Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Online publication date: 08 July 2010

To cite this Article Atiyeh, Hasan K. and Duvnjak, Zdravko(2005) 'Purification of Fructose Syrups Produced from Cane Molasses Media Using Ultrafiltration Membranes and Activated Carbon', Separation Science and Technology, 39: 2, 341 — 362
To link to this Article: DOI: 10.1081/SS-120027562
URL: http://dx.doi.org/10.1081/SS-120027562

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 39, No. 2, pp. 341–362, 2004

Purification of Fructose Syrups Produced from Cane Molasses Media Using Ultrafiltration Membranes and Activated Carbon

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ABSTRACT

The purification of fructose syrups produced from sugar cane molasses media using ultrafiltration membranes, activated carbon, and ion-exchange resins was studied. Polyethersulfone (PES) membrane (10 kDa MWCO) and a thin film composite (TFC) membrane (1 kDa MWCO) were used for decolorization of fructose syrups. When activated carbon (Darco G-60) was used to remove colorants from the broth containing fructose, a color removal efficiency of 98.4% was attained, with 33% (w/v) activated carbon in the fructose syrup. However, the color removal efficiencies were 94.2 and 98.7% with the PES and TFC membranes, respectively. Further treatments of the permeate of the PES membrane

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DOI: 10.1081/SS-120027562 0149-6395 (Print); 1520-5754 (Online)

with 7.5% (w/v) activated carbon resulted in an increase in color removal efficiency up to 99.7%. The color removal efficiency was up to 99.9% when the permeate of the TFC membrane was treated with 3% (w/v) activated carbon. The demineralization of the decolorized product was carried out using ion-exchange resin (Rexyn I-300). Clear and colorless fructose syrup that visibly looked like industrial high fructose corn syrup was produced. These findings are useful for those working on the removal of color and minerals from fructose syrups produced from molasses media.

Key Words: Fructose syrups; Purification; Cane molasses; Ultrafitration; Decolorization.

INTRODUCTION

Fructose is the sweetest natural sugar. It is a constituent of invert sugar and of high fructose corn syrup (HFCS), which are produced industrially in large quantities. Three HFCS containing 42, 55, and 90% fructose are commercially produced. Existing industrial methods use expensive chromatographic methods to produce the 90 HFCS from the 42 HFCS.[1] An alternative to this separation method is a selective microbial conversion of glucose from sucrose media to a product that is easier to separate from fructose than glucose. Recently, the production of fructose and ethanol using *Saccharomyces cerevisiae* ATCC 36858, which possesses a capability to selectively ferment glucose and galactose to ethanol from synthetic media with sucrose[2–4] or raffinose[5] as well as cane[6] and beet[7] molasses media, was studied in this laboratory.

Purification is an important step in the production of high fructose syrups. The high fructose syrups should be colorless, and have no taste other than sweetness, no undesirable odor, and should be clear in appearance.[8] The industrial purification of high fructose syrups normally includes decolorization and demineralization.[1] Decolorization of high fructose syrups is generally carried out using activated carbon, whose main function is color removal but it also helps to remove color precursors and sugar degradation products.[9] Demineralization is commonly performed using ion-exchange resins, which eliminate ionic matters from fructose syrups.

The color of the HFCS produced commercially is about 5 IU, measured according to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) color method 4,[9] or below 25 reference base units (RBU).[10] Color specifications on sugar products for the soft-drink industry in the United States are usually expressed in terms of RBU and range

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from 25 to 40 RBU.[11] The calculation of ICUMSA units is based on the absorbance of sugar solution at 420 nm. The estimation of RBU units is also based on absorbance at 420 nm but includes a correction for turbidity by using the absorbance at 720 nm.

The fructose syrups produced from cane molasses media[6] should have similar characteristics to the industrially produced HFCS. The objective of the present study was to explore ultrafiltration membranes, activated carbon, and ion-exchange resins techniques for the purification of fructose syrups produced from a cane molasses medium.

EXPERIMENTAL

Purification of Fructose Syrups

A pure fructose syrup was produced by removing biomass, ethanol, color and minerals from the broth obtained by fermenting sugar cane molasses media.

Biomass Removal

Biomass was removed from the fermentation broth by centrifugation at 17,000 *g* for 15 min. The supernatant was collected and used for further treatment.

Ethanol Removal

Ethanol was removed from the biomass-free broth using a vacuum evaporator (Buchi RE121 Rotavapor, Switzerland). A water aspirator was used to create vacuum. The temperature of the water bath was 45°C and the temperature of the circulating cooling liquid through the condenser was −14°C. Vacuum evaporation was carried out using about 250 mL of the biomass-free broth in 500-mL round flask rotating at 120 rpm for 30 min. The remaining broth and the collected condensate were analyzed for sugar and ethanol concentrations.

Color Removal

The decolorization of the biomass-free broth, containing either fructose and ethanol as main products or only fructose after ethanol removal, was carried out using activated carbon. In addition, biomass-free broth containing

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only fructose as a main product was used in decolorization tests using membranes followed by activated carbon.

