Molasses Purification and Valorisation

Towards a sustainable production of hydroxymethylfurfural

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Molasses Purification and Valorisation

Towards a sustainable production of hydroxymethylfurfural

by Mikael Sjölin

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Title and subtitle: Molasses Purification and Valorisation – Towards a sustainable production of hydroxymethylfurfural

Abstract:

The industrial transition from petrochemical-based to a more sustainable and circular economy requires the utilisation of renewable biobased raw materials that do not compete with other important industries, such as the food sector, preferably wastes or low value by-products. 5-hydroxymethylfurfural (HMF) is a versatile platform chemical that has great potential for utilisation in various sectors in a future bioeconomy, as it can be produced from sugars.

In this thesis, sugar beet molasses was assessed for the production of HMF. This dark, highly viscous and impure by-product, but yet with a high content of sucrose, could first be hydrolysed using yeast into glucose and fructose, followed by a biphasic and acid-catalysed dehydration step to produce HMF. The challenge is to handle the impurities of the molasses and the by-products that are formed during the dehydration reaction. Hence, separation and purification processes are required.

Initially, membrane filtration was tested and evaluated for the purification of molasses, and its impact on the hydrolysis and dehydration steps was determined. It was found that ultrafiltration of the molasses has a positive impact on the dehydration step with regards to increased conversion of fructose compared to crude molasses. Simultaneously, it also yielded in a higher product selectivity compared to the control sample consisting of a pure sucrose solution. This could possibly be due to the higher salt content in molasses compared to pure sucrose, which can aid the partitioning of HMF to the organic solvent. The reaction rate of the enzymatic hydrolysis was unfortunately not improved by membrane filtration. However, there is a significant difference in productivity between hydrolysis of sucrose in molasses compared to a pure sucrose solution. Results from this study revealed that viscosity, salt concentration and a synergistic effect of ions present in the molasses are the main reasons.

Secondly, by-products from the dehydration step could be removed through adsorption using granular activated carbon in order to purify the HMF product. The HMF was thereafter recovered by an evaporation step. The organic solvent was condensed and could successfully be reused for either extracting more HMF from the aqueous phase to obtain more HMF and increase the overall process yield or being recycled for another dehydration reaction.

The conclusion from this study is that it is possible to produce HMF from sugar beet molasses, but it requires additional separation and purification steps and further development to be efficient and economically competitive.

Key words: sugar beet molasses, 5-hydroxymethylfurfural, membrane filtration, hydrolysis, dehydration, adsorption, purification, solvent regeneration.

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“Do. Or do not. There is no try.”

– Yoda

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Abstract

The industrial transition from petrochemical-based to a more sustainable and circular economy requires the utilisation of renewable biobased raw materials that do not compete with other important industries, such as the food sector, preferably wastes or low value by-products. 5-hydroxymethylfurfural (HMF) is a versatile platform chemical that has great potential for utilisation in various sectors in a future bioeconomy, as it can be produced from sugars.

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Populärvetenskaplig sammanfattning

Klimatkrisen är en av de största utmaningarna mänskligheten står inför under kommande decennier. Den globala uppvärmningen blir allt högre och högre, allteftersom koldioxidhalten i atmosfären ökar. En bidragande sektor är kemikalie- och plastindustrin, vars produkter fortfarande i stor utsträckning baseras på fossila råvaror. Behovet av biobaserade produkter för att främja ett mer hållbart och cirkulärt samhälle blir alltmer påtagligt. Det ultimata hade varit om det hade funnits en plattformskemikalie som både var biobaserad och var universell för många olika användningsområden. Dessutom, om den kunde framställas ifrån avfall eller en lågvärdesbiprodukt på ett säkert och skalbart sätt utan att konkurrera med användningsområden till andra industrier, så som livsmedelsindustrin, hade det varit fantastiskt. Vet ni vad – det finns en sådan kemikalie, och den går att producera ifrån melass från sockerbruk.

Hydroxymetylfurfural, förkortat HMF, går att syntetisera vidare till produkter, allt ifrån biobränslen och tillsatser, till plaster och lösningsmedel. Det går att framställa HMF ifrån diverse bioråvaror, men högst utbyte och selektivitet fås när sockerarten fruktos används. I tidigare projekt på LTH har HMF framställts framgångsrikt, dock ifrån syntetiska startmaterial (d.v.s. ren fruktos) men inte ifrån en riktig lågvärdebiprodukt. Det är just det som behandlas i denna avhandling – nämligen framställningen av HMF från melass.

Problemen med melass är dels att den huvudsakliga sockerarten är sackaros och inte fruktos, och att den innehåller en massa andra ämnen som kan störa den tidigare testade processen. Därför börjar denna föreslagna process med membranfiltrering för att rena melassen. Både ultrafiltrering och nanofiltrering har testats och utvärderats. Vid ultrafiltrering är sackaros en tillräckligt liten molekyl för att slinka igenom membranets porer, men större molekyler så som proteiner, stärkelse eller fiber, hålls tillbaka av membranet. Vid nanofiltrering är det tvärt om. Här hålls sackaros tillbaka av membranet tillsammans med de andra stora molekylerna, och separeras ifrån pyttesmå ämnen som salter och minimala organiska ämnen som delvis går igenom membranet.

Det andra problemet att det är sackaros i melass och inte fruktos, går hyfsat enkelt att åtgärda. Sackaros består nämligen av en glukos- och en fruktosenhet, så genom att dela molekylen på mitten genom att tillsätta vanlig bakjäst under varma förhållanden (55 ºC, så att inte jästen börjar växa och jäsa sockret till etanol), går det att erhålla en blandning av glukos och fruktos. Dock när vi har jämfört reaktionshastigheten mellan delningen av sackaros i en syntetisk lösning med den i melass, så är produktiviteten väsentligt lägre för melass. Detta har vidare undersökts, och slutsatsen är att problemet antagligen beror på en kombination av flera effekter. En generellt hög salthalt, kombination av vissa metalljoner samt hög

viskositet (dvs hur trögflytande vätskor är) påverkar alla det verksamma enzymet i jästen på ett negativt sätt.

Reaktionssteget för att skapa HMF ifrån fruktos, går alldeles utmärkt att göra ifrån melass, och både ett högre utbyte och selektivitet går att erhålla jämfört med fruktos ifrån en ren sackaroslösning. Här kan faktiskt salthalten vara en tillgång, då extraktionen över till ett organiskt lösningsmedel istället för vatten kan gynnas av salter. Dessutom sågs en positiv effekt på utbytet då ultrafiltrerad melass användes, där stora molekyler avlägsnats.

När väl HMF har producerats, behöver den utvinnas ifrån det organiska lösningsmedlet. Detta görs först via dekantering av oljefasen ifrån vattenfasen, därefter kan det organiska lösningsmedlet kokas bort för att erhålla HMF. Problemet är dock att HMF inte heller den är helt ren, utan en del andra biprodukter finns också i lösningsmedlet. Genom adsorption med aktivt kol, går det att separera biprodukterna ifrån HMF i lösningen, och därmed ökar renhetsgraden på produkten. Det organiska lösningsmedlet som kokats bort går att kondensera och användas igen för att antingen recirkuleras tillbaka till reaktionskärlet, alternativt för att laka ut mer HMF ifrån den kvarvarande vattenfasen och på så vis öka det totala utbytet.

Slutligen och kortfattat, denna föreslagna process fungerar för att tillverka HMF ifrån melass. Den kräver bara lite kärlek och omtanke här och där för att bli optimal och lönsam.

List of Papers

Paper I

Sjölin M., Thuvander J., Wallberg O. and Lipnizki F. (2020) Purification of Sucrose in Sugar Beet Molasses by utilizing Ceramic Nanofiltration and Ultrafiltration Membranes. *Membranes*, **10** (1), 5. https://doi.org/10.3390/membranes10010005

Paper II

Sjölin M., Sayed M., Thuvander J., Lipnizki F., Hatti-Kaul, R. and Wallberg O. (2022) Effect of membrane purification and concentration of sucrose in sugar beet molasses for the production of 5-hydroxymethylfurfural. *Chemical Engineering Research and Design*, **179**, 365-373. https://doi.org/10.1016/j.cherd.2022.01.007

Paper III

Sjölin M., Djärf M., Ismail M., Schagerlöf H., Wallberg O., Hatti-Kaul R. and Sayed M. (2023) A comprehensive investigation of sucrose hydrolysis inhibition when using sugar beet molasses and yeast. *Submitted Manuscript*

Paper IV

Sjölin M., Sayed M., Espinoza D., Tallvod S. and Al-Rudainy B. (2023) Regeneration of dimethyl carbonate and recovery of 5-hydroxymethylfurfural used in a biphasic process through activated carbon adsorption and evaporation. *Manuscript*

Author's contribution to the papers

Paper I

I designed the study and performed the experiments, where parts of the ultrafiltration data were obtained with the assistance of students. I analysed, processed and conceptualised the presentation of data. I wrote the first draft of the manuscript, and revised it together with the other co-authors.

Paper II

I designed the study and performed the experiments, some parts together with Dr Mahmoud Sayed. I analysed, processed and conceptualised the presentation of data. I wrote the first draft of the manuscript, and revised it together with the other co-authors.

Paper III

I designed the study and performed the experiments together with Dr Mahmoud Sayed and Maria Djärf, apart from the in silico analysis which was performed by Dr Mohamed Ismail. I analysed, processed and conceptualised the presentation of data together with Maria Djärf. I wrote the first draft of the manuscript, and revised it together with the other co-authors.

Paper IV

I designed the study and performed the experiments together with Dr Mahmoud Sayed, apart from the computer simulation which was performed by Simon Tallvod and Daniel Espinoza. The NMR analysis was performed by Dr Basel Al-Rudainy. I analysed, processed and conceptualised the presentation of data together with Dr Mahmoud Sayed. I wrote the first draft of the manuscript, and revised it together with the other co-authors.

