membrane because this membrane showed the highest observed retention of FOS and lower retention of others saccharides (glucose, fructose and sucrose). The diafiltration experiments were carried out with 2.3 L feed solution of the syrup, at 35 bar pressure, and feed concentration 350 g/L at room temperature with a recirculation flux of 6 L/min. The feed volume was kept constant along the experiment by adding distilled water up to a maximum concentration of FOS in the retentate. During this process the permeate flux increases significantly. This occurs because the fouling of the membrane is negligible and the difference in osmotic pressure decreases during the process. Table 2 shows the results of two filtrations, where the first was performed as a diafiltration up to a concentration about 80%

FOS. In the second filtration, the permeate volume of the first filtration was filtrated and it was obtained a concentration of FOS in the retentate, this retentate volume could be added to a continuous process.

Table 2. Permeate and retentate concentrations using NP030 membrane in the diafiltration process.

	First filtration				Second filtration	
	Conc. Init. (g/L)	Conc. Init. %	Retentate (g/L)	Retentate (%)	Retentate (g/L)	Retentate (%)
FOS	180.3	51.44	250.9	80.11	96.80	51.55
Suc	35.32	10.08	27.09	8.65	21.20	11.29
Gluc	98.02	27.96	25.73	8.22	55.80	29.71
Fruct	36.86	10.52	9.48	3.03	13.99	7.45
Tot Conc	350.5		313.2		187.79	

The Figure 8 shows the sugars concentration versus the cumulative volume of the permeate. Results showed that it was possible to obtain a purity greater than 80% in the first retentate and when permeate was filtered again the concentration of FOS obtained in the second retentate is concentrated, which would minimize losses of product, with the adding the retentate in the first tank.

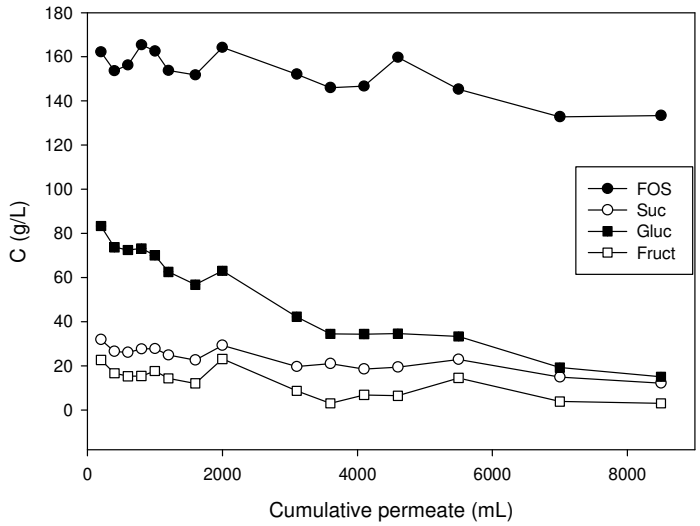

Figure 8. Retentate concentrations of the saccharides versus cumulative permeate flux in the NP030 membrane using tangential cell filtration.

According to the results of the NP030 membrane, one can conclude that the NP030 membrane is able to produce, by diafiltration, FOS solutions with high purity, thus increasing its applicability in foods.

7.4. Conclusions

According to the experiments performed in dead-end stirred filtration cell, was performed a classification of four membranes for their capacity for the purification of FOS. The NP010, NP030, DL and HL membranes were used in tangential cell filtration. From these experiments, the NP030 membrane was selected, since it presented the higher differences between the observed retentions for FOS and sucrose. From filtration results it is apparent that the most appropriate membrane for purifying fructooligosaccharides from mono and disaccharides is the NP030 membrane.

Diafiltration experiments with the NP030 membrane led to a concentrate with 80% of fructooligosaccharides. This study clearly demonstrates the potential of diafiltration using the NP030 membrane for the purification of fructooligosaccharides from mixtures containing mono and disaccharides.

The more regular, as far as pore size is concerned, the polyethersulfone membrane appears to exert better separation characteristics than the polyamide membranes. This is because the compounds to be separated have a small (as far as membrane separations are concerned) molecular size difference that requires a more uniform pore size distribution in order for such separation to be achieved.

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Chapter 8

Mass transfer and transport during purification of fructooligosaccharides by nanofiltration

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Mass transfer and transport during purification of fructooligosaccharides by nanofiltration

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Abstract

In this work, a process has been designed for the purification of fructooligosaccharides obtained by enzymatic transformation of sucrose from sugar cane. The designed protocol includes two nanofiltration stages with the same membrane. The first one is a diafiltration process, at constant volume followed by concentration. The second stage consists in the nanofiltration of the permeate of the first stage to obtain a concentrate similar in its characteristics to the initial solution. The process allows getting purities over 90% in fructooligosaccharides with yields around 80%.

These processes were studied and modeled by taking into account the effects of the resulting osmotic pressure and the changes in resistance due to solute adsorption on the membrane. The transport is modeled by assuming that there are diffusion and convection but hindered by friction in the pore. The steric partitioning, along with an adequate mass balance for the differently sized molecules have also been considered leading to get the pore size distribution of the membrane.

Keywords: Nanofiltration, fructooligosaccharides purification, mass transfer, adsorption, pore size distribution.

8.1. Introduction

Fructooligosaccharides (FOS) are fructose oligomers mainly composed of kestose, nystose and 1-β-fructofuranosyl nystose in which one to three of the fructosyl units are bound at the β-2,1 position of the sucrose [1]. They are better known as neo-sugars and have numerous beneficial and favorable functional properties, including improvement of the intestinal microflora [2-4], non-cariogenic, being diabetic-friendly products, decreasing the serum levels of cholesterol, phospholipids and triglycerides [1] and relieving constipation [5]. FOS have been widely used in bio-industries as sweeteners because of their positive functional properties [6].

FOS are regarded as functional food ingredients that may be employed in product formulation. FOS synthesis from sucrose, although being highly competitive, produces also mono and disaccharides, which are undesirable, and should be removed to improve product quality [7]. Besides, the commercial value of oligosaccharides increases with purity, boosting the interest in recovering oligosaccharides by techniques such as nanofiltration and chromatography, thus obtaining high purity products.

Nanofiltration (NF) membrane separation technology has been applied to the purification of FOS in lab-scale successfully [8, 9]. It is important to predict the results of purification under different operation conditions for the industrial design, including FOS purity, yield, dilute consumption and operation time. For the sake of technical prediction and optimization, quantitative analysis of the transport of saccharide molecules through NF membranes is necessary.

Microfiltration and ultrafiltration, are well established separation processes in the biotechnology and fermentation industry, are used as a way of purifying oligosaccharides from high molecular weight enzymes and polysaccharides. However, these commercial products often contain low molecular weight sugars that, as already mentioned, do not contribute to the beneficial properties of the higher molecular weight oligosaccharides. Nanofiltration appears to be a potential industrial scale method for purification and concentration of oligosaccharides mixtures [8].

The aim of this work was to separate FOS from a mixture of sugars, which contained also glucose, fructose, sucrose by using nanofiltration processes in two stages. The first stage includes a diafiltration process followed by a phase of concentration, reaching a high purity in FOS. In the second stage, the permeate of the diafiltration process is concentrated to increase the yield of the total process. The behaviour of the process has been analyzed by taking into account osmosis and considering the variation of the membrane resistance as a consequence of the adsorption process [10]. The transport through the pores has been studied in terms of diffusion plus convection, both hindered by friction along the pores and partitioning by steric effects [11, 12] at the pore entrance and exit.

8.1.1. Theory

In the filtration process of the molecules of small and medium size there are two fundamental factors that affect to the permeate flux, J_V [13]*:*

1- The osmotic pressure, ΔΠ and

2- The total resistance of the membrane system, $R_{sys}(t)$.

$$J_V = \frac{\Delta P - \Delta \Pi}{\eta_s R_{sys}(t)} \quad (1)$$

where Δp is the applied pressure and η_s is the solution viscosity.

If we assume that the osmotic pressure follows the van't Hoff's law, the osmotic pressure difference generated by all components present in solution, can be calculated as:

$$\Delta \Pi = \sum_{i=1}^{n} \Delta \Pi_i = \frac{R.T}{Mw_i} \left(C_{m,i} - C_{p,i}\right) \quad (2)$$

where $C_{m,i}$ is the concentration on the membrane surface and $C_{p,i}$ the permeate concentration of each component i.

The resistance of the membrane system is the sum of the membrane resistance, R_m plus a series of terms that depend on the fouling by the interaction between the solute and the membrane itself.

$$R_{sys}(t) = R_m + R_f(t) \quad (3)$$

$R_f(t)$ represents the reversible and irreversible fouling and, depending on the acting fouling mechanism, may be due to the formation of a cake, to the adsorption of solute molecules inside the pores or to the pore blocking when the pore size is similar to the molecule.