Decolorization Using Activated Carbon Biomass-Free Broth Containing Fructose and Ethanol

In the first test, 16.5 g of activated carbon (Darco G-60) was added into an Erlenmeyer flask with 50 mL of the biomass-free broth containing fructose and ethanol. The flask was placed into a rotary shaker (Lab-Line Instrument Inc., IL) at 33°C and 200 rpm, and 10 min later, the carbon was removed from the broth by centrifugation at 17,000 *g* for 15 min. The supernatant was analyzed for sugar concentration, pH, conductivity, and color. It was noticed that only about 14 mL of supernatant was obtained after centrifugation. The rest of the broth remained in the carbon. In the second test, 36 mL of deionized water was added to 16.5 g of activated carbon and mixed for 5 min. Then, 50 mL of the broth was added to the flask containing the wetted carbon, mixed for 10 min, and the separated liquid was analyzed as described previously.

Treatment of Biomass- and Ethanol-Free Broth Containing Fructose with Various Amounts of Activated Carbon

In one test, Erlenmeyer flasks with the broth containing fructose, after biomass and ethanol removal, and either 11, 22, or 33% (w/v) of activated carbon were placed in a rotary shaker at 33°C and 200 rpm for 20 min. The carbon was then removed from the broth by centrifugation at 17,000 *g* for 15 min. The supernatant was analyzed for sugar concentration, pH, conductivity, and color. In another test, the broth treated with 11% (w/v) of carbon for 20 min was subsequently treated two more times with fresh carbon.

Treatment of Biomass- and Ethanol-Free Broth Containing Fructose for Various Time Intervals

In this test, 8.25 g of activated carbon was placed in each of five separate Erlenmeyer flasks and 25 mL of the biomass- and ethanol-free broth was then added to each flask to obtain slurry with 33% (w/v) carbon. The flasks were incubated in a rotary shaker for the periods of time between 10 and 120 min at 200 rpm and 33°C. The carbon was then removed from the broth by centrifugation and the supernatant was analyzed for sugar concentration, pH, conductivity, and color.

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Recovery of Fructose from Spent Carbon

In this test, 33 g of activated carbon was placed in an Erlenmeyer flask and 100 mL of the biomass- and ethanol-free broth was added. The flask was then placed into a rotary shaker at 200 rpm and 33°C. After 20 min of incubation, the carbon was removed from the broth, and the supernatant was analyzed for sugar concentration, pH, conductivity, and color. The spent carbon was washed four times, each time with 72 mL of fresh deionized water for 5 min to recover the sugars. After each washing, the carbon was separated from the liquid by centrifugation and the supernatant was analyzed as above. The four washouts were combined and added to the treated broth. The mixture was then passed through five columns containing ion-exchange resins to remove minerals, as is described later.

Decolorization Using Membranes Followed by Activated Carbon

Two different ultrafiltration membranes were used for the removal of colorants from the fructose broth. One of the membranes was made of polyethersulfone (PES) and it is commercially known as PES-HO51 (Osmonics Inc., USA). This membrane has a molecular weight cut off (MWCO) of 10 kDa. The other membrane was a thin film composite (TFC) membrane. This membrane was made by the Industrial Membrane Research Institute (IMRI) at the University of Ottawa, Canada. It had a thin, selective coating layer made of brominated sulfonated poly (2,6-dimethyl-1,4-phenylene oxide) (SPPOBr) over PES-HO51 that served as a support. This membrane has a MWCO of 1 kDa and was negatively charged. These membranes were used in the setup described in Fig. 1. After biomass and ethanol removal, the broth containing fructose was filtered through a 0.45-μm, surfactant-free, nitrocellulose membrane (Millipore Corporation, Massachusetts) to remove any suspended solids. The permeation cell containing the membrane to be tested was filled with 100 mL of the filtered broth. The cell was placed on a magnetic stirrer and connected to a compressed nitrogen cylinder. The ultrafiltration experiments were carried out at 13.6 atm and room temperature (23°C). The permeate was collected in a graduated cylinder that was kept in an ice bath to reduce the possibility of sugar consumption.

The flux rate of the permeate was calculated according to the following equation:

$$F = \frac{V_P}{t_P A} \tag{1}$$

where F is the flux rate of the permeate, V_P is the permeate volume, t_p is the

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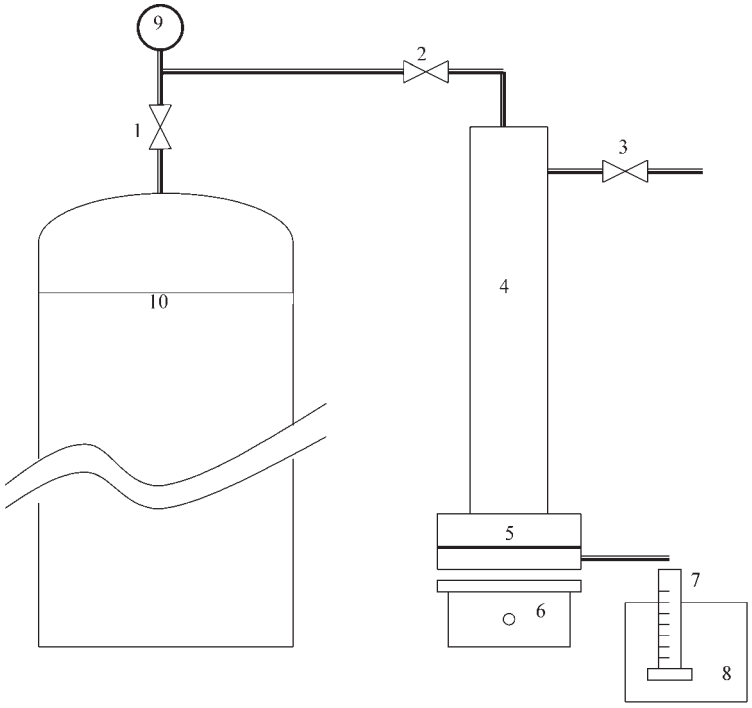