Other publications

Kängsepp P., Väänänen J., Örning K., **Sjölin M**., Olsson P., Rönnberg J., Wallebeck F., Cimbritz M., Pellicer-Nàcher, C. (2016) Performance and operating experiences of the first Scandinavian full-scale Discfilter installation for tertiary phosphorus polishing with preceding coagulation and flocculation. *Water Practice and Technology,* **11** (2), 459-468. https://doi.org/10.2166/wpt.2016.040

Kängsepp P., **Sjölin M**., Mutlu A.G., Teil B., Pellicer-Nàcher, C. (2020) First full-scale combined MBBR, coagulation, flocculation, discfilter plant with phosphorus removal in France. *Water Practice and Technology,* **15** (1), 19-27. https://doi.org/10.2166/wpt.2019.081

Hliavitskaya T., Plisko T., Bildyukevich A., Lipnizki F., Rodrigues G., **Sjölin M**. (2020) Modification of PES ultrafiltration membranes by cationic polyelectrolyte Praestol 859: Characterization, performance and applications for purification of hemicellulose. *Chemical Engineering Research and Design*, **162**, 187-199. https://doi.org/10.1016/j.cherd.2020.08.008

Hliavitskaya T., Plisko T., Svetlana P., Bildyukevich A., Lipnizki F., Rodrigues G., **Sjölin M**. (2020) Development of antifouling ultrafiltration PES membranes for concentration of hemicellulose. *Journal of Applied Polymer Science*, **138** (17), 50316. https://doi.org/10.1002/app.50316

Burts K., Plisko T., Bildyukevich A., Rodrigues G., **Sjölin M**., Lipnizki F., Ulbricht M. (2022) Development of polysulfone ultrafiltration membranes with enhanced antifouling performance for the valorisation of side streams in the pulp and paper industry. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **632**, 127742. https://doi.org/10.1016/j.colsurfa.2021.127742

Burts K., Plisko T., **Sjölin M**., Rodrigues G., Bildyukevich A., Lipnizki F., Ulbricht M. (2022) Development of Antifouling Polysulfone Membranes by Synergistic Modification with Two Different Additives in Casting Solution and Coagulation Bath: Synperonic F108 and Polyacrylic Acid. *Materials*, **15** (1), 359. https://doi.org/10.3390/ma15010359

Sjölin, M., Herrlin, H., Al-Rudainy, B., Birgersson, S., Stålbrand, H., Bonjour, O., Jannasch, P., Berlin, M., Lipnizki, F., Wallberg, O. Purification of galactoglucomannan from steam pre-treated spruce using ultrafiltration, diafiltration and precipitation. *Unpublished manuscript.*

Sjölin, M., Toragaravalli, P., Ramesh, R., Johansson, K., Lipnizki, F., Thuvander, J. Concentration of potato fruit juice from the starch industry using nanofiltration membranes as pretreatment process upstream an evaporator for energy savings. *Unpublished manuscript.*

Correlated conference presentations

Mikael Sjölin, Johan Thuvander, Ola Wallberg, Frank Lipnizki. 'Purification and Retention of Sucrose in Sugar Beet Molasses by Utilizing Ceramic Nanofiltration Membranes', *Engineering with Membranes 2019*, Poster presentation, Båstad, Sweden.

Mikael Sjölin, Johan Thuvander, Ola Wallberg, Frank Lipnizki. 'Purification of sucrose in Sugar Beet Molasses by Utilizing Ceramic Nanofiltration and Ultrafiltration Membranes', *Forum for Membrane Filtration in Food Processing 2019*, Oral presentation, Silkeborg, Denmark.

Mikael Sjölin. 'Using membrane filtration for purification of waste and by-products from the food industry for polymer synthesis', *Food Science Sweden Conference 2019*, Oral pitch presentation, Alnarp, Sweden.

Mikael Sjölin, Johan Thuvander, Mahmoud Sayed, Frank Lipnizki, Rajni Hatti-Kaul, Ola Wallberg. 'Ultrafiltration and nanofiltration for the purification of sugar beet molasses utilised in the 5-hydroxymethylfurfural formation process', *International Congress on Membranes and Membrane Processes 2020*, Poster Presentation, online virtual conference.

Mikael Sjölin, Mahmoud Sayed, Ola Wallberg, Frank Lipnizki. Membrane filtration for the purification of sucrose in sugar beet molasses utilised in the 5-hydroxymethylfurfural production process' *Network Young Membrains 2021*, Pitch and Poster presentation, Lund, Sweden.

Mikael Sjölin, Mahmoud Sayed, Johan Thuvander, Rajni Hatti-Kaul, Ola Wallberg, Frank Lipnizki. 'Impact of sugar beet molasses purification by ultra- and nanofiltration on the 5-hydroxymethylfurfural production', *Filtech 2022*, Oral Presentation, Cologne, Germany.

Mikael Sjölin, Mahmoud Sayed, Ola Wallberg, Frank Lipnizki. 'Nanofiltration and ultrafiltration of sugar beet molasses used for the production of hydroxymethylfurfural', Euromembrane 2022, Poster Presentation, Copenhagen, Denmark.

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Abbreviations

BET	Brunauer-Emmett-Teller
CFV	Crossflow velocity
CM	Crude molasses
DLS	Dynamic light scattering
DMC	Dimethyl carbonate
FDCA	2,5-furan dicarboxylic acid
FTIR	Fourier-transform infrared spectroscopy
GHG	Greenhouse gas
HCM	High concentration molasses
HMF	5-(hydroxymethyl)furfural
HPLC	High-performance liquid chromatography
LCM	Low concentration molasses
LLE	Liquid-liquid extraction
MW	Molecular weight
MWCO	Molecular weight cut-off
NF	Nanofiltration
NFM	Nanofiltered molasses
PET	Polyethylene terephthalate
PEF	Polyethylene furanoate
PWF	Pure water flux
PSD	Particle size distribution
TMP	Transmembrane pressure
TN	Total nitrogen
TS	Total solids
UF	Ultrafiltration
UFM	Ultrafiltered molasses
UV	Ultraviolet
VIS	Visual light
VR	Volume reduction
Z-AVE	Average particle size

1. Introduction

We are facing a climate crisis. Human activities have, since the Industrial Revolution, contributed to a tremendous increase of CO_2 in the atmosphere. Since the 18th century, the CO_2 level has increased by 50%, which is more than the natural increase for the past 20 000 years [1]. Carbon dioxide is a greenhouse gas (GHG) that is responsible for a rise in the global average temperature. As an effect of this, the planet's climate and ecosystems are changing and the Arctic ices are melting, which leads to an increase of the sea level [2]. To stop this escalating trend, most nations in the world (196 when the goals were negotiated) pledged to reduce the emissions in an attempt to limit the climate change through the Paris Agreement in 2015 [3]. The goal of this agreement is to limit the increase of the global temperature to maximum 2 ºC to avoid irreversible effects of the climate crisis and, if possible, even below 1.5 ºC. The European Union presented a strategy in order to implement the Paris Agreement, called The European Green Deal, which includes a set of policy initiatives to reach climate neutrality and to make the European Union economy sustainable by 2050 [4, 5]. The inclusion of the European Green Deal objectives into the National Energy and Climate Plans happened with the EU Climate Law in 2021 [6]. Similarly, the United Nations' 2030 Agenda, established in 2015, outlines 17 sustainable development goals aimed at fostering sustainable industries, environments and societies [7]. Nonetheless, according to the International Energy Agency, both current global initiatives and aspirations are not anticipated to achieve the climate objectives, based on the energy-related sectors [8].

It has been proposed that the transition towards a bioeconomy, where fuels, energy, chemicals and materials are derived from biomass rather than oil, will serve as a key factor in progressing towards a sustainable society. The establishment of a robust bioeconomy is the core of the European Union's sustainability strategy [9].

1.1 The chemical and plastic industry

Today's chemical industry is heavily dependent on fossil resources. It accounts for around 16 % of the total annual emissions of GHG [10]. Many processes are highly energy-intensive and release a lot of GHG to the atmosphere. As the fossil reserves are depleting, and to minimise the emissions to reach the 2 ºC goal of the Paris Agreement, the chemical industry must consider alternative sustainable options to stop the global warming.

We are extremely dependent on the chemical sector for products related to all aspects of our lives [11]. The chemical sector stands for approximately 11 % and 8 % of the global demand for primary oil and natural gas, respectively [12]. Global demand for plastics has surged over the past decade due to their versatile applications, with primary markets in packaging, building/construction and automotive sectors [13]. In 2016, the global plastic production reached 335 million tons, whereof about 18 % was produced in Europe [13]. Escalating demand, coupled with insufficient recycling and growing virgin plastic production, shows the urgency of identifying biobased and bio-renewable alternatives to oil-based feedstocks as a crucial step forward for the chemical and plastic industry [14-16].

To reach the utopic scenario of net zero emissions in the future, the chemical production also needs to shift to renewable fossil-free feedstocks like organic wastes, biomass residues, recycled materials [17], and even by-products from other industrial sectors – like the food industry. The use of biobased by-products and converting them into usable feedstocks would reduce the carbon footprint and simultaneously contribute to product valorisations [18].

1.2 Molasses and the sugar industry

Sugar is one of the main staple foods in the world today. The global sugar production of 2023/24 is expected to reach 187.9 million tons, with the largest producing regions being Brazil, India and the European Union [19]. The two main crops for sugar production are sugar beets and sugar canes, where around 75 % of the global annual production comes from sugar canes [20]. In the Nordic countries, it is primarily sugar beets, containing around 20 % sucrose, that are grown due to the colder climate [20]. Örtofta Sockerbruk (owned by Nordic Sugar AB) is currently the only sugar refinery in Sweden, with an annual production of around 250 000 tons sugar, and the majority of the sugar beets processed is grown in the Scania region [21, 22].

The sugar process can vary some from refinery to refinery, but the main principles are the same. An overview of a typical sugar refinery can be seen in Figure 1.1 [23].