Although $R_f(t)$ has different origins, we can try to correlate it to the amount of foulants deposited or adsorbed on the membrane itself. For a diafiltration system, it is possible to perform a mass balance for the process, because the decrease of the mass of solute in the feed tank is the mass of solute permeating plus the mass of solute deposited on the membrane [10]. Thus for the i-th component,

$$V_f \cdot \frac{dC_{r,i}}{dt} + \frac{dV_p}{dt} C_{p,i} + \frac{dm_{ads,i}}{dt} = 0 \quad (4)$$

where V_f is the feed volume, t is time, $C_{r,i}$ is the concentration of the solute i in the retentate, V_p is the permeate flux and $m_{ads,I}$ is the mass of the i-th component adsorbed or deposited on the membrane.

Eq. (4) can be rewritten by taking into account that:

1) the volume flow J_V is

$$\frac{dV}{dt} = A_m J_V \quad (5)$$

where A_m is the membrane area

2) the true retention, R_i is

$$R_i = 1 - \frac{C_{p,i}}{C_{m,i}} \quad (6)$$

3) and the adsorbed mass of the i-component can be related with the concentration on the membrane surface, assuming that the adsorption rate follows a first-order kinetics, defined by the rate constant $k_{ads,i}$:

$$\frac{dm_{ads,i}}{dt} = k_{ads,i} \cdot C_{m,i} \quad (7)$$

Then, Eq. (4) reads as:

$$\frac{dC_{r,i}}{dt} = -J_V \cdot \frac{A_m}{V_f} \cdot C_{m,i}(1 - R_i) - \frac{k_{ads,i}}{V_f} \cdot C_{m,i} \quad (8)$$

Moreover, we can assume that the resistance due to fouling is proportional to the total adsorbed mass:

$$R_f(t) = K_R . \sum_{i=1}^{n} m_{ads,i}(t) = K_R . \sum_{i=1}^{n} \left(m_{0,i} + \int_0^t k_{ads,i} . C_{m,i} . dt \right) \quad (9)$$

where K_R is the proportionality constant and $m_{0,i}$ is the initial mass adsorbed for t=0.

The concentration on the membrane active layer, $C_{m,i}$ can be calculated from *the Film Theory* and the mass transfer coefficient, $K_{m,i}$ [14].

$$J_V = K_{m,i} \ln \frac{C_{m,i} - C_{p,i}}{C_{r,i} - C_{p.i}} \quad (10)$$

The mass transfer coefficient can be evaluated by means of an appropriate correlation of dimensionless numbers for the system and process conditions.

$$Sh_i = A \cdot Re^{\alpha} . Sc_i^{\beta} \quad (11)$$

where *Sh* is Sherwood number, *Re* is Reynolds number, and *Sc* is Schmidt number. They are defined as:

$$Sh_i = \frac{K_{mi} . d_h}{D_i} , \quad Re = \frac{d_h . v \rho_s}{\eta_s} \quad \text{and} \quad Sc_i = \frac{\eta_s}{\rho_s D_i} \quad (12)$$

where d_h is the hydraulic diameter of the membrane channel, v the average tangential velocity on the membrane surface, ρ_s is the solution density and D_i the diffusivity of each solutes.

Using the above equations in a diafiltration process for FOS purification, we can:

1) analyze the relative importance of the effect of osmotic pressure and the increase of resistance as function of time,
2) test the applicability of the model and
3) calculate the constant of adsorption, $k_{ads,i}$, to study the importance of the fouling with due to each solute.

The solute flux through the pores can be evaluated via the extended Nernst-Planck Equation that, for uncharged solutes, only considers diffusion and convection phenomena for the transport through the membrane. Both mechanisms are hindered by the pore friction [11].

$$J_i = -D_{p,i}\frac{dC_i}{dx} + K'_{c,i}C_i\frac{J_V}{A_k} \quad (13)$$

J_i is the flux of the i-solute

$$J_i = \frac{J_V C_{p,i}}{A_k} \quad (14)$$

and

$$D_{p,i} = K_{d,i}\, D_{\infty,i} \quad (15)$$

The hindrance factors: K_{id} and K'_{ic} are taken as functions of the ratio $\lambda = r_i/r_{p,}$ (in cylindrical geometry r_p is the pore radius). These hindrance factors were calculated here by using the correlations proposed by Dechadilok and Deen [15].

The partitioning coefficient, here due solely to steric effects, is:

$$\frac{C_{0,i}}{C_{m,i}} = \frac{C_{\Delta x,i}}{C_{p,i}} = \phi_i \quad (16)$$

Then, Eq. (13) can be integrated from the feed concentration at the pore entrance, $C_{0,i}$, to the permeate concentration (at the pore ending), $C_{\Delta x,i}$, and using Eqs. (14) to (16), the rejection of the solute i is calculated by:

$$R_i = 1 - \frac{C_{p,i}}{C_{m,i}} = 1 - \frac{K'_{c,i}\phi_i}{1-(1-K'_{c,i}\phi_i)e^{-Pe'_i}} \quad (17)$$

where Pe'_i is the Peclet number for the i-component, which is the ratio of convective to diffusive terms in the transport equation

$$Pe'_i = \frac{K'_{c,i}\, J_v}{D_{p,i}}\left(\frac{\Delta_x}{A_K}\right) \quad (18)$$

The Hagen-Poiseuille equation allows correlating thickness and membrane porosity, by assuming cylindrical pore geometry, as:

$$\frac{\Delta_x}{A_K} = \frac{1-r_p^2}{L_w 8\eta_p} \quad (19)$$

where L_w is the hydraulic permeability of the membrane, r_p the pore radius and η_p, is the water viscosity inside the pore, that can be related with the viscosity of free water by the next equation:

$$\eta_p = \left[\frac{\left(1-\frac{d_w}{r_p}\right)^4}{\eta_0} + \frac{d_w}{r_p} \frac{\left(4-6\frac{d_w}{r_p}+4\left(\frac{d_w}{r_p}\right)^2-\left(\frac{d_w}{r_p}\right)^3\right)}{10\eta_0} \right]^{-1} \quad (20)$$

The experimental results of rejection versus the permeate volume flux can be fitted to obtain the mean pore radius of the membrane. Because the membrane has a pore size distribution and the rejection of uncharged solutes is a sieving process, each solute will provide a pore radius proportional to its own size that corresponds to a certain fraction of pores. In order to evaluate the pore size distribution, it can be assumed that for each molecular weight, there is a fraction of totally retaining pores, while the rest of them allow a free pass of the molecules. Then, we can write the mass balance for each molecular weight as: [16].

$$\begin{cases} J_{W,t} = J_V(1 - R_i) \\ J_W = J_V(1 - C_{pi}) \end{cases} \quad (21)$$

where $J_{w,t}$ and J_w are pure water flux passing through the transmitting and the total pure water flux respectively.

Thus, $J_{w,t}$ /J_w versus the pore radius gives the accumulated fraction of flux passing through the non-rejecting pores. The derivative of this function should thus provide the fraction of flux-carrying pores for each permeating molecule of a given molecular weight. In order to obtain a continuous behavior, an analytical function can be used to interpolate the $J_{w,t}$ /J_w versus the molecular weight M_w, such that its derivative can easily be calculated. For the purpose of fitting well the experimental data it seems appropriate to use a logicial curve, with horizontal asymptotes at $J_{w,t}/J_w$ = 1 and 0, as follows [17]:

$$\frac{J_{W,t}}{J_W} = \frac{1}{1+\left(\frac{r_p}{B}\right)^C} \quad (22)$$

where B and C are constants to be evaluated by fitting Eq. (22) to the experimental results. In this way, the differential pore size distribution ($d(J_{w,t}/J_w)/dr_p$ vs. dr_p) can straightforwardly be obtained.

8.2. Material and Methods

8.2.1. Chemicals

Analytical grade purity glucose and fructose were purchased from Panreac Quimica S.A (Spain). The used sucrose was obtained from a food company (Acor) made from sugar beet. A commercial mixture of fructooligosaccharides (Orafti®P95) was acquired from BENEO-Orafti – Belgium (FOS 93.2 %, 6.8% other sugars). With these sugars was prepared a syrup, this syrup was called FOS-model, the concentration is similar to that obtained from enzymatic synthesis, and consists of 50-52% of fructooligosaccharides, 20-22% of glucose, 11-12% of sucrose and 10-13% of fructose, and its concentration was determinated by chromatography (HPLC).

8.2.2. Enzyme production

The enzyme was produced by the new microorganism, coded LEB-V10, from the Laboratory of Bioprocess Engineering, (UNICAMP-Brazil), isolated by Maugeri and Hernalteens [18], maintained on agar slants (glucose 2%, yeast extract 0.5%, malt extract 1%, Na_2HPO_4 0.2%, agar 2%) at 5^0C. Cultivation of the strain for enzyme production was carried out in liquid culture (glucose 2%, peptone 2%, yeast extract 1% and K_2HPO_4 0.5%, pH 4.5) at 30^0C and 150 rpm for 24 h. For enzyme production, 20 ml of inoculum were transferred to a 500 ml flask containing 200 ml of growth medium and cultured at 30ºC for 18.5 hours at 250 rpm. The fermentation medium consisting in (per liter): 65 g of sugar from cane molasses and 100 g of corn steep liquor. After cultivation, the cells were separated by centrifugation (5ºC) and the enzyme recovered as follows: ethanol was added to the supernatant solution at –20ºC up to a final concentration of 70% (v/v). The process was performed in a stirred reactor at 2^oC, under mild agitation. The precipitate was centrifuged, recovered and re-dissolved in sodium acetate buffer (0.05 M, pH 4.5).