Figure 1. Schematic diagram of the decolorization process using a membrane system: (1–2) valves; (3) pressure relief valve; (4) permeation cell; (5) membrane; (6) magnetic stirrer; (7) permeate collector; (8) ice bath; (9) pressure gauge; (10) nitrogen cylinder.

permeation time, and A is the effective membrane area. The effective area of each membrane was $9.73 \times 10^{-4}\,m^2$.

The permeate and the retentate of the PES and TFC membranes were analyzed for pH, conductivity, sugar contents, and color. The permeate of the PES membrane was further treated twice with 3% (w/v) and once with 1.5% (w/v) activated carbon. The permeate of the TFC membrane was treated once with 3% (w/v) activated carbon. The permeate was analyzed after each carbon treatment stage, which was carried out in a rotary shaker for 20 min at 200 rpm and 33°C.

Minerals Removal

The broth, after color removal by carbon and recovery of sugars by washing the spent carbon with fresh water, was deionized using a mixture of

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cation and anion exchange resins (Rexyn I-300) that were packed into a plexiglass column. About 286 mL of the diluted broth containing 44.5 g/L fructose was used, which was prepared by mixing 25 mL of the treated broth with activated carbon, 63 mL from the first washout, 67 mL from the second washout, 69 mL from the third washout, and 69 mL from the fourth washout. Using a peristaltic pump, the diluted broth was pumped through five columns of resins at a rate of approximately 8.5 mL/min. Each column was packed with 28 mL of resins. The broth collected after each column treatment was analyzed for pH, conductivity, sugar contents, and color.

Analysis

Sugars and Ethanol Determination

Glucose, fructose, galactose, sucrose, fructo-oligosaccharides, raffinose, and glycerol were measured using a 600E system controller Water's high performance liquid chromatograph (HPLC) with a Water's 410 differential refractometer as the detector. A Sugar-Pak I column (Waters, Massachusetts) operated at 75°C, with deionized water containing approximately 50 mg/L EDTA-disodium-calcium salt as the mobile phase flowing at 0.5 mL/min, was used. Ethanol concentration was determined enzymatically using alcohol dehydrogenase.[12]

Conductivity and pH Determination

The broth conductivity, in millimhos, was measured using a VWR model 2052 conductivity meter (Mississauga, Ontario). The pH measurements were conducted using an Orion Research digital ionalyzer 501 (Orion Research Inc., Massachusetts).

Color Determination

Color removal was determined spectrophotometrically by measuring the absorbance of the broth at 420 nm and 25°C as recommended by the ICUMSA method 4.[13] The broth was filtered through a 0.45-μm, surfactant-free, nitrocellulose membrane (Millipore Corporation, Massachusetts). The absorbance of the broth was measured against deionized water as a reference, using a Beckman DU 640 spectrophotometer. Samples were diluted before the absorbance measurement to be in transmittance range between 20 and 80%. The pH of the samples was adjusted to 7.0 ± 0.2 with HCl or NaOH. The color of the sugar solution is calculated according to the

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following equation:

$$IU = \frac{A_{420}}{bc} \times 1000 = \frac{-\log T_s}{bc} \times 1000 \quad (2)$$

where IU is the ICUMSA color units, A_{420} is the absorbance at 420 nm, T_s is the transmittance at 420 nm, b is the path length of light (cm), and c is the solids content (g/mL). The path length of the light (i.e., cell width) was 1 cm. In the ICUMSA method 4, the solids content is determined refractometrically and converted to density units using standard tables.[13] However, the determination of solids content refractometrically has its limitations, especially for low-grade materials such as molasses, due to the presence of a significant proportion of salts and nonsugars, which are of high specific gravity compared to the sugar in the solution.[14] Therefore, in the present study, the solids content in the broth was determined by HPLC as the summation of the concentrations in g/mL of raffinose, sucrose, glucose, fructose, and glycerol.

RESULTS AND DISCUSSION

Ethanol Removal

After biomass removal from the fermentation broth, the biomass-free solution that contained 46 g/L ethanol and 116 g/L fructose was used to remove ethanol, color and minerals. Ethanol was removed from this solution by vacuum evaporation. Between 94 and 98% of ethanol was recovered from three different runs. The ethanol concentration in the collected condensate was about 184 g/L. The fructose concentration in the remaining broth, after ethanol removal, increased from about 116 to 153 g/L.

Color Removal

The biomass-free broth with or without ethanol was black. The absorbance and transmittance curves of the broth were similar for broths with and without ethanol in the visible light wavelength region (400 to 800 nm). The transmittance was below 8% in both broth mixtures in this region, which indicates the significant intensity of colorants present.