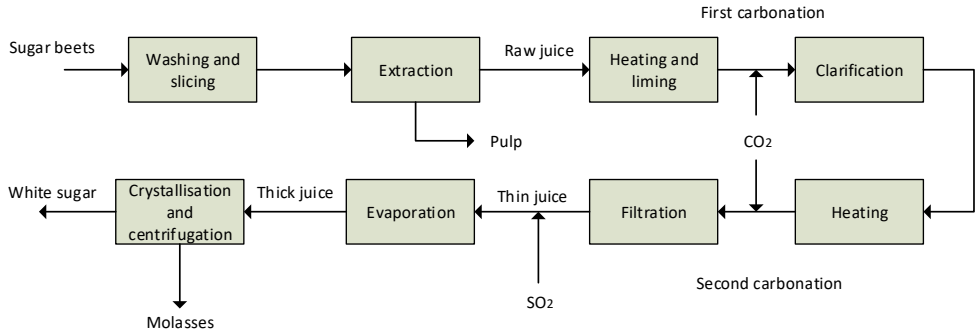


Figure 1.1. Schematic overview of the sugar refinery process from sugar beets, adapted from [23].

After transport of the beets to the sugar plant, they are washed and then sliced into pieces, called cossettes, for easier extraction of sugar [24]. These cossettes are then extracted in water, heated to a temperature of 70 ºC. This so-called raw juice is separated from the beet pulp and contains about 15 % sucrose and 1-2 % of impurities [20, 25]. These impurities comprise, for instance, polysaccharides, proteins and starch and give the final product unwanted colour and taste [23, 26]. To remove the impurities, the raw juice is subjected to heating, liming and clarification processes with sulfidation and carbonation, as well as filtration [24, 25]. This purified liquid is called thin juice, which is subsequently evaporated from about 14-16 ºBrix to 70-75 ºBrix [26]. This concentrated liquid is called thick juice, and it was partly used in *Paper III*. After evaporation, the sucrose is crystallised and separated through centrifugation. The remaining liquid, which contains too many impurities to enable further crystallisation, is called molasses.

Molasses is highly viscous, has a dark colour and also a very distinctive taste and smell. The annual production volume of beet molasses in Europe is around 6 million tons, according to the Organisation of Economic Cooperation and Development (OECD) [27]. In the European Union today, molasses is mainly used for ethanol production (around 70 %), with some for animal feed and yeast production [28]. The use of beet molasses as animal feed is rather low due to its lower palatability compared to cane molasses. Even though it is a low-value by-product, it still contains a significant amount of sucrose (around 50 %) [29]. Table 1.1 shows the composition of molasses (data from *Paper I* and *Paper II*), and a complementary elemental analysis can be seen in Table 1.2 (data from *Paper III*), where the elemental composition of thick juice from the sugar refinery is also shown for comparison of the amounts of impurities per g of sucrose. Molasses is also known to contain both betaine and polyphenolic compounds [30, 31]. The amount and type of impurities are problems not only to the sugar refinery process but also to other types of industrial sectors. Beet molasses also contains more sucrose and less inverted sugar compared to cane molasses [28].

Table 1.1. Sugar beet molasses raw material composition (data from *Paper I* and *II*).

Compound	Content (% of Total Solids)
Sucrose	60.0
Ash	13.0
Organic nitrogen-based compounds	10.1
Nitrogen salts	1.9
Glucose and fructose	0.2
Raffinose	0.3
Lactic acid	4.2
Acetic acid	1.4
Oligomeric and polymeric glucose	1.5
Unidentified	7.4

Table 1.2. Elemental analysis of molasses and thick juice, normalised per gram of sucrose (data from *Paper III*).

Element	Analysis wavelength (nm)	Crude Molasses (mg $g_{sucrose}^{-1}$)	Thick Juice (mg $g_{sucrose}^{-1}$)
Ca	317.933	2.17	0.15
Cd	228.802	0.00	0.00
Cu	327.393	0.01	0.00
Fe	238.204	0.01	0.00
K	766.490	67.62	5.74
Mg	285.213	0.06	0.00
Na	589.592	9.50	0.60
P	213.617	0.52	0.07
Pb	220.353	0.00	0.00
S	181.975	7.22	0.56
Zn	206.200	0.02	0.01

Molasses has been identified as challenging to be utilised as a biobased chemical feedstock due to its physico-chemical properties and recovery, which makes separation technologies and upstream processing have a key role for further process development [32].

1.3 Scope and outline of this thesis

The overarching scope of this doctoral thesis was to investigate the possibility of using a low-value industrial by-product for the production of the platform chemical 5-hydroxymethylfurfural (HMF) – namely, sugar beet molasses. The molasses was pretreated and hydrolysed, and HMF was produced, purified and recovered, combined with an integrated solvent regeneration process.

The different process steps were evaluated individually, from pretreatment to downstream processing, and an overview of the process is shown in Chapter 2, which also provides some background information about the HMF process. In Chapter 3, the membrane filtration and concentration through evaporation are presented, where the phenomenon of possible aggregations is also discussed. Chapter 4 delves into the enzymatic hydrolysis of sucrose to fructose and glucose and the inhibition of the reaction rate that is faced when using molasses. A deeper investigation was attempted to target what it is in the molasses that inhibits the hydrolysis process. Chapter 5 focuses on the dehydration reaction and the impact the differently pretreated molasses types have on the process yield and selectivity. In Chapter 6, the recovery and purification process of HMF through adsorption and evaporation is assessed. The results on solvent regeneration and reusability are also presented. The main findings of the research are compiled in Chapter 7, together with suggestions of future prospects to be performed as an extension of this work.

On this process journey, many knowledge gaps have been covered and novelties been addressed, featuring everything from testing of a 200 Da ceramic nanofiltration membrane for retaining sucrose to unravelling the mysteries of enzymatic inhibition of molasses, the optimisation of an adsorption process for the HMF downstream processing and the development of a solvent regeneration and recycling process.

2 Overview of the HMF production process

HMF is a molecule of great potential and interest, due to its high functionality and applicability for the production of different chemicals (Figure 2.1). In 2010, the United States Department of Energy selected HMF as one of the top ten most promising bio-based product opportunities from carbohydrates [33]. It can be further synthesised into compounds and products used for biofuels, polymers, pharmaceuticals, additives, resins, solvents and precursors, and it is therefore considered a very important biobased platform chemical for a sustainable society in the future [34-36]. HMF is characterised by the furan ring, an aldehyde and a hydroxyl as functional groups, and it is these potential functionalities that make it so interesting and versatile for the chemical industry.

2.1 HMF as a platform chemical

By exchanging the functional groups of HMF to other types for instance, carboxylic acids, one can create plenty of different compounds with various properties. Adding ring-opening reactions and polymerisation-derived compounds to that list, the HMF potential is enormous. An overview of the many possibilities of HMF is depicted in Figure 2.1.

Through oxidation of the HMF, multifunctional compounds, such as 2,5-diformyl furan, 5-formyl-2-furancarboxylic acid, 5-hydroxymethyl-2-furancarboxylic acid and 2,5-furandicarboxylic acid (FDCA), can be produced, which can be polymerised and used in the plastic industry [37]. FDCA is of special interest, since it is acknowledged as a vital renewable building block, particularly as a biobased alternative to terephthalic acid in polyethylene terephthalate (PET) and various other polyester materials [10, 38-40]. By substituting PET with polyethylene furanoate (PEF), an estimated reduction of greenhouse gas emissions by 30-50 % is anticipated, alongside potential enhancements in material properties [10, 38, 41, 42].

A comprehensive life cycle assessment of corn starch-based PEF suggests a GHG reduction of 45-55 % (using fossil-based ethylene glycol) or 68-82 % (with biobased ethylene glycol) compared to fossil-based PET [41]. Moreover, when

lignocellulosic biomass is employed as a feedstock, further reductions are expected [43]. Economic analysis supports PEF production from sources like wheat straw as a competitive PET alternative [44].

From HMF, it is also possible to produce adipic acid, which can be used as a monomer for nylon production and the biodegradable polyester polybutylene adipate terephthalate [45]. HMF serves also as a precursor for caprolactone (used in biodegradable polycaprolactone), 2,5-bishydroxymethylfuran, a promising bio-based resin compound, tetrahydrofuran and γ-valerolactone (both bio-based organic solvents), as well as 2,5-dimethylfuran (a bio-based fuel additive) [46]. Levulinic and formic acid can also be formed through rehydration (hydrolysis) of HMF under acidic conditions [47].

2.2 HMF production

HMF can be produced from hexoses through hydrothermal dehydration, which is Brønsted acid-catalysed [48]. Due to its tautomer configuration in water, fructose (furanose) is preferred over glucose (pyranose) [49]. This can be done either with heterogeneous or homogeneous catalysts [35]. Heterogeneous catalysts, with Brønsted acid sites, have demonstrated potential reaction routes, such as metal-supported catalysts (Sn, Al, Fe, Cr) and resin-based catalysts [50, 51]. However, as these heterogeneous catalysts often operate at high temperatures and in aqueous solutions, the formation of humins [52, 53] as by-products is an issue, as it causes pore blocking inside the catalyst pore structure [54]. They also require extensive preparation procedures, and the stability over time can be compromised.

Alternatively, it is easier to use a mineral-based acid (homogeneous catalyst), such as H_2SO_4 or HCl, which are known to result in high conversion of the hexoses [49, 55, 56]. Homogeneous acid catalysts have the downside of causing corrosion and requiring expensive separation processes for the product recovery [57]. However, with the suitable process concept and material choices and with some process optimisation, this should hopefully be financially viable to produce at large scale.

The issue with by-products (humins, more thoroughly explained in Chapter 6) in the aqueous solution remains, regardless of the catalyst type. Research has shown that biphasic systems result in increased product yields and selectivity, primarily because HMF is extracted into an organic solvent as it is produced, and the humin formation is thereby limited and reduced (to which extent depends on the solvent type) [47, 56]. Moreover, it has been observed that a continuous process outperforms a batch process, due to the shorter time of exposure of the reaction to high temperatures [56, 58].