8.2.3. Fructooligosaccharides synthesis

FOS were produced in stirred reactors containing sucrose 50% (w/v) from Synth® (Brazil) and enzyme (6.5 UI/mL) in 50mM sodium acetate buffer (pH 4.5), at

50^{0}C for 41h. Samples were taken and the enzyme inactivated by heating in boiling water for 5 minutes. The FOS yield was 55.4% and the resulting mixture contained 277 g/L of total FOS, 141.15 g/L of glucose, 48.8 g/L of sucrose and 33.2 g/L of fructose. This solution was called FOS-synthesis. The chromatographic analysis has allowed determining that FOS are mainly composed of a mixture of kestose (GF_2), nystose (GF_3) and 1-β-fructofuranosyl nystose (GF_4) [12, 2, 19] where G corresponds to a glucose molecule and F to a fructose.

8.2.4. Membranes

Six different polymeric flat NF membranes have been tested: NP010, NP030 from Microdyn-Nadir, NF270 from Dow-Filmtec (it was kindly supplied by the manufacturer as flat sheets) and DL, HL and DK from DESAL-Osmonics.

Once the selection was completed the two stage nanofiltration purification experiments were conducted on a spiral wound NP030 membrane with the characteristics are shown in Table 1.

Table 1. Nominal data of the membranes used.

Membrane	MWCO (Da)	Water permeability (10^{-11} m/Pa·s)	Max. pressure(bar)	Max. temperature (ºC)	pH range	Rejection (%)
NP010	1000	1.38-2.80	40	95	0-14	25-55 Na_2SO_4
NP030	400	0.28-0.50	40	95	0-14	80-95 Na_2SO_4
NF270	400	3.62[1]	41	45	3-10	85 $MgSO_4$
DL		2.12	40	50	3-10	96 $MgSO_4$
HL		2.67	40	50	3-10	98 $MgSO_4$
DK		1.51	40	50	3-10	98 $MgSO_4$

[1]Test measures with $CaCl_2$

8.2.5. Filtration devices

8.2.5.1. Membrane selection essays

The tests were conducted in laboratory with a 250 g/L sugar solution prepared from FOS-synthesis: First, in a stirred cell that allowed treating a small

solution volume (200 mL), and later with a tangential flux cell processing 2 L of solution FOS-Model.

For the first experiment, a STERLITECH™ HP4750 Stirred Cell was used. The system consisted in a cylindrical container with a membrane located at the base and a magnetic stirrer that simulates the convection in a crossflow process. The pressure difference was applied by a change in the internal atmosphere, with application of nitrogen gas. In this cell, the membrane area is 14.6 cm^2. The operation conditions were 25 bar of pressure, supplied by the N_2 and the stirred velocity was 1100 rpm.

Tangential experiments have been performed by using a flat sheet crossflow module with a single channel of length L=110mm, wide W=60mm and height H=0.5mm, resulting in a membrane area of 0.0066m^2. The pressure difference was 18 bars and the velocity of recirculation was 0.55m/s.

8.2.6. Purification processes

The experimental setup consisted of a 4.5 L feed tank with a coil for temperature control, a pump (Hydra Cell, model G03, Wanner Engineering, Inc., Minneapolis, USA), a spiral wound membrane (SPIRA-CEL ® OY NP030 2440D 1 - 44 mil parallel spacer) (Microdyn Nadir, Germain) with an effective membrane area of 1.8 m^2. Two pressure gauges at the membrane inlet and outlet to measure the transmembrane pressure, a needle valve located after the membrane and two flowmeters to measure the retentate and permeate flux (see Figure 1 where a scheme of this esperimental set up).

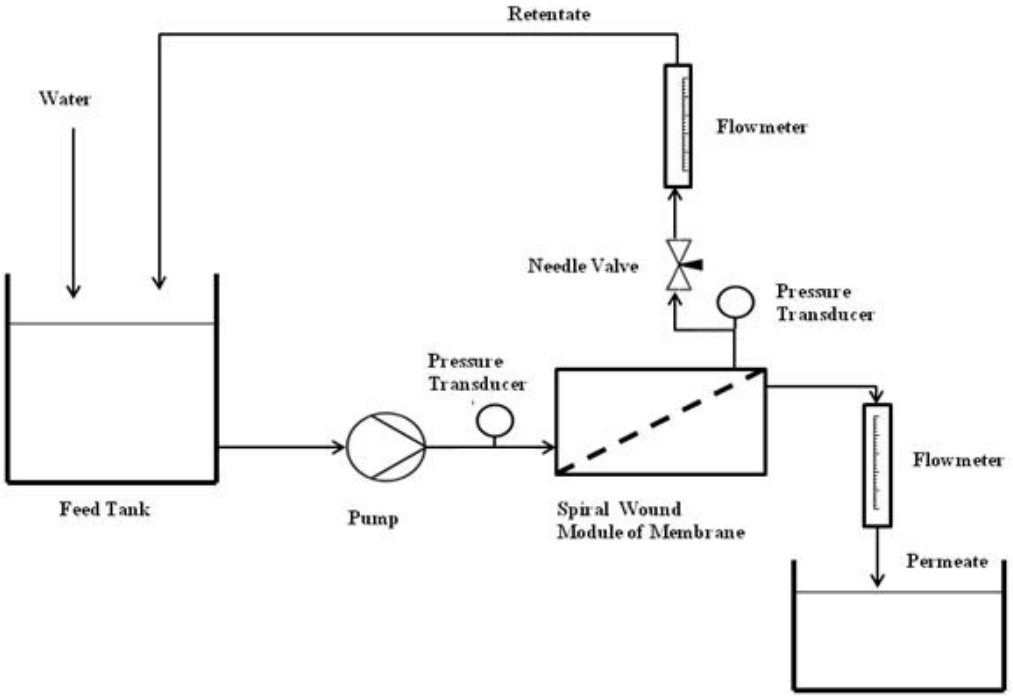

Figure 1. Experimental setup for the diafiltration process.

The membranes were conditioned by applying pressure to them, until reaching a steady state, with demineralized water as feed, at an intermediate pressure according to their pressure limits (40 bar). The solution obtained from the enzymatic process that has a concentration of 500 g/L is diluted to 250 g/L to reduce the effect of osmotic pressure in the process of diafiltration. The operating conditions were: a pressure of 4 MPa, a recirculation flow of 5 L/min and a temperature of 298 K. Under these conditions, and assuming that viscosity and density are similar to those for water, it can be set the operating parameters of the system as shown in Table 2.

Table 2. Fixed variables for the NF in the spiral wound (membrane filtration properties).

Parameter	Value
A_m (m^2)	1.8
d_h (m)	1.1×10^{-3}
μ_s (Kg/ms)	8.9×10^{-4}
ρ_s (Kg/m^3)	998

The feed volume (4.5 L) was kept constant along the experiment by adding distilled water. The time-course of the permeate flux was measured along the experiments. Samples of permeate and retentate were withdrawn at different times and analyzed by chromatography. When the solution has the desired purity in FOS, the addition of water was stopped and the solution concentrated until the solution of the tank was nearly exhausted. This first trial was performed with the FOS-model solution, to study the feasibility of the process before performing it with the FOS-synthesis solution.

The second stage involves a process of concentration of the permeate obtained in the previous process, for subsequent addition to the next batch processing. For that, 43.5 L of permeate are concentrated in the same system of nanofiltration at the same pressure and velocity of circulation until the concentration of FOS in the retentate is similar to the original solution in the diafiltration process.

These two stages can be integrated as shown in the Figure 2 to allow the batch concentration and purification of FOS obtained in the enzymatic process.