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Color Removal by Activated Carbon Biomass-Free Broth Containing Fructose and Ethanol

When a biomass-free broth was contacted for 10 min with 33% (w/v) of activated carbon, about 72% of the broth used in the treatment remained in the carbon after its separation by centrifugation (Table 1). In addition, a decrease in fructose and ethanol concentrations in the remaining broth by 5 and 25%, respectively, was also noticed. This was due to the redistribution of fructose and ethanol between the treated broth and the carbon. Only 26% fructose and 21% ethanol were recovered with the broth after the carbon treatment. In an attempt to reduce the amount of broth remaining in the carbon, the carbon was mixed with water prior to the addition of the broth. The percentages of recovered fructose and ethanol in the broth after the wet carbon treatment nearly doubled compared to the values obtained without carbon wetting (see Table 1). However, the addition of water to the carbon prior to the treatment resulted in diluting the treated broth by 58%. Furthermore, the fructose and ethanol concentrations in the treated broth were lower than expected by 9 and 17%, respectively, because of the adsorption of these compounds onto the carbon.

The conductivity of the treated broth using pre-wetted carbon slightly decreased, while it increased by 30% when the carbon was not wetted

Table 1. Data of treatment of a biomass-free broth with 33% (w/v) activated carbon.

	Untreated broth	Treated broth	Treated broth[a]
Amount (mL)	50.0	14.0	47.0
pH	4.95	5.73	5.74
Conductivity (millimhos)	18.1	23.5	17.5
Fructose (g/L)	113.5	107.4	60.3
Fructose recovery[b] (%)	—	26.49	49.90
Ethanol (g/L)	45.8	34.3	22.0
Ethanol recovery[b] (%)	—	20.96	45.17
Total solids (g/L)	126.4	117.6	66.4
Color (IU)	500,475	3,088	2,153
Color removal[c] (%)	—	99.4	99.6

[a]Activated carbon was mixed with 36 mL of deionized water before the addition of 50 mL of untreated broth.
[b]Values are calculated based on fructose and ethanol concentrations in the broth before carbon treatment.
[c]Values are calculated based on the color of the broth before carbon treatment.

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(see Table 1). The slight decrease in the conductivity is due to the dilution effect by the water used with the wet carbon treatment. However, the increase in the conductivity of the treated broth without prior carbon wetting could be due to the redistribution of the minerals between the treated broth and the carbon.

It was reported that activated carbon has neither ash removal nor buffering capacity.[15–17] Therefore, sugar liquors treated with carbon usually show a pH drop by about 1.0 unit from an initial pH value between 7.5 and 8.5 depending on the clarification method used before carbon treatment. However, in the present study, the pH of the treated broth increased after carbon treatment (Table 1). This could be due to higher ash content and colorants in the broth produced by fermentation of cane molasses than in raw sugar liquors. To test whether the activated carbon contributed to the increase in the pH and conductivity of the broth after the treatment, 100 mL of deionized water was contacted with 33 g of activated carbon for 20 min. The pH and conductivity of deionized water after carbon treatment increased from 7.2 to 7.8 and 6.5 to 91 μmhos, respectively. These results explain the increase in the pH of broth after carbon treatment but do not justify the increase in the conductivity (see Table 1).

The broth that contained fructose and ethanol used in this study has 500,475 ICUMSA color units (see Table 1), while raw sugar liquors before carbon treatment usually have color in the range of 2000 ICUMSA.[17] This shows a large difference between the broth used in this study and raw sugar liquors used in the sugar industry. It was reported that about 1 g of activated carbon is required per 100 g of syrup solids for each stage of decolorization of HFCS[9] and for decolorization of sugar liquor from a feed with a color of approximately 1200 to 120 IU.[17] In the present study, more than 99% of color was removed from the broth, after 10 min of contact with 33% (w/v) activated carbon with and without prior wetting (see Table 1). The color of the treated broth changed from black to light brown. The fructose content in the treated broth, with and without prior carbon wetting, was above 95% of the total sugar concentration. Koren and Duvnjak[18] used 3% (w/v) activated carbon (Darco G-60) to completely decolorize a light brown syrup containing about 111 g/L fructose, 6 g/L glucose, and 47 g/L ethanol without any loss of these compounds in the carbon. This syrup was produced from a mixture of diluted HFCS and Jerusalem artichoke juice by a microbial selective conversion process. The absorbance of their syrup, which was measured at 460 nm, decreased from 0.577 to 0.004 as a result of carbon treatment. In terms of color units calculated using Eq. (2) with absorbance at 460 nm instead of 420 nm, their syrup before carbon treatment had 4931 color units. For comparison, the broth used in the present study had an initial color of 310,349 color units when measured at 460 nm, which is 62 times higher than the syrup used by Koren and Duvnjak.[18]

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Treatment of Biomass- and Ethanol-Free Broth Containing Fructose with Various Amounts of Activated Carbon

Considering that the activated carbon adsorbed a significant amount of ethanol, a biomass- and ethanol-free broth was also used in the decolorization process with various amounts of activated carbon. The results showed that the amount of broth retained by the carbon increased with an increase in the percentage of activated carbon and, consequently, the percentage of recovered fructose in the treated broth decreased (Table 2). In addition, the fructose concentration in the treated broth decreased by 7% when it was treated with 33% (w/v) carbon. The fructose content in the treated broth was about 91% of the total sugar concentration, regardless of the number of treatment steps or amount of carbon used. The fructose content in the treated broth was similar to the untreated broth. The pH and conductivity of the treated broth increased with an increase in the carbon concentration. The color removal efficiency also increased with an increase in the concentration of activated carbon in the broth (Table 3).