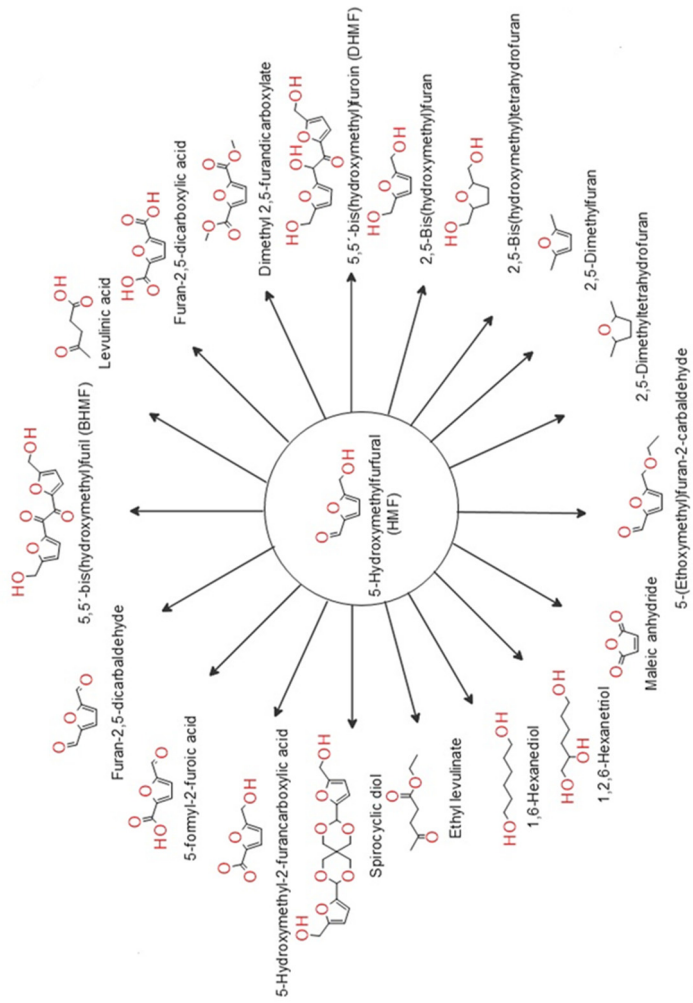


Figure 2.1. The very versatile platform chemical HMF and the potential compounds it can be converted to. Picture drawn by Dr. Mahmoud Sayed.

The HMF process used in this work involved an acid biphasic system in batches, where both the dehydration and the extraction occurred in the same container. It comprised molasses (or a pure fructose solution in *Paper IV*) with HCl (around pH 1.5) as catalyst and dimethyl carbonate (DMC) as an organic solvent. A ratio of 3:1 of DMC:water and the choice of solvent type were based on the results of Sayed et al. (2020) [56].

An overview of the process steps and the different flows can be observed in Figure 2.2. Here, an option on how to utilise some of the glucose is also highlighted, through an isomerisation step. However, this has not been tested yet (and therefore not further discussed in this thesis), but in theory, more fructose could be produced and thereby increase the overall process yield and hopefully also the productivity.

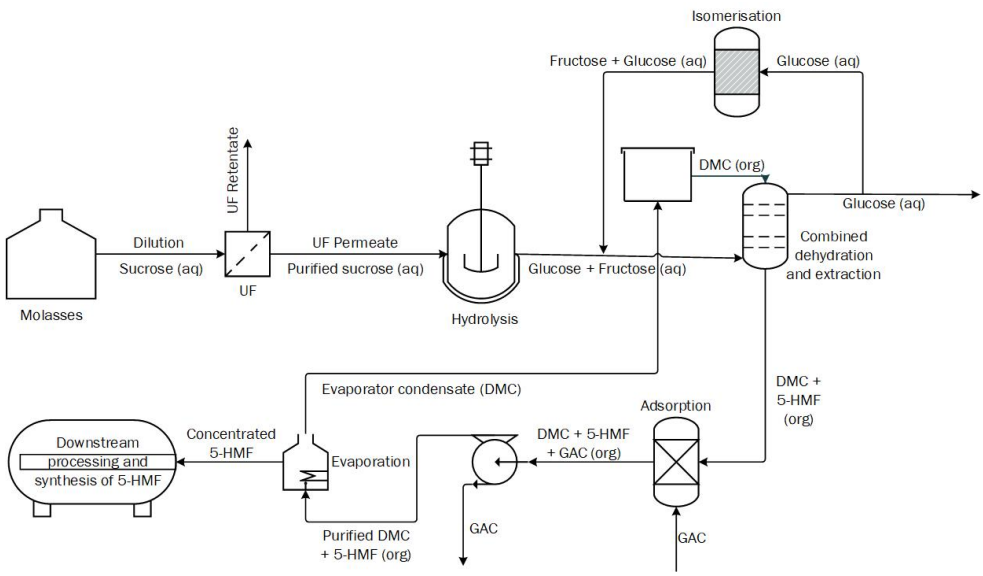


Figure 2.2. A process overview of the production of HMF from molasses as one integrated process.

2.3 Process overview

This thesis is based on four papers, where each paper focuses on different parts of the HMF process. The process steps that were studied in this thesis and which parts each respective paper focuses on are schematically shown in Figure 2.3.

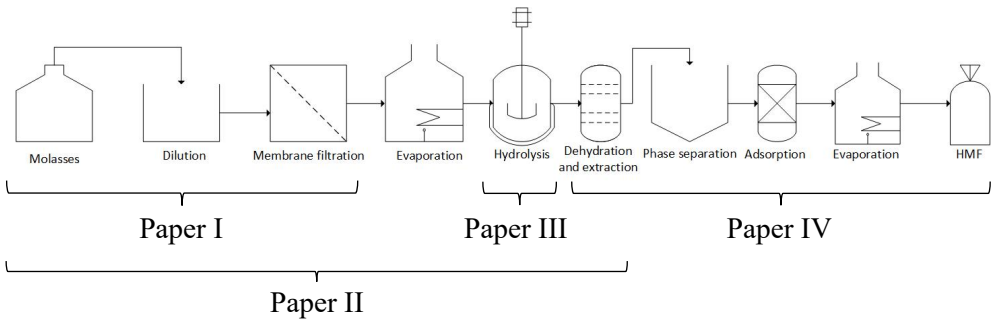


Figure 2.3. An overview of which process steps that were investigated in each respective Paper.

Paper I is partly a pre-study to the membrane filtration performed in Paper II, to determine ideal process parameter settings prior to performing a concentration study, and partly examining and explaining the significant differences in retention between different concentrations of molasses.

In *Paper II*, two differently pretreated (membrane filtered) types of molasses were assessed for improvement of the hydrolysis step and the dehydration reaction, compared to both crude molasses (reference) and also a pure sucrose solution (control).

In Paper III, the study of the hydrolysis step was deepened, especially trying to get a better understanding of the enzymatic inhibition when hydrolysing molasses.

The product recovery and possibility of solvent (DMC) regeneration are assessed in *Paper IV*, which also features the development of an adsorption process to increase the purity of recovered HMF.

3 Membrane filtration and concentration

Membrane filtration is a unit operation that separates substances in gases or liquids. A membrane is a semipermeable barrier that allows small compounds to pass through but retains larger compounds [59]. The barrier material can consist of either polymeric films, such as polysulfone, polyether sulfone, cellulose acetate and polyamides, or ceramic materials, including Al_2O_3, TiO_2, ZnO_2 and SiC. The commercially most common types of membrane processes are the pressure-driven processes: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), which can be categorised by pore size/molecular weight cut-off (MWCO) and typical operating conditions (Table 3.1) [60, 61].

Table 3.1. Typical process conditions and characteristics of pressure driven membrane processes [60, 61].

Membrane process	Operating TMP (bar)	Pore size (nm)	Nominal MWCO (Da)	Application example
MF	< 2	> 100	-	Cell separation
UF	1-10	1-100	> 1 000	Protein purification
NF	3-40	0.5-2	200-1 000	Water softening
RO	10-100	< 1	< 200	Desalination

In crossflow filtration (as used in this thesis, see Figure 3.1), the incoming feed is divided into two streams: a permeate that contains the lower molecular-weight (MW) fraction of the feed that has passed through the membrane and a retentate that contains the remaining fraction of the feed that has been retained by the membrane [59]. The capacity of a membrane is measured in flux (J), which is the volumetric permeate flow through the membrane per m^2 and unit time. The driving force is the difference in applied pressure between the feed and permeate side, called transmembrane pressure (TMP), and the flux-reducing parameters are osmotic pressure (Π), viscosity (μ) and various types of resistances (R_i) (Equation 3.1):

$$J = \frac{TMP - \Pi}{\mu\,(R_m + R_f + R_{cp})} \tag{3.1}$$

where R_m, R_f and R_{cp} are the filtration resistance of the membrane, fouling and concentration polarisation, respectively.

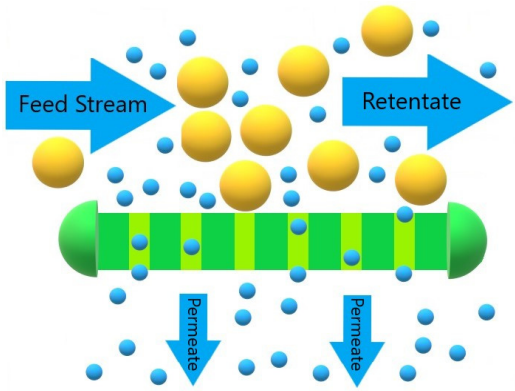


Figure 3.1. The basic principle of crossflow filtration.