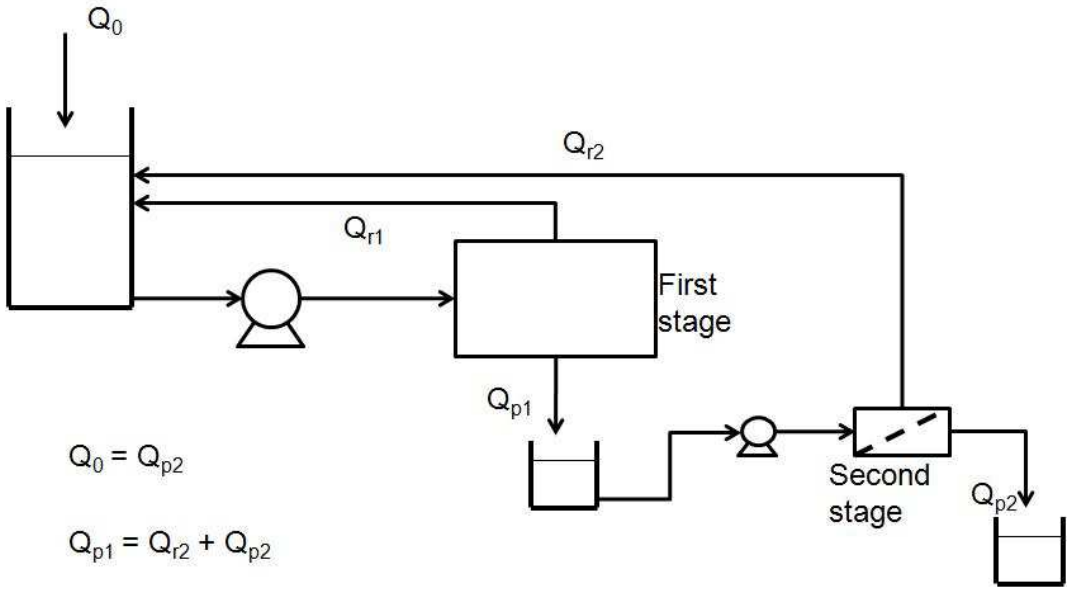

Figure 2. Scheme of FOS purification with two-stage nanofiltration. Q_0 is the water flux in the diafiltration process.

8.2.7. Analytical methods

Identification and quantification of the sugars (sucrose, glucose, fructose, and FOS (GF_2 and (GF_3+GF_4)) was achieved by an HPLC Shimadzu LC-9A. Chromatography was performed on a Shodex KS 801 guard column at 22-24°C, using software Class-VP. Sugars were eluted in distilled water at a flux rate of 0.8 mL/min and the injected volume was 20 µL.

8.3. Results and Discussion

8.3.1. Membrane selection

Hydraulic permeability was measured for the 6 membranes. Results are shown in Table 3. In principle, a higher permeability makes the membrane more interesting for the process. The observed retention for FOS should exceed a 50 % and to be significantly below this value for the rest of sugars.

Table 3. Hydraulic permeability of the studied membranes.

Membranes	Permeability (m/Pa.s)
NP010	1.012×10^{-12}
NP030	6.89×10^{-15}
NF270	2.68×10^{-13}
HL	6.84×10^{-14}
DL	2.86×10^{-13}
DK	7.51×10^{-14}

First, it was tested the six membranes with the stirred cell. A FOS-synthesis solution of 250 g/L of total sugars was filtered. The results (see Table 4) allowed to select two potential candidates, NP030 and NP010, since the other membranes presented sucrose and FOS retentions that were too similar.

Table 4. Results for retention of the different sugars, in the stirred cell.

	R_{obs} (%) FOS	R_{obs} (%) Sucrose	R_{obs} (%) Glucose	R_{obs} (%) Fructose
NP010	81	50	13	1
NP030	87	58	9	1
NF270	96	84	27	20
HL	97	89	17	12
DL	99	92	12	7
DK	98	93	15	14

The two selected membranes were tested in a tangential filtration cell which is closer to a process in a spiral wound module. Experiments were conducted with total recirculation of both permeate and retentate. Figure 3 shows the corresponding results. Note that at the end of the process:

a. The observed retention of FOS for both membranes is above 50% (R_{obs}=64% for NP010 and R_{obs}=72% for NP030).
b. The sucrose retention is also similar and below 50%.
c. Retentions of glucose and fructose are slightly lower for NP030.
d. The final retention of FOS for membrane NP010 is reached after a gradual increase over time from 40 % at the beginning of the process.
e. The membrane with larger difference of retention between FOS and the rest sugars is the NP030.

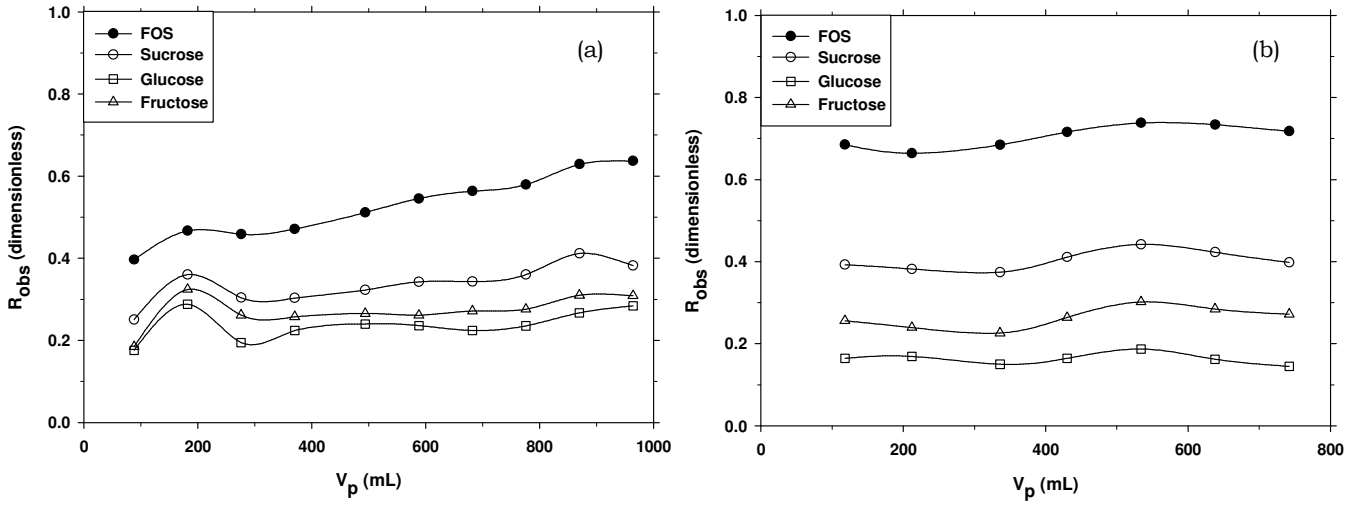

Figure 3. Observed retention versus the permeated volume in the tangential cell. (a) NP010 membrane and (b) NP030 membrane.

Based on these results, membrane NP030 was chosen as the most suitable for the process despite of the fact that membrane NP010 has a higher hydraulic permeability, more than two orders of magnitude. Membrane NP010 has been dismissed because:

a. It shows more fouling causing an increase of the FOS retention over time.
b. It has lower final retention of FOS.
c. There would present a possible decrease in FOS retention in the diafiltration process since its concentration would decrease in the feed solution.
d. Finally, the glucose retention, R_{obs}=28% is slightly higher that for the NP030 membrane (R_{obs}=15%) (Glucose is the sugar with the highest concentration in the initial solution ~ 30 %).

8.3.2. Diafiltration process and batch concentration

To analyse the feasibility of the process, a diafiltration process with the FOS-model solution, with concentration 250 g/L, was conducted. Results showed that it was possible to obtain a purity of 90% in the first retentate and when permeate was filtered again the concentration obtained in the second retentate is similar to the initial solution, which would minimize losses of product.

The detailed study of the evolution of system variables in the process has been carried out with a diluted solution (250 g/L) of FOS-synthesis to reduce the effects of osmotic pressure. When in the diafiltration process, a retentate concentration around 90% in FOS is obtained, the water addition is stopped to concentrate the solution. The time evolution of the permeate flux J_V is shown in Figure 4. In the diafiltration process the retentate concentration decreases (especially for smaller molecules) with the time and the same doesn't happen to osmotic pressure. Thus according to Eq. (1), J_V increases despite the increasing the resistance due to adsorption on the membrane surface. In the process of retentate concentration, osmotic pressure increases and thus J_V decreases.

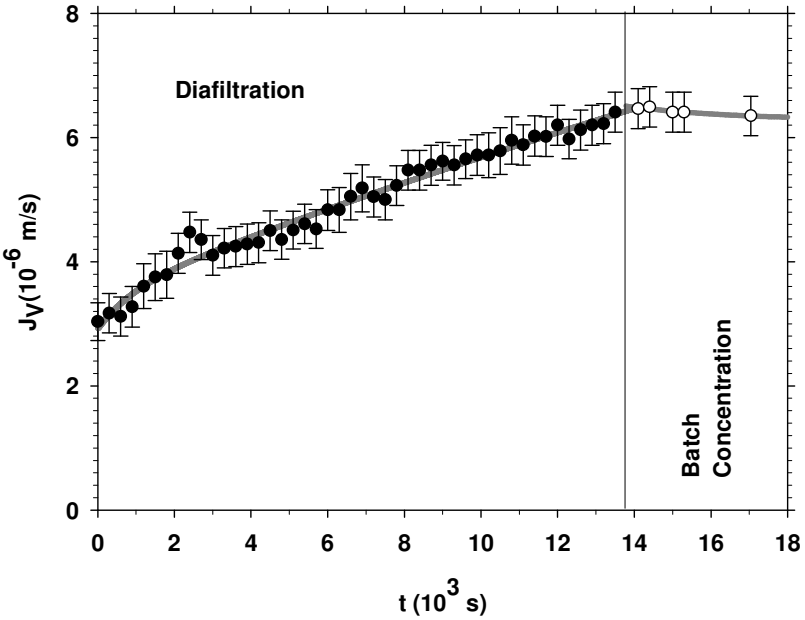

Figure 4. Time evolution of the permeate flux, J_V. Experimental (symbols) and visual trend (solid lines).