Treatment of Biomass- and Ethanol-Free Broth Containing Fructose for Various Time Intervals

The contact time in the range from 10 to 120 min, between the broth and 33% (w/v) of activated carbon did not have any effect on the pH,

Table 2. Data of treatment of a biomass- and ethanol-free broth with various amounts of activated carbon.

Carbon used (%, w/v)		Amount (mL)	pH	Conductivity (millimhos)	Fructose (g/L)	Fructose recovery[a] (%)
11	Untreated broth	20.0	4.89	21.80	154.1	—
	Treated broth	14.7	5.21	24.80	146.5	69.84
22	Untreated broth	30.0	4.89	21.80	154.1	—
	Treated broth	14.5	5.48	26.80	144.8	45.40
33	Untreated broth	50.0	4.89	21.80	154.1	—
	Treated broth	14.0	5.70	28.90	143.2	26.01

[a]Values are calculated based on the fructose concentration in the broth before carbon treatment.

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Table 3. Color removal after treatment of a biomass- and ethanol-free broth with various amounts of activated carbon.

Carbon used (%, w/v)		Total solids (g/L)	Color IU	Color removal[a] (%)
—	Untreated broth	180.3	479,254	—
11	Treated broth	169.6	237,694	50.4
22	Treated broth	167.1	58,655	87.8
33	Treated broth	163.3	7,599	98.4

[a]Values are calculated based on the color of the broth before carbon treatment.

conductivity, and the fructose concentration of the treated broth. However, the decolorization efficiency increased from 97.5 to 99.0% when the contact time was extended from 10 to 120 min. Since about 98% of color was removed in the first 20 min of treatment, instead of waiting for another 100 min to remove an additional 1% of colorants, 20-min time-periods were applied in further tests.

Color Removal by Activated Carbon Followed by Washing the Carbon to Recover Fructose

Considering that the fructose recovery in the carbon-treated broth was low, it was necessary to increase it by washing out fructose from the spent carbon. The carbon from the treated broth was washed four times, each time with 72 mL of fresh deionized water, to recover fructose. The total fructose recovered, after the four washes of the spent carbon with water, was 95% (Table 4). The remaining 5% may have been firmly bound to the carbon. This is in agreement with reported data that there was always 2 to 6% sucrose retained by the carbon after treatment of sucrose solutions regardless of the wash water volume.[16] In the present study, mixing all the washouts with the treated broth resulted in diluting the broth by three times (see Table 4).

The total solids content of the broth used in the decolorization process was 169 g/L (Table 5). This is much lower compared to about 45% dissolved solids in the HFC syrups[9] and 65 Brix sucrose solutions in the sugar industry.[17] In the present study, 98% of the color in the broth was removed (see Table 5). Color materials were firmly adsorbed on the carbon and less than 2% of them were transferred to the water from the spent carbon after four washing steps.

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Table 4. Data of treatment of a biomass- and ethanol-free broth with 33% (w/v) activated carbon followed by fructose recovery.

	Amount (mL)	pH	Conductivity (millimhos)	Fructose (g/L)	Fructose recovery[a] (%)	Fructose in mixture[b] (g/L)
Untreated broth	100.0	5.00	21.90	152.7	—	—
Treated broth	28.0	5.70	27.90	137.0	25.11	—
1st washing[c]	72.0	5.70	20.60	73.7	59.86	91.4
2nd washing[c]	72.0	5.81	13.38	39.9	78.67	69.9
3rd washing[c]	72.0	5.83	7.86	20.8	88.49	55.4
4th washing[c]	72.0	5.87	4.94	12.9	94.55	45.7

[a]Values are calculated based on the fructose concentration in the broth before carbon treatment.
[b]Values are calculated based on the addition of all washouts to the broth after carbon treatment.
[c]pH and conductivity of the deionized water used to wash the spent carbon were 7.2 and 6.5 μmhos.

Color Removal by Membranes Followed by Activated Carbon

Considering that a large amount of activated carbon was required for the decolorization of broth, and that the final solution, after addition of washouts, contained a low fructose concentration, a membrane technique was tested to

Table 5. Color removal after treatment of a biomass- and ethanol-free broth with 33% (w/v) activated carbon followed by fructose recovery.

	Total solids (g/L)	Color IU	Color removal[a] (%)
Untreated broth	169.0	518,435	—
Treated broth	147.2	9,461	98.2
1st washing[b]	81.1	3,998	—
2nd washing[b]	43.6	2,871	—
3rd washing[b]	23.0	1,754	—
4th washing[b]	14.2	1,249	—

[a]Values are calculated based on the color of the broth before carbon treatment.
[b]pH and conductivity of the deionized water used to wash the spent carbon were 7.2 and 6.5 μmhos.