There are a few different types of fouling, including pore blocking, adsorption of compounds to the surface, cake formation and occasionally even gel formation if the retained compounds are of such nature [62]. Depending on the presence of different compounds in the solution, these types of fouling occur to varying degrees. Thus, different cleaning strategies must be applied, based on fouling and compound types, to recover the filtration flux [63, 64]. Pure water flux (PWF) is often used to evaluate the severity of fouling and determine the efficiency of various cleaning agents. With PWF, only water is filtered, and by measuring PWF for a pristine membrane at certain process setpoints and comparing it with the PWF after fouling and again after cleaning, it is possible to obtain a percentage of the membrane's filtration capacity. When the solutes are not bound to the surface but instead form a stagnant boundary layer on the membrane surface with a higher concentration of the retained compounds, the resistance phenomenon is called concentration polarisation [65]. This is illustrated in Figure 3.2. This means that the concentration at the actual membrane surface (c_m) is higher compared with the bulk (c_b) of the feed flow. The pressure-independent flux behaviour here can be described as in Equation 3.2, where k is the mass transfer coefficient [66]. Since we only can sample from the bulk for practical reasons, a difference arises between the observed retention (R_{obs}) and the true retention (R_{true}) (Equation 3.3 and 3.4). In this thesis, including *Paper I* and *Paper II*, all retention values presented are observed retentions.

$$J = k\ ln\left(\frac{C_m - C_b}{C_b - C_p}\right) \tag{3.2}$$

$$R_{true} = 1 - \frac{c_p}{c_m} \tag{3.3}$$

$$R_{obs} = 1 - \frac{c_p}{c_b} \tag{3.4}$$

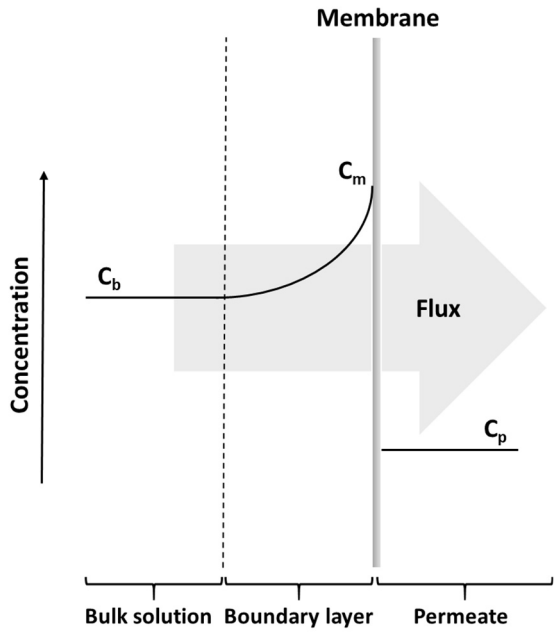


Figure 3.2. Illustration of concentration polarisation during membrane filtration.

The concentration polarisation can be limited and controlled by various means - for instance, introducing shear forces near the surface, such as baffles (which the spacers provide in spiral wound modules), rotating units, vibrating units, ultra-sonication or air sparging [62, 67]. Conventionally, the easiest approach (if module type and pump allow) is to increase the crossflow velocity (CFV) and thus shear force. Higher CFVs result in higher Reynolds numbers, increasing the turbulence of the flow and, consequently, the potential flux. The Reynolds number for flows in a pipe (in this case, a tubular membrane) is calculated according to Equation 3.5:

$$Re = \frac{\rho \, CFV \, d}{\mu} \tag{3.5}$$

where ρ is the fluid density, d is the diameter of the tubular membrane and μ is the viscosity of the feed.

It is also possible to enhance the flux by increasing the TMP. However, the flux has limits, as well (see Figure 3.3), and at a certain point, it no longer linearly increases with the TMP (called critical flux) [68], and even further, there is no increase, regardless of the additional driving force (called limiting flux) [69]. As explained in Equation 3.1, when TMP increases, the fouling and concentration polarisation become more severe, as well, leading to smaller increments in flux or none at all. There are two types of critical flux: the strong form and the weak form [70]. The

strong form of critical flux is the point at which the flux deviates from the PWF, and the weak form is the point at which the flux deviates from linearity as TMP increases (Figure 3.3) [70]. The strong and the weak forms of critical flux are effected primarily by reversible and irreversible fouling, respectively [71].

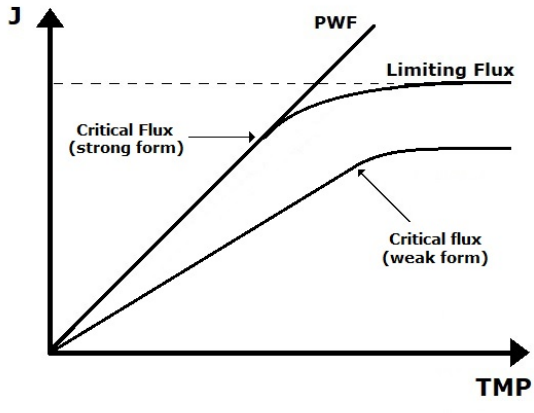


Figure 3.3. Flux curves showing the critical flux (strong and weak form) and the limiting flux.

Generally, there are many advantages of membrane processes over other separation processes, such as evaporators, dryers and centrifuges. Membrane processes are energy-efficient and selective, can operate at low temperatures and continuously with a low footprint and are easy to scale up from pilot scale to full scale or by process retrofitting [72]. Furthermore, specifically for ceramic membranes, which is the type of membrane used in *Paper I* and *Paper II*, are known for being both temperature- and chemical-resistant, achieve high filtration fluxes and have a mechanical process robustness advantage, compared with polymeric membranes. The main drawback is that they generally are more expensive than polymeric membranes [73].

In this part of the process study, molasses was filtered by nanofiltration and ultrafiltration, but prior to each filtration experiment, it was necessary to dilute the molasses to allow it to be pumped and to operate the filtration at acceptable flux levels.

3.1 Nanofiltration of molasses

In this work, a ceramic tubular NF membrane with a 200 Da MWCO by Fraunhofer IKTS was used to separate low-MW compounds from sucrose on a lab scale. Its active layer comprised TiO_2 on an Al_2O_3 support, with a filtration area of 48.4 cm^2 (length 250 mm, d_{outer} 10 mm, d_{inner} 7 mm). Sucrose has a MW of 342 Da and thus can be partially retained by the NF membrane, even though the MWCO of the membrane and the MW of sucrose are similar in size. The retention can be affected to some extent by the operation conditions, and thus, a parameter study was performed.

3.1.1 Parameter study

In *Paper I*, the TMP and the CFV were varied for two different concentrations of molasses: 0.5 % molasses (LCM) and 10 % molasses (HCM). The results regarding flux and various retentions are shown in Figure 3.4 and Figure 3.5 a-f, respectively.

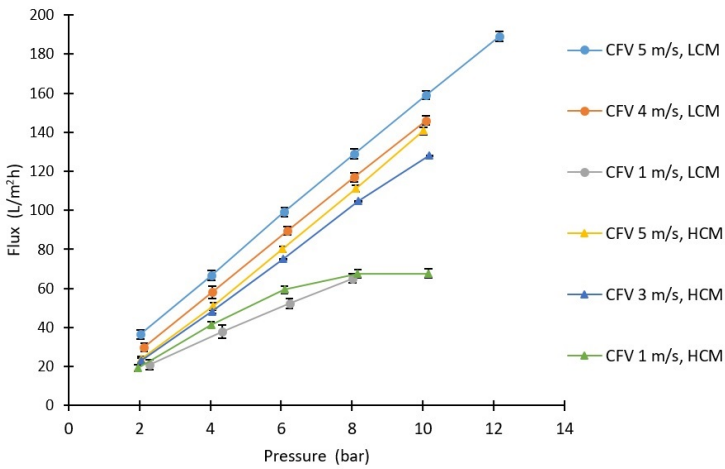


Figure 3.4. Changes in filtration flux during the parameter study of the NF membrane with two concentrations of molasses (LCM and HCM). Data from *Paper I*.

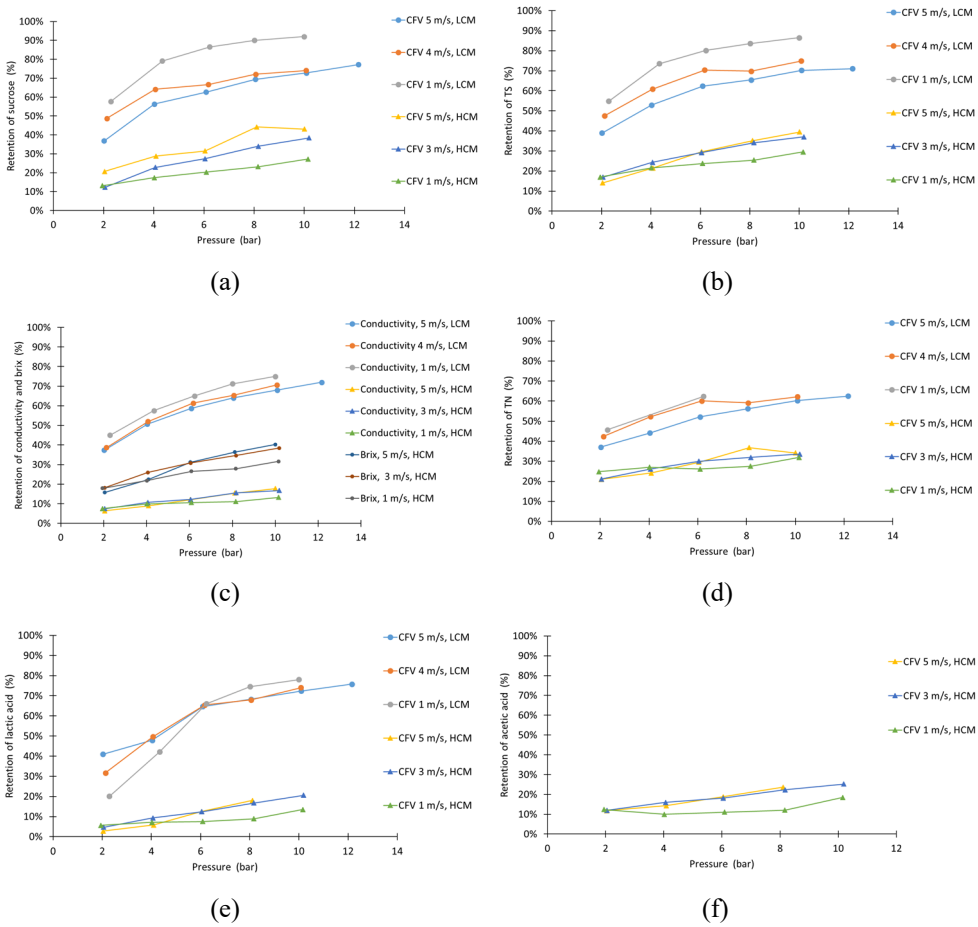


Figure 3.5. Retention of (a) sucrose, (b) total solids (TS), (c) conductivity and brix, (d) total nitrogen (TN), (e) lactic acid, and (f) acetic acid during the parameter study of the NF membrane with two concentrations of molasses (LCM and HCM). Data from *Paper I*.