In the retentate, the concentration of all sugars decreases with the time, since none is completely retained. However, the concentration of small sugars tends rapidly to very low values during the process, as shown in Figure 5. Note that when molecular size decreases, the concentration in the retentate increases.

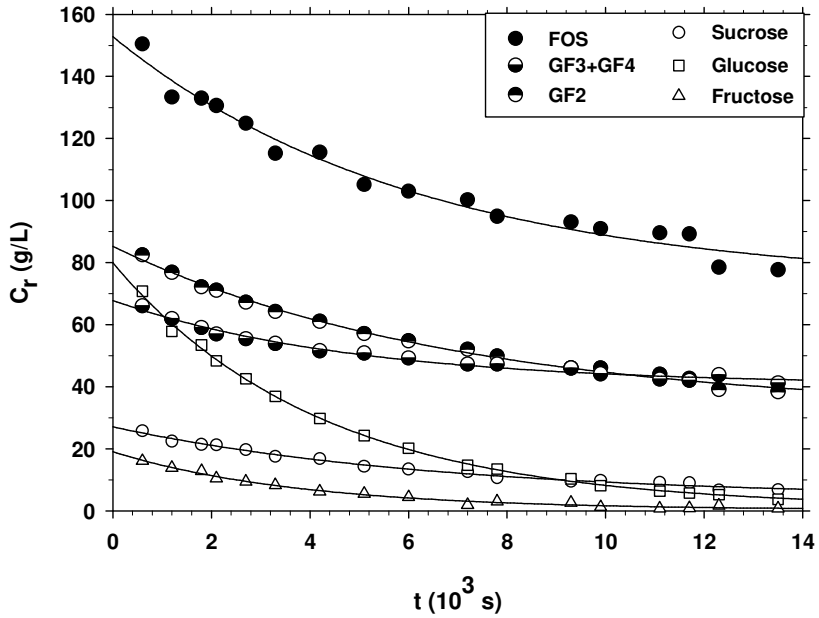

Figure 5. Time evolution of the concentration of the different sugars in the retentate during the diafiltration process. Experimental (symbols) and visual trend (solid lines).

Figure 6 shows the time evolution of the observed retention for the studied sugars in the process of diafiltration. It shows that the total retention of FOS is intermediate between GF_2 (lower) and GF_3+GF_4 (highest). In the first stage, the observed retention showed a slight increase as a result, a possible pseudomembrane formation on the surface by deposition of solutes. The larger molecules reach soon a fairly stable value of the observed retention. This indicates a constant value of the concentration ratio. The smaller molecules tend to very high retentions for large times. This happens because concentration in the feed solution tends to zero and, in this case, R_{obs} approaches 1 [20].

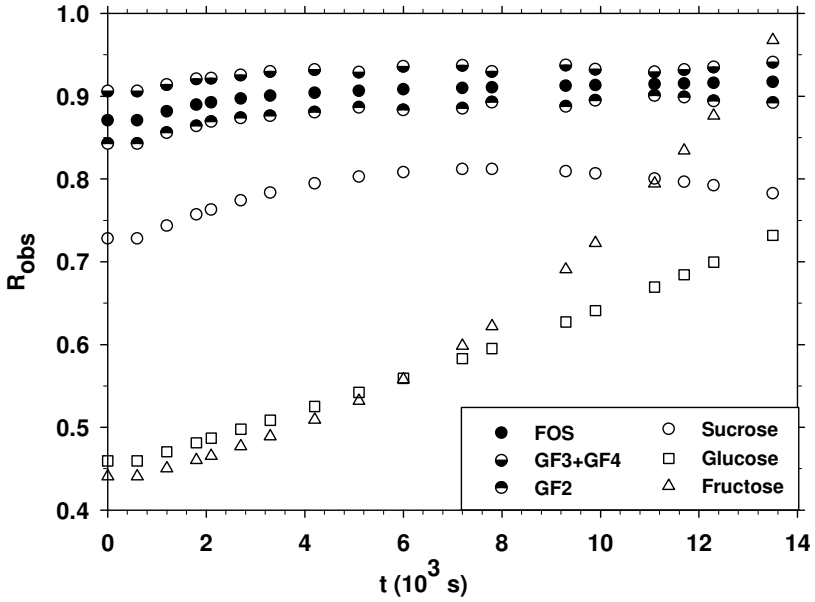

Figure 6. Time evolution of observed retention for the different sugars in the diafiltration process.

We can use the model based on mass balance, expressed by Eqs (4) to (9), to analyse the importance of adsorption on the surface of the membrane. The application of Eq. (8) allows determining the constant of adsorption of each sugar. The concentration on the surface of the membrane is calculated by The Film Theory (Eq. (10)). The mass transfer coefficient outside membranes can be calculated by Eq. (11), which considered the effects of spacer materials between membranes in spiral wound, and is suitable when 100<*Re*<1000. [21]. Under these conditions we can write Eq. (11) as:

$$\mathrm{Sh_i} = 0.065.\,\mathrm{Re}^{0.875}.\,\mathrm{Sc_i}^{0.25} \qquad (23)$$

Table 5 shows the solute properties used. The density and viscosity of the solution was considered equal to that of water. The solute radius has been calculated considering the molecules as spheres [10] and the diffusion coefficients have been estimated by the Stokes-Einstein equation [22].

Table 5. Value of parameters for each component.

Parameter	GF_4	GF_3	GF_2	Sucrose	Glucose	Fructose
Mw (g/mol)	828	666	504	342	180	180
ρ_i (Kg/m^3) [23]	1592	1590	1542	1587	1562	1600
$r_{p,i}$ (m)	$5.91x10^{-10}$	$5.50x10^{-10}$	$5.06x10^{-10}$	$4.40x10^{-10}$	$3.57x10^{-10}$	$3.55x10^{-10}$
D_i (m^2/s)	$4.15x10^{-10}$	$4.46x10^{-10}$	$4.85x10^{-10}$	$5.57x10^{-10}$	$6.86x10^{-10}$	$6.92x10^{-10}$

Table 6 shows the values of the mass transfer coefficient and Reynolds, Schmidt and Sherwood numbers calculated from data in Tables 2 and 5 for recirculation flux of 5 L/min, for each solute present in the solution.

Table 6. Values of the dimensionless numbers and K_m for each solute in the operation conditions of the system.

Parameter	GF_4	GF_3	GF_2	Sucrose	Glucose	Fructose
Re (dimensionless)	100.3	100.3	100.3	100.3	100.3	100.3
Sh_i (dimensionless)	24.94	24.50	23.99	23.17	22.00	21.95
Sc_i (dimensionless)	2149	2000	1839	1601	1300	1289
K_m (10^6 m/s)	9.4	9.9	10.6	11.1	13.5	13.7

Figure 7 shows Eq. (8) so that the absolute value of the slopes of the lines represent the constants of adsorption of each solute, $k_{ads,i}$. All lines have a correlation coefficient greater than 0.97, which means that the assumption of adsorption kinetics of first-order in Eq. (7) was adequate. The mixture of FOS (GF_3 + GF_4) was evaluated as a whole because the HPLC column did not completely separate the two peaks. In this case, the concentration of both these species for the initial and final instants was assumed to be the geometric mean value of both species.

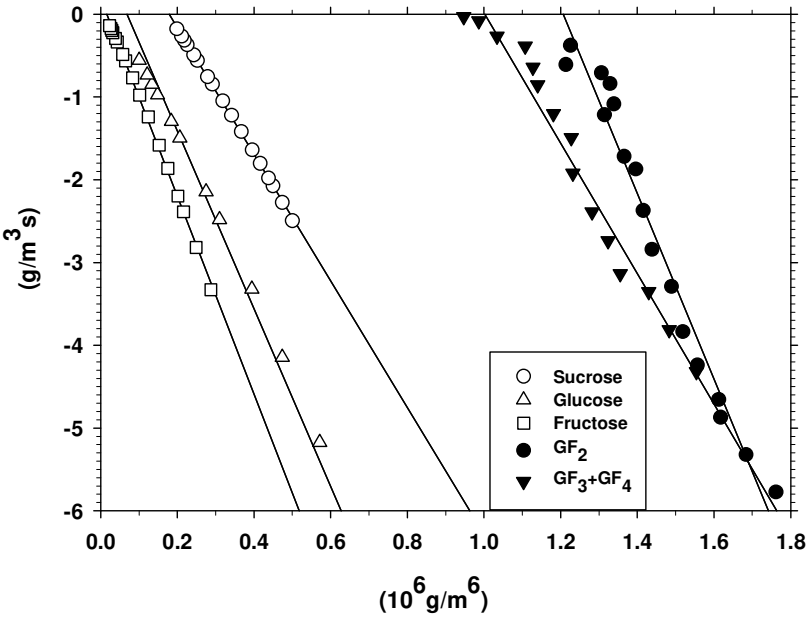

Figure 7. Linear fit to obtain the adsorption constant of the different sugars by using Eq. (8).