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decrease the amount of activated carbon to decolorize the broth and increase the concentration of fructose in the product.

Prior to the beginning the ultrafiltration tests with the PES and TFC membranes, the broth was filtered through a 0.45-μm nitrocellulose membrane to remove suspended solids and so to reduce the fouling effects of suspended solids on the ultrafiltration membranes. The flux of the broth through the 0.45-μm nitrocellulose membrane was 274 L/m^2 h in a 0.75 h of the test. However, the permeate flux rates of the PES and TFC membranes were 1.95 and 0.29 L/m^2 h during 9.5 and 25 h of the permeation time, respectively. The low flux rate through the PES and TFC membranes is due to their small pore size. The concentration of fructose, the conductivity, and pH of the filtered broth using 0.45-μm nitrocellulose membrane did not significantly change (Table 6). There were no significant changes in the pH or fructose concentration in the permeate or retentate of both membranes either. However, the conductivity of the permeates of both membranes was about 19% higher than that of the untreated broth, while the conductivity of the retentates was slightly lower than that of the untreated broth. After the carbon treatment of the permeates of both membranes, the pH and conductivity of the treated broth increased (see Table 6). This is consistent with the results obtained when only carbon was used for the color removal from the broth (see Tables 1 and 2).

No significant changes in the color of the broth filtered through the 0.45-μm nitrocellulose membrane were noticed, while the decolorization efficiencies of the PES and TFC membranes were 94 and 99%, respectively (Table 7). The higher decolorization efficiency of the TFC membrane compared to the PES membrane is due to its smaller pore size and its chemical characteristics. Considering that the TFC membrane was negatively charged and that large portion of the sugar colorants are also negatively charged,[15,16] that also contributed to the better decolorization efficiency of the membrane. However, the color of the treated broth using the membrane separation is still considerably high compared to the color of HFCS, which is about 5 IU.[9]

In the present study, the flux rates of the PES (10 kDa) and TFC (1 kDa MWCO) membranes with a broth of 487,590 IU were more than three times lower than those reported for a PES membrane of 5 kDa MWCO[19] with 50 Brix raw sugar solutions, which usually have color in the range of 2000 to 3000 IU.[17,20] However, the decolorization efficiencies obtained in the present study were 9 to 14% higher than those of the PES 5 kDa MWCO membrane.

Membranes with MWCO between 5 and 300 kDa have also been used for decolorization of sugar liquors.[19–22] In a study to decolorize a 50 Brix raw sugar cane solution of initial color of 3200 IU using mineral membranes with 15 and 300 kDa MWCO, the decolorization efficiencies were 39 and 20%, respectively.[20] The decolorization efficiencies increased to 58 and 50%, respectively, when a flocculating agent was added to the sugar solution before

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Table 6. Data after treatment of a biomass- and ethanol-free broth with membranes followed by activated carbon.

	Amount (mL)	pH	Conductivity (millimhos)	Fructose (g/L)	Fructose recovery[a] (%)
Untreated broth	280.0	4.85	21.6	148.0	—
Filtered broth[b]	200.0	4.83	21.7	146.5	—
Permeate of 10 kDa PES	18.0	4.85	25.7	147.6	—
Retentate of 10 kDa PES	82.0	4.84	21.0	142.4	—
Permeate of 10 kDa PES (1st treatment 3% (w/v) carbon)[c]	13.5[d]	5.00	26.5	144.2	87.95
Permeate of 10 kDa PES (2nd treatment 3% (w/v) carbon)[c]	11.0[d]	5.16	27.2	142.5	86.94
Permeate of 10 kDa PES (3rd treatment 1.5% (w/v) carbon)[c]	9.0[d]	5.31	27.3	142.1	94.48
Permeate of 1 kDa TFC	7.0	4.91	26.0	154.6	—
Retentate of 1 kDa TFC	43.0	4.76	21.4	146.4	—
Permeate of 1 kDa TFC (treatment 3% (w/v) carbon)[c]	4.4[d]	5.10	26.5	150.1	85.45

[a]Values are calculated based on the fructose concentration in the permeate before each carbon treatment step.
[b]0.45-μm nitrocellulose membrane was used for filtration.
[c]Initial amounts of permeates of the PES membrane used before 1st, 2nd, and 3rd treatments were 15.0, 12.5, and 9.5 mL, respectively. For the TFC membrane, the initial amount of permeate was 5.0 mL.
[d]Amount after treatment with activated carbon.

membrane separation. The flux rates of permeate of the 15 and 300 kDa MWCO membranes, with or without flocculation, were 25 and 65 $L/m^2 h$, respectively. The flux rate of the permeate of a mineral membrane with 300 kDa MWCO was about 200 $L/m^2 h$ when it was used to remove the turbidity of a 14 Brix sugar juice.[21] The turbidity of the sugar juice was almost completely removed, however, the color of the juice decreased from 11,600 IU by only 2%. Decloux and coworkers[22] reported that the decolorization efficiency of a 30 Brix raw sugar cane syrup with a color of 3720 IU was 64% using a mineral ultrafiltration membrane with a permeate flux rate of 47 $L/m^2 h$. The differences in the flux rates and decolorization

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Table 7. Color removal after treatment of a biomass- and ethanol-free broth with membranes followed by activated carbon.