As shown in Figure 3.5, the retention of all types of compounds was significantly higher for LCM than for HCM. This is further discussed in Section 3.3. In Figure 3.4, it is only when operating at the lowest CFV and HCM that the critical and limiting flux was reached, for the investigated pressure region. As expected, the higher the TMP and the higher the CFV was, the higher the flux became (see Equation 3.1 and 3.5). CFV correlates directly with the turbulence of the flow, wherein a higher CFV leads to a higher Reynolds number. Therefore, it was with the highest CFV and at 10 bar pressure that a concentration study with the NF membrane was performed (*Paper II*).

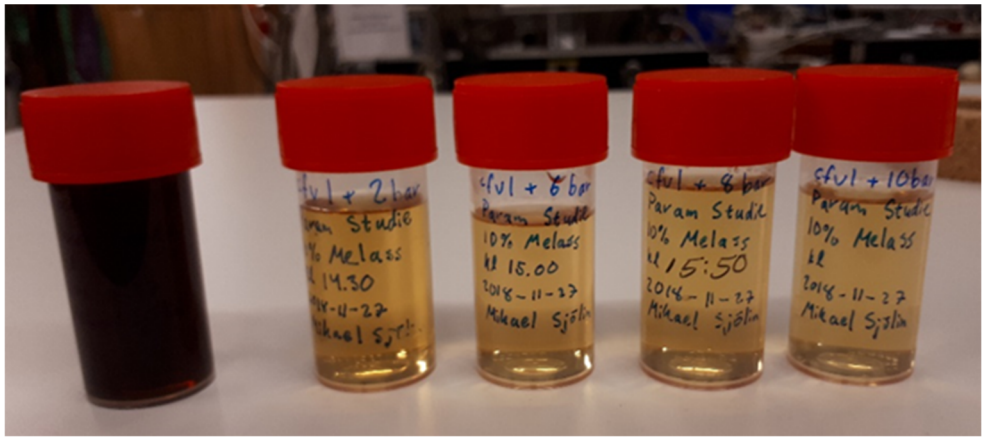


Figure 3.6. Samples of feed (to the left) and four permeate samples (to the right) obtained from the parameter study using the NF membrane.

3.1.2 Concentration study

The concentration study was performed in recirculated batch mode, in which the retentate was recirculated continuously to the feed tank and permeate was removed over time to enable retained compounds to be concentrated in the feed solution. Volume reduction (VR) is the volumetric amount of permeate that is removed from the process over time in relation to the original feed volume and was calculated according to Equation 3.6 [74]:

$$VR = \frac{V_p}{V_0} \tag{3.6}$$

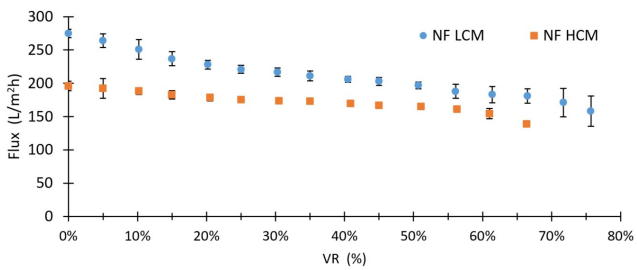


Figure 3.7. Influence of volume reduction (VR) on flux with 0.5 % (LCM) and 10 % (HCM) concentration. Data from *Paper II*.

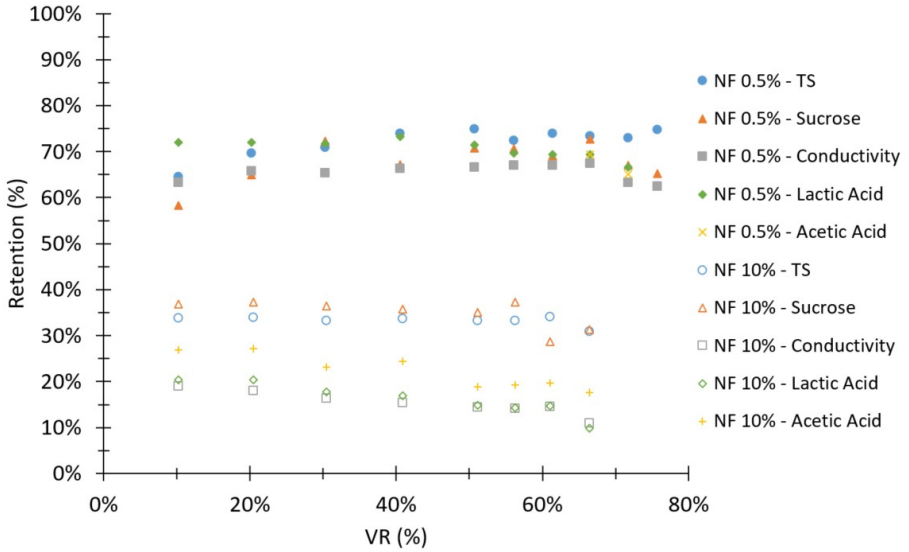


Figure 3.8. Retention of various compounds during the concentration study of the NF membrane and two concentrations of molasses (LCM and HCM). Data from *Paper II*.

The flux declined as the VR increased (Figure 3.7), due to higher concentrations in the bulk, implying higher osmotic pressure and likely greater fouling and concentration polarisation over time. Relatively high VR could be reached without any major losses in flux, and if even more low-MW compounds would be desired to be removed, a diafiltration step could be an alternative process to consider after the initial concentration. However, considering the retentions of the NF process, a diafiltration step would result in significant product loss and low process yield. The retentions of various compounds (Figure 3.8) again demonstrate a significant difference between LCM and HCM (see Section 3.3).

To apply a membrane process on a larger scale, one must consider that the membranes can be cleaned after usage. Table 3.2 shows the changes in PWF that occurred after the parameter and concentration studies for a pristine membrane, a fouled membrane and after three cycles with chemical cleaning agents. Although significant fouling developed on the membranes, it was possible to clean them with the cleaning strategy of alkaline-acid-alkaline, as proposed by Trägårdh (1989) [63]. Acid cleaning agents are good in removal of inorganic fouling, as they reverse the precipitation and solubilise the salts from the molasses. The first alkaline cleaning agent removed various types of organic fouling, as did the second alkaline cleaner after inorganics had been removed, but the latter also adjusts the surface charges of the membrane [63, 64].

Table 3.2. Changes in PWF of the NF membrane in the parameter and concentration studies. Data from *Paper I and II*.

	Pristine membrane (L/m^2 h bar)	Fouled membrane (L/m^2 h bar)	Alkaline cleaning 1 (L/m^2 h bar)	Acid cleaning (L/m^2 h bar)	Alkaline cleaning 2 (L/m^2 hbar)
Parameter study, LCM	35±1.9	5±1.8	24±1.5	20±2.0	33±1.6
Parameter study, HCM	34±1.2	17±1.6	30±1.8	31±1.6	38±1.7
Concentration study, LCM	33±1.6	12±2.0	26±1.8	23±1.7	36±2.4
Concentration study, HCM	38±1.7	23±1.6	33±1.8	35±1.8	40±1.9

3.2 Ultrafiltration of molasses

The UF was performed using a ceramic tubular membrane with an MWCO of 10 kDa from Atech Innovations (UF type 37/3.8) to separate high-MW compounds from sucrose. The UF membrane consisted of TiO_2 as active layer on an Al_2O_3 support, with a total filtration area of 0.53 m^2 (1.2 m long tube with 37 channels, each with an inner diameter of 3.8 mm).

3.2.1 Parameter study

The parameter study of the UF membrane process was also performed using two concentrations of molasses: 1 % (LCM) and 10 % (HCM). The filtration fluxes for these two concentrations are shown in Figure 3.9, and the average retentions are summarised in Table 3.3.

Here, the critical flux point appears to have been exceeded (as no linearity between pressure and flux was observed, compared with Figure 3.4), and even the limiting flux seems to have been reached for one of the parameter combinations (LCM, CFV 1 m/s and 2.7 bar) in the investigated interval. The retentions (Table 3.3) showed a difference between sucrose and ultraviolet (UV)-absorbing compounds and, to some extent, also total nitrogen (TN). This demonstrates the possibility of sucrose purification from large molecular complexes, such as proteins. Although, a slight retention of sucrose was also observed (0-16 %), leading to a loss in the overall process yield. During UF, a clear difference between the retention of LCM and HCM can also be observed. This indicates that something happens with either the affinities and/or sizes of the compounds present in the molasses at different concentrations. This phenomenon is further discussed in Chapter 3.3.

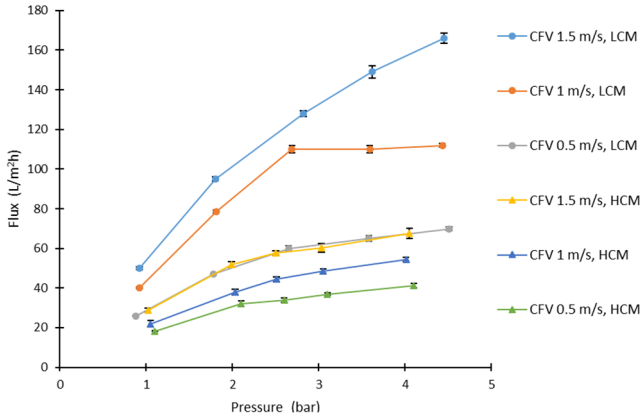


Figure 3.9. Changes in filtration flux in the parameter study of the UF membrane with two concentrations of molasses (LCM and HCM). Data from *Paper I*.