The kinetic constants of adsorption are represented in Figure 8 as a function of the molecular weights. This constant is higher for small and large molecular weights than for intermediate ones. This behavior can be explained as follows:

a. Due to their size, small molecules have access to both the outer as the inner membrane surface. That is, they can be absorbed over a large area. This process in which molecules adhere to the inner wall of the pores is known as "Standard Blocking Model" [24] (see Figure 8). In the diafiltration process, the concentration of small molecules in the retentate declines rapidly. Adsorption is only important for short times (see Figure 5).

b. Due to their size, large molecules cannot penetrate inside the pores; they tend to accumulate in the outer surface of the membrane. The magnitude of the accumulated layer will depend on the kinetic constant of the process, k_{ads}, and on the balance between deposition by the convective effect and the drag induced by the tangential flux. This fouling mechanism is known as "Cake Filtration Model" [24] (see Figure 8). In this case the balance between convective and shear

forces produces a slight increase in the accumulation because the recirculation velocity is constant while J_v increases with time (see Figure 4). By contrast, the effect of adsorption equilibrium should be decreasing because the concentration of these species is decreasing (see Figure 5) with time.

c. Middle-sized molecules have the ability to pass through the pores or to be adsorbed to the outer surface but do not have much room to adsorb on the inner surface of the membrane but rather they block the pore, preventing the deposition of other molecules. That is, the adsorption constant is smaller than for the other molecules in the mixture. This mechanism in which the molecules attach to the outer surface and/or block the pores is known as "Complete Blocking and Intermediate Blocking Models" [24] (see Figure 8).

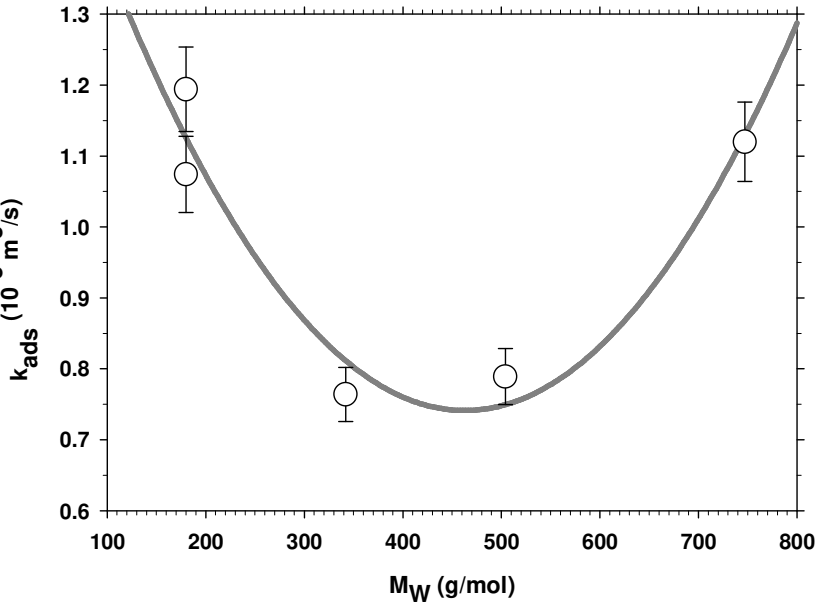

Figure 8. Adsorption constant as a function of the molecular weight and adsorption mechanisms proposed for each case.

If regardless of the mechanism that occurs in each case there is a proportionality between the adsorbed mass and the resistance due to fouling, $R_f(t)$. Eq. (9) allows finding out this proportionality constant. To do this, $R_{sys}(t)$ and the osmotic pressure decrease are calculated by using Eqs. (1) and (2), respectively.

Thus, with Eq. (3) it can be calculated $R_f(t)$ since R_m was calculated by measuring the permeability of the membrane to distilled water operating at different transmembrane pressures. The slope of the plot J_p vs ΔP gives the permeability of the membrane L_p (1.8519 x 10^{-12} m/Pa). The membrane resistance R_m (7.6 x 10^{14} m^{-1}) was calculated from Eq. (1) when R(t) = 0 and $\Delta \Pi = 0$.

Figure 9 shows the evolution of $R_f(t)$ versus time for the filtration process. As can be seen, the resistance due to deposition is negligible even in the initial moments in which their value is lower than the resistance of the clean membrane. As occurs in diafiltration, concentration in the retentate species decreases and the molecules detach from the surface of the membrane doing the resistance due to fouling almost negligible. This allows to corroborate the membrane material is appropriate for this process of purification.

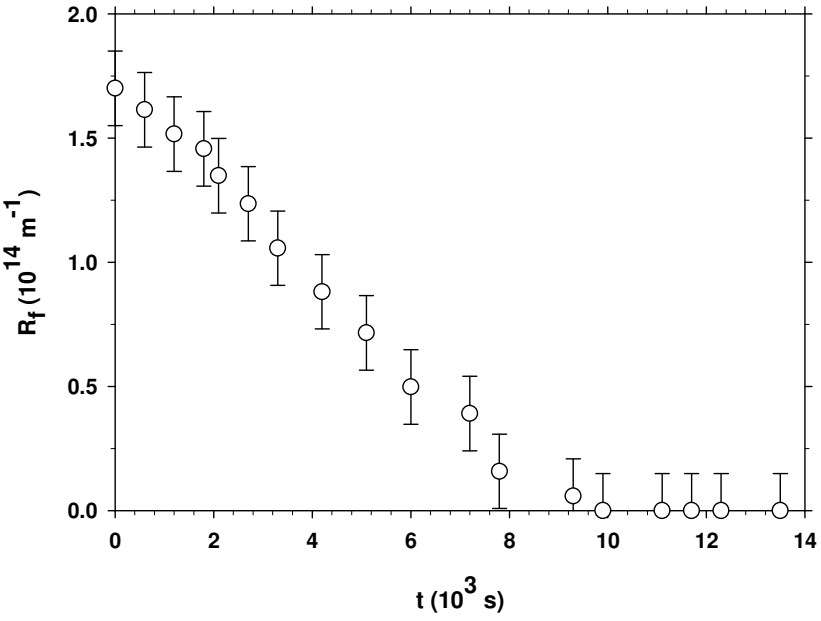

Figure 9. Time evolution of the resistance due to fouling during the diafiltration process.

As it was assumed, the representation of $R_f(t)$ as a function of mass on the membrane surface at any moment ($m_{ads}(t)$ -m_0) shows a linear behavior (Figure 10). The value of K_R=8.0×10^{15} $m^{-1}kg^{-1}$ is in the same order as that obtained by other authors in similar experiments [10].

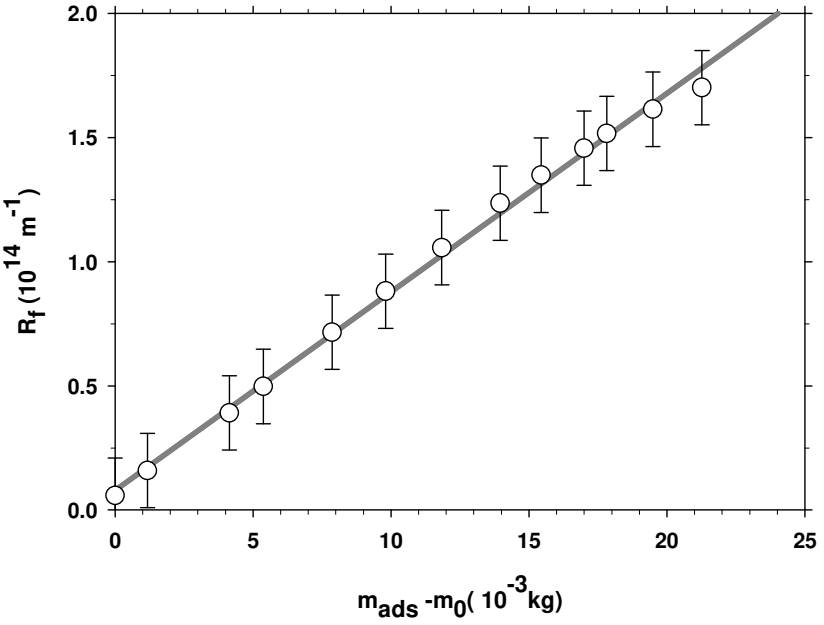

Figure 10. Resistance due to fouling as a function of the adsorbed mass in the membrane.