	Total solids (g/L)	Color IU	Color removal[a] (%)
Untreated broth	171.6	487,590	—
Filtered broth[b]	169.7	501,944	—
Permeate of 10 kDa PES	172.6	28,465	94.2
Retentate of 10 kDa PES	167.5	631,216	—
Permeate of 10 kDa PES (1st treatment 3% (w/v) carbon)	167.7	7,384	98.5
Permeate of 10 kDa PES (2nd treatment 3% (w/v) carbon)	165.6	2,314	99.5
Permeate of 10 kDa PES (3rd treatment 1.5% (w/v) carbon)	164.9	1,422	99.7
Permeate of 1 kDa TFC	183.6	6,460	98.7
Retentate of 1 kDa TFC	170.5	598,047	—
Permeate of 1 kDa TFC (treatment 3% (w/v) carbon)	178.0	535	99.9

[a]Values are calculated based on the color of the broth before filtration.
[b]0.45-μm nitrocellulose membrane was used for filtration.

efficiencies between the various kinds of membranes are attributed to differences in the type and nature of the feed and operating conditions used with these membranes. The nature of colorants (i.e., natural or formed during processing) and their intensity in sugar liquors also influence color removal efficiency. Natural colorants are characterized by a low molecular weight (below 0.9 kDa), while those produced during sugar processing cover a wide range of molecular weights from below 1 to above 150 kDa.[22] Chen[23] reported that the predominant coloring components in raw sugar and molasses are represented by a molecular weight range of 3 to 30 kDa.

Taking into account that in the present study the color of the treated broth using membrane separation is still considerably high, further treatment of the permeates of both membranes was done with activated carbon. The permeate of the PES membrane was treated with 7.5% (w/v) total carbon in three steps, while the permeate of the TFC membrane was treated once with 3% (w/v) carbon. The results showed that about 87% of the fructose was recovered after each of the first and second treatments of the permeate of the PES membrane

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with 3% (w/v) carbon, while a higher fructose recovery was noticed after the treatment with 1.5% (w/v) carbon (see Table 6). A fructose recovery of 85% was obtained after the treatment of the permeate of the TFC membrane with 3% (w/v) carbon (see Table 6). The incomplete fructose recovery after the carbon treatment was due to the loss of treated permeates in the carbon and the adsorption of fructose. After the treatment with 3% (w/v) carbon, the percentage of permeates of both membranes remaining in the carbon was about 11%. However, it was 5% after the treatment with 1.5% (w/v) carbon for the permeate of the PES membrane. In addition, a slight decrease in the fructose concentration in the permeates of both membranes after each carbon treatment was noticed (see Table 6).

The total fructose recovered, after the carbon treatments of the permeates of the PES and TFC membranes, were 74 and 85%, respectively. These results are considerably higher than those obtained when the broth was treated only with activated carbon prior to recovering fructose by washing the spent carbon that lead to the washing steps dilution of the treated broth.

The color removal efficiencies from the permeate of the PES membrane, after each treatment with 3% (w/v) carbon, were 74 and 69%, respectively (see Table 7). However, when the permeate of the TFC membrane was treated with 3% (w/v) carbon, the decolorization efficiency was 92%. This could be due to the differences in the nature and types of colorants that are present in the permeates of both membranes. The color removal from the broth, after its treatment with the membranes and carbon, was above 99% (see Table 7). This showed that the use of membranes and carbon for color removal results in a higher degree of decolorization than if only carbon was used. Furthermore, the use of membranes drastically decreased the amount of carbon needed for the decolorization process and increased the fructose recovery without diluting the treated broth.

The color of the permeate of both membranes after carbon treatment changed from brown to almost colorless. The fructose content in the broth, after membrane separation and carbon treatment, was greater than 91% of the total sugar concentration. A considerable drop in the raffinose concentration was noticed with the use of membranes and carbon. No raffinose was noticed in the permeate of the TFC membrane (data not shown). This could be due to the agglomeration of molasses colorants, which hindered raffinose flow through the membrane, even though this sugar has a lower molecular weight than the TFC membrane MWCO. This is in agreement with Vercellotti and coworkers[24] data, which showed that sugar cane molasses colorants agglomerated and formed a fouling layer on a 15 kDa MWCO ceramic membrane during the processing of a 15 Brix sugar liquor. They also found that materials such as starch, dextrans, and possible complexes of polysaccharide and colorants were implicated in membrane fouling. It was

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suggested that these materials prevented smaller molecules from passing through the membrane.

Minerals Removal

The conductivity of the broth after the carbon treatment remained in the order of 10^4 μmhos. The demineralization of fructose syrups and other sugar liquors can be carried out using a cation-anion multiple bed system or a combination of anionic and cationic resins in a mixed bed unit.[9,15] In this study, mineral removal was carried out using a mixture of anion and cation exchange resins (Rexyn I-300). About 286 mL of biomass- and ethanol-free broth, after carbon treatment and addition of water washouts of the spent carbon, was passed through a 28-mL bed of Rexyn I-300 resin. The resin in the column was saturated after one passage of the broth. The broth from the first column was then passed through four other similar resin beds.