Table 3.3. Comparison between the retention of various compounds during the parameter study of the UF of LCM and HCM, respectively. Data from *Paper I*.

			Retention (%)		
	Sucrose	**TS**	**TN**	**UV**	**Conductivity**
UF, LCM	0-16	9-18	17-28	38-48	10-31
UF, HCM	0-16	5-11	8-15	22-37	5-20

3.2.2 Concentration study

Two concentration studies were performed: one for LCM and one for HCM. As expected, the flux decreased somewhat as the VR was increasing (Figure 3.10), likely due to membrane fouling over time (Table 3.4). Regardless, both processes reached acceptable flux levels in the investigated interval. Furthermore, Figure 3.11 shows possibilities of sucrose purification, as the retention of UV-absorbing compounds was higher than the retention of sucrose. However, the concentration also resulted in a sucrose yield of 72 % and 78 % by filtering LCM and HMC, respectively.

Retentions of total nitrogen (TN) could indicate proteins in the molasses solutions. Therefore, a membrane cleaning strategy with first an enzymatic cleaning agent (Ultrasil 53), followed by an alkaline cleaning agent (Ultrasil 10) for the removal of remaining organic compounds, was adopted. The results regarding PWF can be seen in Table 3.4, where the PWF for pristine, fouled and cleaned membranes are summarised. The recovery in PWF, by comparing with the PWF of the pristine membrane, was high, which concludes that the chosen cleaning strategy was successful.

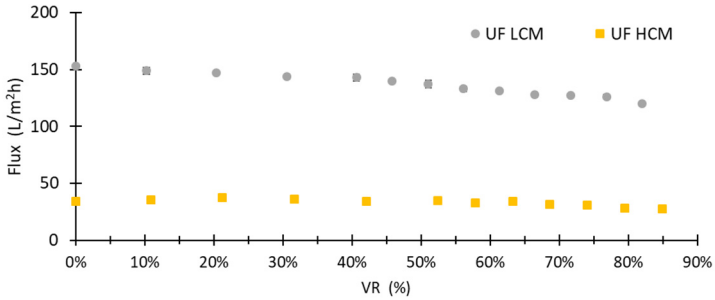


Figure 3.10. Influence of VR on filtration flux in the concentration study of the UF membrane with two concentrations of molasses (LCM and HCM). Data from *Paper II*.

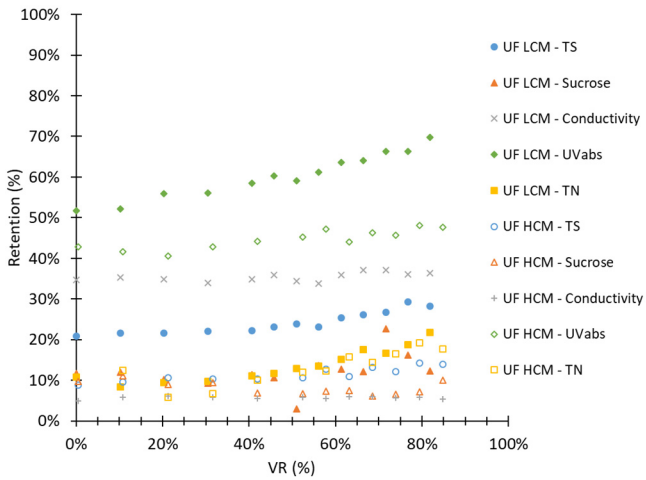


Figure 3.11. Retention of various compounds during UF of LCM and HCM, up to VR of 85 %. Data from *Paper II*.

Table 3.4. Changes in PWF of pristine, fouled and cleaned UF membranes in the parameter and concentration studies. Data from *Paper I and II*.

	Pristine membrane (L/m²hbar)	**Fouled membrane (L/m²hbar)**	**Enzymatic cleaning (L/m²hbar)**	**Enzymatic cleaning 2 (L/m²hbar)**	**Alkaline cleaning (L/m²hbar)**
Parameter study, LCM	63±1.1	17±0.7	29±0.3	39±1.2	56±1.4
Parameter study, HCM	57±2.2	25±1.4	66±2.2	-	-
Concentration study, LCM	57±1.6	23±2.0	38±1.8	-	47±2.4
Concentration study, HCM	60±1.7	21±1.6	43±1.7	-	56±1.8

3.3 Dynamic light scattering

The difference in retention between LCM and HCM for both the UF and NF process (*Paper I and II*) was an interesting observation, and it was decided to investigate this further. Through dynamic light scattering (DLS), it is possible to see differences in particle sizes present in the solution, and a rough indication of the particle size distribution (PSD) can be obtained.

By changing the concentration of molasses and measuring the average particle size (Z-AVE), the results shown in Figure 3.12 were obtained. Initially, by decreasing the concentration, the Z-AVE decreases, but at some point, it begins to increase again. This indicates a potential aggregate formation, which could possibly be created as the ionic strength of the liquid decreases, enabling larger water-molecule complexes to form [75]. It is yet unknown what compounds in the molasses that form these aggregates and if there are different types of physiochemical phenomena causing these observations [76]. It could also be protein precipitation as an effect of dilution [77], or protein swelling, similar as in a study by Cicuta and Hopkinson (2001), where their proteins' sizes changed by varying the ionic strength and pH [78]. The time factor could also affect the aggregate formation, a parameter that is excluded in this type of comparison. Even lower concentrations than the analysed region would have been interesting to see, but the low count rate of the analysis in these regions makes further dilutions very uncertain.

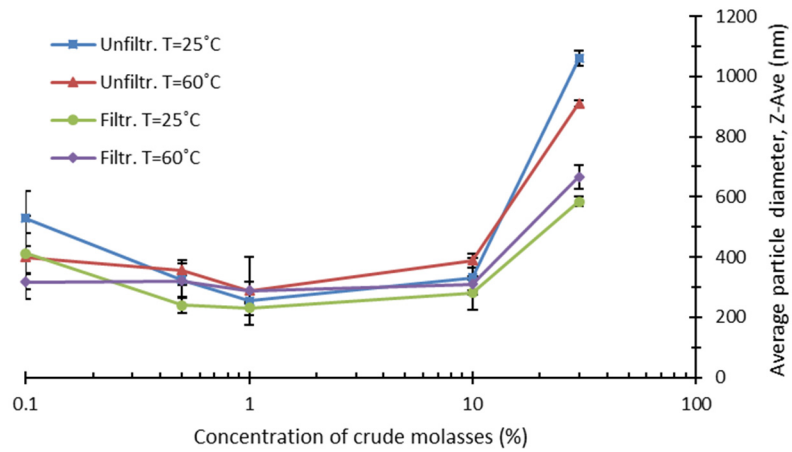
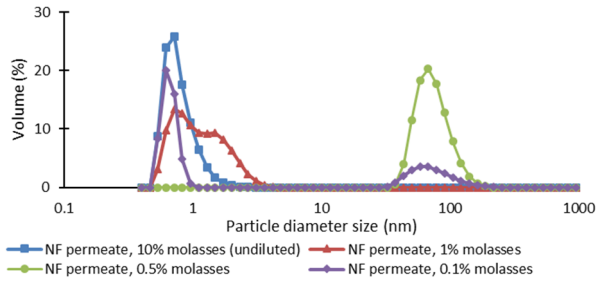


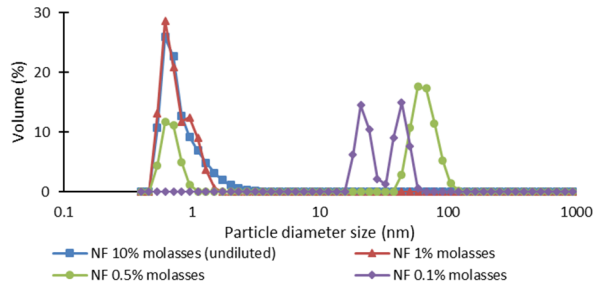
Figure 3.12. The changes in average particle size (Z-AVE) for different concentrations of molasses, at both room temperature and 60 ºC, and when being filtered and not with a 0.2 µm syringe filter. Data from *Paper I*.

To gain further insight into this phenomenon, the permeate from the NF concentration study was also analysed with different dilutions (meaning only small

compounds were present). A PSD of a couple of different concentrations can be seen in Figure 3.13 below. These results should be considered very indicative and not absolute, as the absolute values of the peaks varied somewhat when repeating the measurements, but the trends are the same. It appears that some peaks appear in the region of higher-MW compounds, when the dilution approaches levels comparable with LCM.



(a)



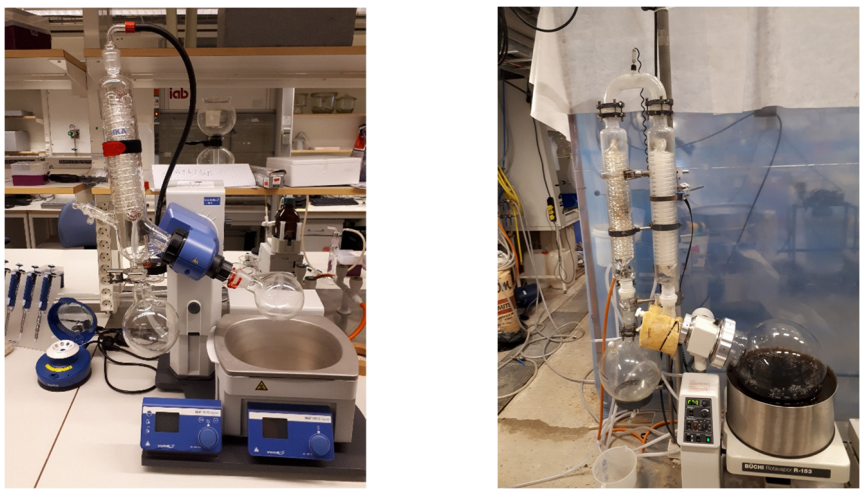
(b)

Figure 3.13. The particle size distribution (PSD) of nanofiltration permeate at different dilutions, where (a) was measured at room temperature and (b) at 60 ºC.