The Nernst Planck equation can be used in order to analyse the transport process of solutes through the membrane, and to compare the size of them with the average pore size. Using Eq. (13) to (20), the mean pore size of the membrane can be evaluated from the true retention values, R_i versus J_V. Although other authors introduce the effect of the applied pressure gradient for the same system [12], their works show that the introduction of this effect does not change the results significantly. In a previous work, it was observed that the introduction of the effect of pressure on this type of system leads to changes in retention below a 2% [25, 26]. Figure 11 shows the true retention values as a function of permeate flux and the model fitted lines. The deviation between the R_i data and the fit may be due to the effect of adsorption/desorption of molecules which modifies the structure of the membrane and it is not taken into account by this model. The pore size results obtained with each molecule are shown in Table 7. Although in most cases, this model has been tested in conditions of total recycling of permeate and retentate [27], recent works, where the model is applied with different operation modes (total recycling, retentate concentration or continue diafiltration), show that the parameters are independent of operating conditions [28].

Table 7. Data for the pores radius.

	M_w (g/mol)	$r_{p,i}$ (nm)	$<r_p>/r_s$
FOS	666	0.69	0.96
GF_3+GF_4	747	0.71	0.91
GF_2	504	0.66	1.04
Sucrose	342	0.61	1.18
Glucose	180	0.55	1.46
Fructose	180	0.56	1.47

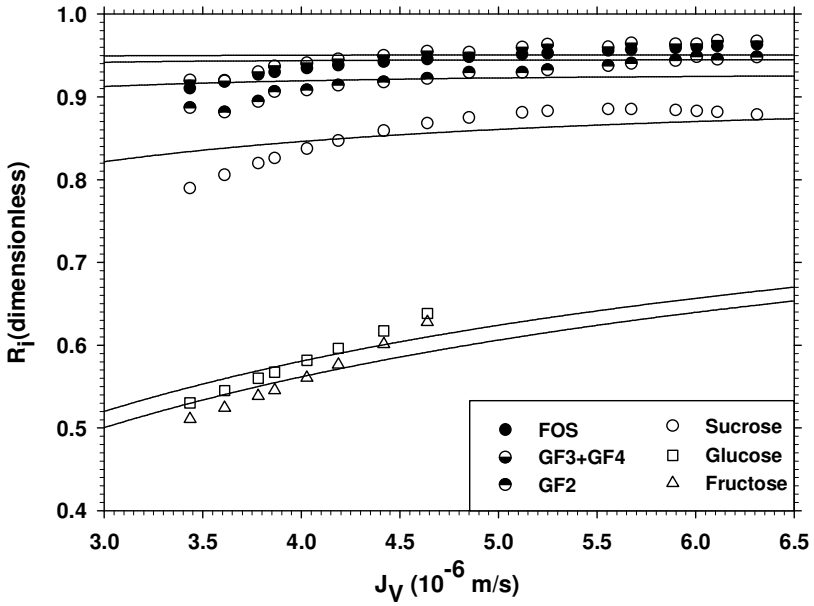

Figure 11. True retention (R_i) versus permeate flux (J_v) and fit of the model of mass transfer through the membrane.

With the true retention values and J_v, using Eq. (21) it can be obtained the cumulative distribution of pore sizes. The flow ratios obtained from experimental data have been fitted to the Eq. (22) to obtain an analytic expression for the cumulative distribution (Figure 12.a). The analytical or numerical differentiation allows getting the differential distribution (Figure 12.b). This function is almost symmetrical. Therefore, they can be fitted to a Gaussian function which leads to a mean pore size and a standard deviation: $<r_p>$ = 0.52 nm, σ = 0.08 nm.

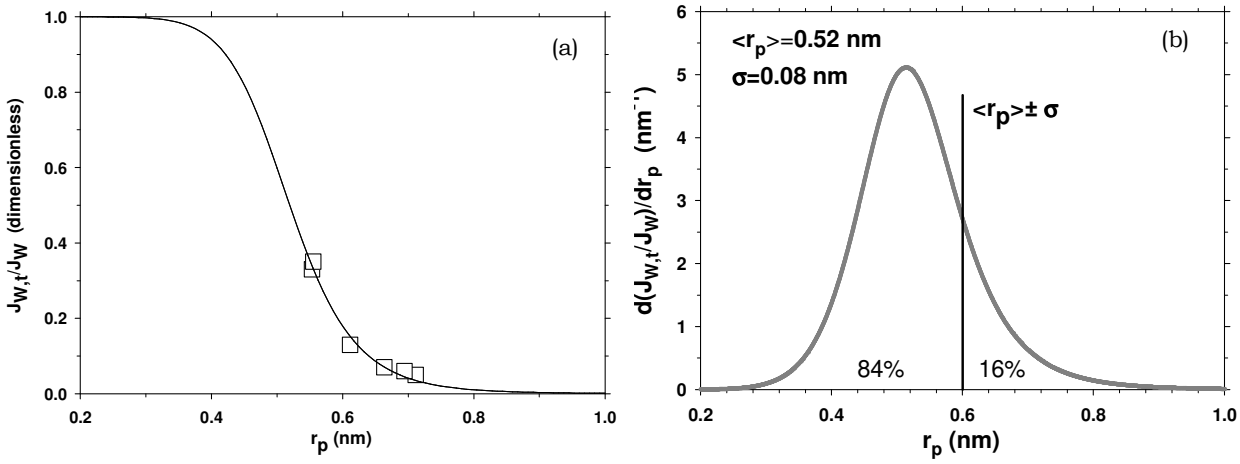

Figure 12. (a) Flux ratio, $J_{w,t}/J_w$, versus pore size and fitted curve according to Eq. (22) (b) Pore size differential distribution of the membrane.

In the last column of Table 7, we compare the mean pore size and the molecule size. It can be seen that, as in Figure 8, the larger molecules are slightly higher than the mean pore size. This is why, it is difficult for them to penetrate into the pores and tend to accumulate on the surface. The molecules of medium size are between 4% and 18% smaller than the mean pore size and should pass through the pores without difficulty, but if they are adsorbed inside they block the pore. The smallest molecules are about 50% smaller than the pore size, so if they are adsorbed, at least in internal areas where the pore broadens, they can coat the inside wall and allow the other molecules to pass. However, as this is a distribution, a portion of the large molecules can pass through the pores larger than the mean value (50% of pores). Figure 12.b shows that 16% of pores are larger than (<r_p> + σ) = 0.6 nm, and are wider than the largest molecule GF_4 (see Table 4).

One of the objectives of the study of this process is to find a method for purification and concentration of FOS. The degree of purification in the first stage (see Figure 2) consisting of diafiltration and batch concentration can be calculated as [9]:

$$Pu\% = \frac{C_r^i}{C_r^{Total}} \cdot 100 \qquad (24)$$

Where C_r^i is the concentration in the retentate of component that it is evaluated and C_r^{Total} is the total concentration. Figure 13 shows the time evolution of the purity of each spice in the retentate. The first stage is diafiltration keeping constant the volume because, as has been shown in previous studies, no significant differences there are in the process with VVD (variable volume diafiltration) [9]. When it has reached the desired degree of purity, the addition of water is stopped and then the solution is concentrated (Batch concentration) until the volume is low enough. The Figure 13 shows that the behavior of the species is appropriate: FOS increased until values of 90% whereas the rest of sugars reduce their proportions until symbolic values. The final process of Batch concentration does not affect the purity of final product.

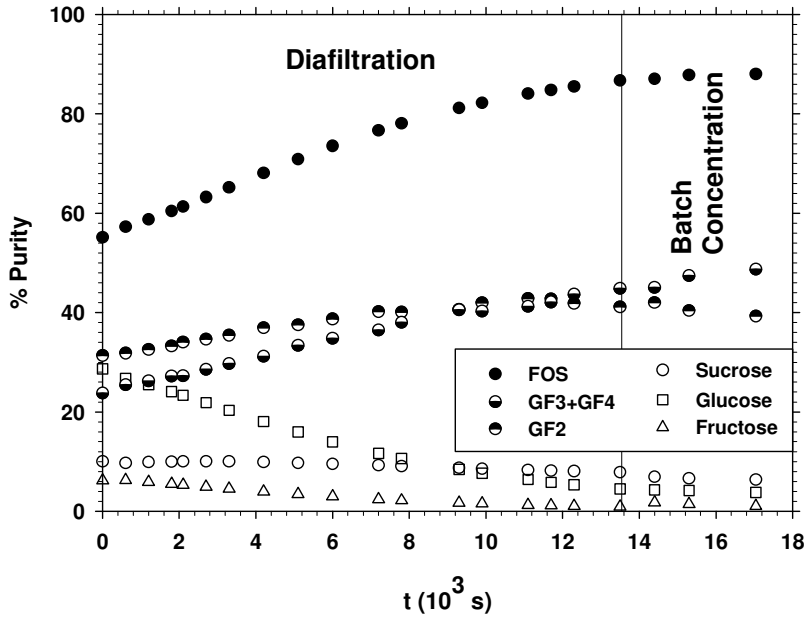

Figure 13. Time evolution of the purity of the substances present during the diafiltration and Batch concentration.