In this test, the conductivity decreased from 1.47×10^4 to 6 μmhos after passing the broth through the five beds (Fig. 2). The average flow rate of the broth through all beds was about 8.5 mL/min. The pH of the broth increased from 5.8 to 10.8 after the third bed. With further treatment of the broth, the pH decreased to 7.1 after the fifth bed. These high pH values probably caused the noticed decrease in the fructose concentration due to its degradation at high pH values.[9,17] The glucose concentration was not significantly affected. To

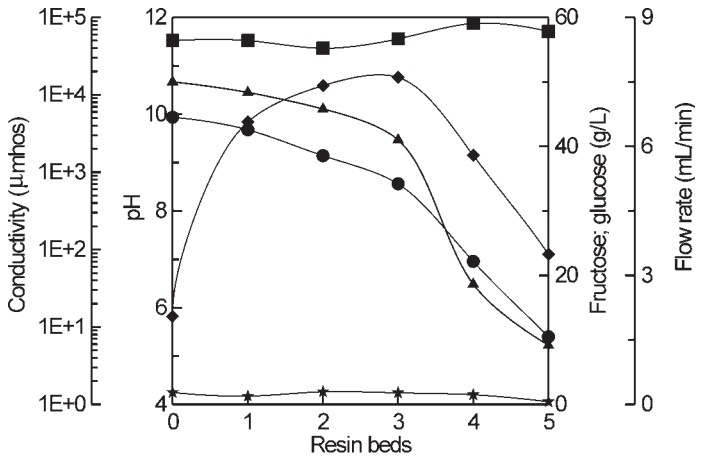

Figure 2. Deionization of the fructose broth with a mixture of anion and cation exchange resins: (▲) conductivity; (◆) pH; (●) fructose; (★) glucose; (■) flow rate.

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prevent the fructose degradation, optimizing the ratio of the amounts of anion to cation exchange resins would be desirable to maintain the pH of sugar liquors between 7 and 8 during the demineralization process.[25]

A 25% fructose loss was reported during processing of fructose syrups produced from Jerusalem artichoke, primarily during the ion-exchange treatment.[8] Koren and Duvnjak[18] also reported a loss of fructose, glucose, and ethanol by 13.6, 12.5, and 25.5%, respectively, after deionization of a product containing 78 g/L fructose, 6 g/L glucose, and 55 g/L ethanol produced from a mixture of HFCS and Jerusalem artichoke juice.

The broth before deionization had 5519 IU (Table 8). The color of the broth after passing through the first ion-exchange bed increased by 47%, which could be due to the formation of new colorants in the treated broth as a result of increasing the pH from 5.8 to 9.8 (see Fig. 2). It was reported that alkaline degradation products of fructose and to a lesser extent, glucose, particularly at pH levels above 7.5 contributed to additional colorants.[9,17] However, in the present study, the color of the broth decreased after the second resin bed. This could be explained by the findings of Blanchard and Geiger,[9] who reported that some color compounds that are not adsorbed by carbon are ionized and, therefore, retained by ion-exchange resins. They found that up to 70% of nitrogenous materials, such as amino acids, in the HFCS were removed by cation exchange resins.

The color of the broth in this study changed from black to nearly colorless after decolorization and deionization treatments. The fructose content in the treated broth was above 96% of the total sugar concentration. The color of the broth after demineralization was 667 IU. Although the purification process at this stage is not economical, these preliminary results

Table 8. Color removal after passing the treated broth, after the addition of washouts, through five beds of ion exchange resins.

	Total solids (g/L)	Color IU	Color removal[a] (%)
Untreated broth	169.0	518,435	—
Treated broth after addition of washouts	49.4	5,519	98.9
1st bed	46.4	8,147	98.4
2nd bed	42.6	3,032	99.4
3rd bed	38.5	908	99.8
4th bed	26.2	500	99.9
5th bed	12.8	667	99.9

[a]Values are calculated based on the color of the untreated broth.

showed that color and minerals could be removed from the sugar cane complex molasses broth.

CONCLUSION

This study showed that the decolorization of the fructose syrups produced from cane molasses media required a large amount of activated carbon due to the large amounts of colorants in these solutions. More than 98% of the colorants were removed from the fructose syrups after treatment with 33% (w/v) of activated carbon. The decolorization efficiency was not significantly altered by increasing the contact time between the broth and the activated carbon. Incorporating a membrane separation technique followed by activated carbon treatment for syrup purification improved the color removal and considerably decreased the amount of carbon needed. The fructose content in the treated syrups, either with activated carbon or membrane separation followed by activated carbon, was more than 90% of the total sugar concentration. The demineralization of fructose syrups using a mixture of anion and cation exchange resins resulted in an almost complete removal of minerals.

ACKNOWLEDGMENTS

The authors thank Dr. Takeshi Matsuura and Mr. Chaoyang Feng from the Industrial Membrane Research Institute (IMRI) at the University of Ottawa for providing the membranes used in this study. They also thank Mr. L. Léveillé from Lallemand Inc., Montreal, for supplying the cane molasses used in this study.

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Received January 2003
Revised May 2003