8.3.3. Nanofiltration process of permeate

In order to recover a part of the FOS that has permeated during the diafiltration stage it is proposed a filtration process of permeate obtained in the first stage, as shown in Figure 2. Figure 14 presents the time evolution of the flow in this second stage. In order to evaluate the reliability of the model used in the previous process, in this stage the permeate flux was calculated from Eq. (1) using the

resistance, $R_f(t)$, calculated by Eq. (9) with K_R value obtained in the diafiltration process. The prediction of the model is presented in Figure 14. As shown, the correlation experimental values and predicted ones is fairly good. The variability of the simulated data is due to the error in the experimental values of concentration, which are necessary to calculate $R_f(t)$.

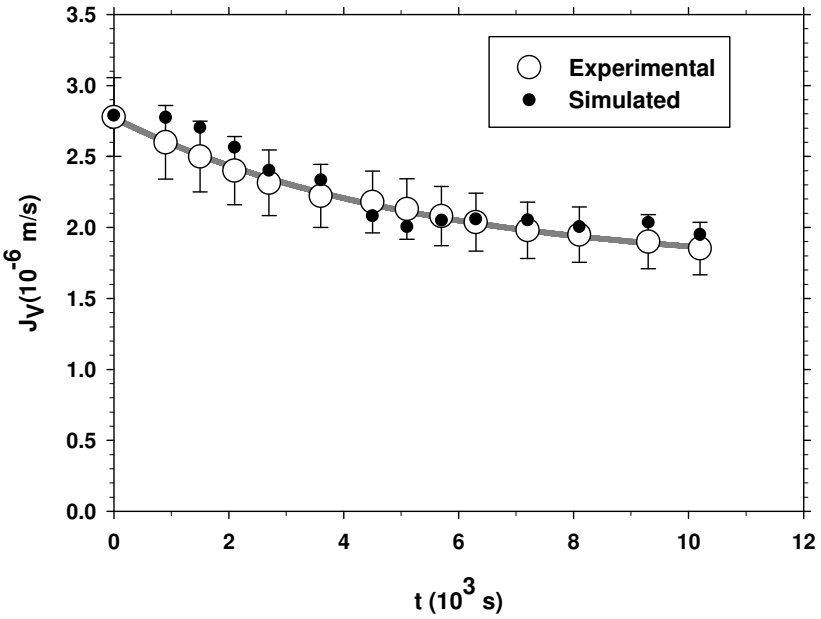

Figure 14. Time evolution of experimental and simulated permeate flux during the second stage of nanofiltration.

Table 8 collects the values of concentrations, mass and purity of each species, the initial and final volumes of concentrate and retentate of this stage. Analysing the retentate data, it can be noticed at this stage that there is a substantial enrichment in the compounds of interest (FOS) versus the simple sugars. The final retentate has a total concentration of 206 g/L, which is very similar to the initial concentration of the first stage (250 g/L) and with the relative proportions of the different compounds very similar (see Figure 13 for t = 0). The permeate data show lower concentrations for all compounds and a high percentage of glucose, which advises against a third filter stage, except for a byproduct rich in simple sugars.

Table 8. Data for the second filtration stage.

	Initial retentate, V_o=43.5 L			Final retentate, V_f=3.4 L			Final permeate, V_f=40.1 L		
	C_r^i (g/L)	m_r^i (g)	Pu_r^i (%)	C_r^i (g/L)	m_r^i (g)	Pu_r^i (%)	C_p^i (g/L)	m_p^i (g)	Pu_p^i (%)
FOS	13.1	568.0	53.5	131.3	445.7	63.8	3.0	122.3	33.7
GF_3+GF_4	5.4	234.6	22.1	59.3	201.2	28.8	0.8	33.5	9.2
GF_2	7.7	333.4	31.4	72.1	244.5	35.0	2.2	88.8	24.5
Sucrose	2.6	113.1	10.6	21.8	73.8	10.6	1.0	39.3	10.8
Glucose	7.1	308.9	29.1	42.3	143.4	20.5	4.1	165.5	45.5
Fructose	1.7	71.8	6.8	10.5	35.5	5.1	0.9	36.3	10.0

8.3.4. Global yield of the process

By taking into account the known initial concentrations of the first stage (see Figure 5, t = 0) and the treated volume (V_f= 4.5 L), the initial mass of each of the compounds has been estimated. In the same way, the mass of each substance in the retentate at the end of the process can be determined: diafiltration plus batch concentration. Finally in Table 8, is shown the mass recovered in the retentate and lost in the permeate of the second stage of nanofiltration. From these data, the yields (Y) have been calculated as:

$$Y\% = \frac{m^i_{initial} - m^i_{permeate}}{m^i_{initial}} \cdot 100 \quad (25)$$

The results are given in Table 9 for the initial stage (diafiltration + batch concentration) and for the overall process. Note that the yields obtained for the first stage are similar to those given by other authors with similar systems [9] and that the second stage retrieves much of the FOS increasing the yield to values above 80% with purities of 90%.

Table 9. Process yields.

	First stage Y%	Overall process Y%
FOS	15.0	81.7
GF_3+GF_4	21.1	88.7
GF_2	10.2	76.1

8.4. Conclusions

The nanofiltration is an appropriate process for the purification of neutral solutes that are differentiated mainly by their size. The use of a two-stages nanofiltration system as designed in this work has permitted:

- Reducing the effects of osmotic pressure and fouling due to the effect of diafiltration on the first stage.
- Recover part of FOS permeated in the first stage.
- Get purities above 90% in FOS with yields of around 80%

Referring to the osmotic pressure model with variable resistance in the membrane by adsorption of solutes, it has been shown that:

- It fits well to the studied process.
- There is a proportionality between the mass adsorbed and the resistance due to fouling.
- The model can predict the flux once the constant of proportionality between mass and resistance is known for the system.

It is possible to determine the pore size distribution of the membrane from an appropriate model from data on flux and retention for a complex solution.

These models could be used to predict the most appropriate operating conditions (concentration, pressure, recirculation flux, etc.) for a shorter time of operation and increasing yield.

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Capítulo 9

Conclusões Gerais

9. Conclusões Gerais

As principais conclusões deste trabalho sobre a purificação de frutooligossacarídeos obtidos de síntese enzimática através de colunas de leito fixo e membranas de nanofiltração são enumeradas a seguir:

Na etapa de adsorção dos açúcares em reatores de mistura, observou-se que o carvão ativo apresenta bastante afinidade pelos frutooligossacarídeos. A zeólita e a celite tiveram uma adsorção bastante pronunciada de glicose. Através dos resultados obtidos experimentalmente observou-se que temperaturas mais elevadas favorecem a etapa de adsorção dos açúcares.

Através da modelagem matemática dos dados experimentais obtidos em reatores de mistura, considerando-se a cinética de adsorção e o fenômeno de transferência de massa, selecionou-se a zeólita NaX por apresentar as maiores taxas de adsorção e menor resistência à transferência de massa.

Nos experimentos realizados em colunas de leito fixo com zeólita NaX obteve-se uma pureza em torno de 90% dos frutooligossacarídeos, sendo as eficiências de separação (ES) entre frutooligossacarídeos e glicose de 6,86, utilizando etanol 60% (v/v) como eluente a 45^{0}C. Baseando-se nesses resultados, concluiu-se que colunas de leito fixo empacotadas com zeólitas X, são promissoras na etapa de purificação de misturas de açúcares, principalmente misturas compostas por frutooligossacarídeos.

Em ensaios com colunas de leito fixo com carvão ativo, foram obtidas purezas de 80% de frutooligossacarídeos, com recuperação de 97,8% do açúcar injetado na coluna, e eficiência de separação de 3,99±0,07 entre frutooligossacarídeos e glicose utilizando etanol 15% (v/v) a 50^{0}C, demonstrando que as colunas empacotadas com carvão ativo podem ser utilizadas numa etapa de separação e purificação de açúcares. Observa-se que o carvão ativo possui maior afinidade pelos frutooligossacarídeos, sendo que na coluna de leito fixo o tempo de residência dos frutooligossacarídeos foi maior que os demais açúcares.

Na seleção de membranas de nanofiltração observou-se que a membrana NP030, foi a que apresentou maior diferença de retenção observada entre frutooligossacarídeos e glicose e, sendo a membrana indicada para um processo de purificação através de nanofiltração.

Na definição de uma metodologia para purificação da mistura de síntese foram realizados dois processos de nanofiltração com a membrana NP030. No primeiro, foi realizado um processo de diafiltração a volume constante, seguido de uma etapa de nanofiltração do permeado do primeiro processo, até a obtenção de uma concentração semelhante à solução inicial. Neste processo foram conseguidas purezas acima de 90% de frutooligossacarídeos com rendimento de 80%.

Neste trabalho definiu-se dois métodos de purificação e separação de frutooligossacarídeos obtidos por síntese enzimática, sendo um deles utilizando colunas de leito fixo com carvão ativo ou zeólita NaX e o outro método através de um processo de diafiltração utilizando membrana de nanofiltração. Com essas metodologias é possível a obtenção de uma solução de frutooligossacarídeos mais pura, e sua posterior aplicação na formulação de produtos alimentícios.

Finalmente, nada impede que essas técnicas de purificação e separação sejam compartilhadas, visando aumentar eficiência e economia de produção. Para isso, uma análise mais detalhada se faz necessário.