3.4 Evaporation

Prior to the hydrolysis and dehydration processes, it was desired that the concentration of sucrose to be high enough for these process steps to be efficient. The nanofiltered molasses (NFM) was concentrated in a 0.5 L Rotavapor (Figure 3.14 a), and the HCM was concentrated in a 10 L Rotavapor (Figure 3.14 b). The NFM was evaporated at 70 ºC with an initial pressure of 110 mbar and a final pressure of 65 mbar, due to boiling point elevation as the NFM got more concentrated. The ultrafiltered molasses (UFM) was evaporated at 60 ºC, with a pressure change from 90 mbar to 57 mbar during the concentration process.

An alternative to the evaporation process is to use a lower dilution of the molasses prior to the membrane processes. If more concentrated molasses could be filtered, there could be energy savings to gain, as membranes are generally more energy-efficient compared to evaporators. However, a more concentrated feed would likely lead to lower fluxes, as the viscosity is higher (which directly affects the filtration flux, in accordance with Equation 3.1), and likely, more fouling and concentration polarisation would occur on the membrane surface. Additionally, the osmotic pressure would also be higher, at least for the NF process, as both sugars and some salts are retained, which also affects the flux in a negative way. A lower flux means more membrane area is required to handle a certain flow or productivity. Larger filter installations result in higher capital investments (CAPEX) and, to some extent, also higher operational expenditures (OPEX), which in the end might not be too different from a hybrid membrane-evaporator solution. However, this has to be further investigated before any conclusions can be drawn, accompanied by a techno-economic analysis of the system options.



(a) (b)

Figure 3.14. The Rotavapors used to evaporate (a) the NFM and (b) the UFM.

4 Sucrose hydrolysis

In order to provide hexoses, and preferably fructose, to the dehydration process to form HMF, the sucrose in the molasses needs to be hydrolysed.

Hydrolysis is the breakdown of a polymer into smaller units by addition of water [79]. For sucrose, this can be either done by using an acid or enzymatically for breaking up the α-1,β-2-glycosidic bond between the two hexoses [80-82].

For acid hydrolysis of sucrose, typically, a strong acid is used, combined with high temperatures [80, 83], but it is also possible to do with weaker acids [83, 84]. It has also been shown that heterogeneous catalysts with strong acid sites can also hydrolyse sucrose [85]. However, some sugar compounds are degraded into undesired by-products during acid hydrolysis [83, 86], which makes this method less selective than enzymatic hydrolysis.

There are a couple of different types of enzymes that can be used for the hydrolysis of sucrose: invertase (a disaccharidase, for example, β-D-fructofuranoside fructohydrolase) and sucrose synthase (for example, D-fructose-2-glucosyl transferase) [87-89]. Invertases are grouped up into two different types, depending on their activity: acidic with pH optimum between 4-5.5 and alkaline/neutral types with pH optimum between 7-8 [88]. The acidic invertase is the main type of enzyme used in this work to hydrolyse sucrose. It exists naturally in baker's yeast (*Saccharomyces cerevisiae*), and it has an optimum activity temperature between 40-60 °C [90-92]. The invertase enzyme is a heavily glycosylated octamer protein (see Figure 4.1 for the structure) with both open- and closed-types of active sites, which enables it to hydrolyse not only sucrose but also smaller oligosaccharides [92].

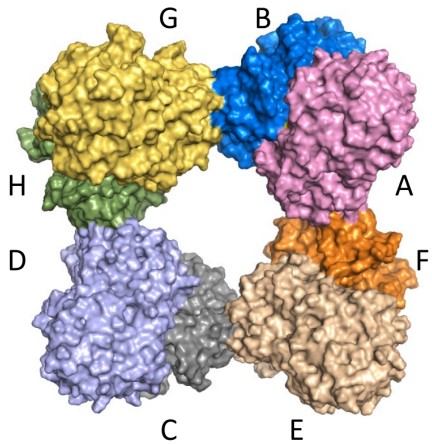


Figure 4.1. The surface 3-D structure of invertase with the eight subunits (A-H, octamer) in different colors. Figure provided by Dr Mohamed Ismail.

4.1 Inhibition during hydrolysis of sucrose in molasses

The use of invertase for synthetic solutions or relatively pure process streams to hydrolyse sucrose is a simple process. However, when using invertase to hydrolyse sucrose in molasses, the activity was lower (*Paper II* and *Paper III*) [93, 94]. This means that there is something in the molasses that inhibits the activity of the invertase enzyme, either a chemical compound or an unfavourable physical property.

There are several different types of inhibition. It is unlikely to be a substrate inhibition, as the higher the concentration of the substrate (sucrose) is, the higher the reaction rate is (and not the opposite). Neither should it be a product inhibition, as the hydrolysis is complete (100 % conversion) after some time. The inhibition just reduces the reaction rate. Enzymatic reactions can also be classified as competitive, non-competitive and un-competitive [95]. It is unlikely that it is a competitive type of inhibition, as the concentration of both raffinose and oligo-/polymeric glucans are much lower than the concentration of sucrose (see Table 1.1 for the molasses composition, in Chapter 1). It is possible to be either a non-competitive type (which is where the inhibitor can bind to the enzyme and block or change the enzyme protein configuration, preventing sucrose to bind in) or un-competitive type of inhibition (where the inhibitor attacks the substrate-enzyme complex, once sucrose is docked at the active site). However, it is hard to tell for certain which mechanism is dominating in this case [96]. In practise, the degree of inhibition is determined by investigating differences in process productivity and efficiency, compared to a model solution.

The reaction productivity and efficiency were defined as follows:

$$Q_p = \frac{P - P_0}{t - t_0} \tag{4.1}$$

$$Q_{p/x} = Q_p \times \frac{1}{X} \tag{4.2}$$

$$Y_{p/x} = \frac{P - P_0}{X} \tag{4.3}$$

$$Conv. = \left(1 - \frac{S}{S_0}\right) \times 100 \tag{4.4}$$

where Q_p (g/L h) represents the volumetric productivity rate, P signifies product concentration, P_0 denotes the initial product concentration, t represents a selected point in time, t_0 corresponds to the starting time of the reaction, $Q_{p/x}$ (g_p/g_{cells} h) denotes the specific productivity relative to cell mass, X represents concentration of cells, $Y_{p/x}$ (g_p/g_{cells}) is defined as the specific product yield based on the concentration of cells, S refers to the concentration of substrate, S_0 corresponds to the initial substrate concentration and the conversion of substrate is expressed as a percentage.

Interestingly, in *Paper II*, without any pretreatment of the molasses (other than membrane filtration for the NFM), the difference in specific reaction rates (Equation 4.2) between pure sucrose (PS) and crude molasses (CM) changed over the experiment time, see Table 4.1. In this study, it was also found that using NF as a pretreatment process has a positive effect on the hydrolysis rate. However, in *Paper III*, by pH adjustment of the CM and NFM to pH 4.5, the differences are significantly lower. Furthermore, the final specific yield (Equation 4.3, $Y_{p/x,\ 120\ min}$) was also significantly improved by the pH adjustment. The initial promising results by using NF (*Paper II*), is after pH adjustment (*Paper III*) negligibly low. This may be explained as an effect of the difference in buffering capacity between the NFM and CM, as the NFM contains less buffering salts. For future scale-ups of the process, it is probably less expensive to adjust the pH of molasses to pH 4.5 than to install an NF process for upstream pretreatment to the enzymatic hydrolysis step. Regardless, the pH needs to decrease to < 2 for the dehydration reaction. To allow the enzymes to operate at higher activity, the overall pH adjustment of the process could optimally be performed in two steps: first to 4.5 for the hydrolysis and then to 1.5 for the dehydration reaction.

Table 4.1. Specific volumetric productivity changes over time and the difference between PS, NFM and CM, at 55 ºC, but at different pH and catalyst dose. Data from *Paper II and III*.

	$Q_{p/x,10min}$ (g_p/g_{cells} h)	$Q_{p/x,60min}$ (g_p/g_{cells} h)	$Q_{p/x,120min}$ (g_p/g_{cells} h)	$Y_{p/x,120min}$ (g_p/g_{cells})
PS (10 mg cells / mL, no pH adjustment)	164.7±1.3	29.3±1.3	14.3±0.5	28.5±1.1
CM (10 mg cells / mL, no pH adjustment)	13.7±1.6	8.8±1.2	6.7±0.4	13.3±0.8
NFM (10 mg cells / mL, no pH adjustment)	30.3±13.6	14.5±3.0	10.8±1.7	21.5±3.5
PS (3 mg cells / mL, pH 4.5)	333.1±8.2	97.5±0.1	52.8±0.2	105.7±0.5
CM (3 mg cells / mL, pH 4.5)	221.5±6.7	74.2±7.5	43.8±1.0	87.5±2.0
NFM (3 mg cells / mL, pH 4.5)	190.3±2.9	69.1±1.4	42.3±1.4	84.6±2.8

4.2 Inhibition investigation

Even though the reaction rates are very similar after pH adjustment to pH 4.5, there is still a difference between PS and CM. A key question is therefore: What is it in the molasses causing this difference? This was comprehensively investigated in *Paper III*, and the key findings are presented in the following sections.

4.2.1 Viscosity

One possible explanation is the viscosity of the solutions, since it directly influences the mobility of the enzymes and the sucrose substrate in the solution [97]. The various investigated feed solutions can be categorised into two main categories (see Table 4.2): low-viscosity feed solutions (PS, TJ) and high-viscosity feed solutions (CM, NFM, UFM). Figure 4.2 shows how the two categories of sucrose feeds are hydrolysed. The low-viscosity solutions (PS and TJ) are hydrolysed faster than the high-viscosity solutions (CM, UFM and NFM), with average specific productivity after 30 minutes ($Q_{p/x,30min}$) of 162.9 g_p/g_{cells} h and 97.6 g_p/g_{cells} h, respectively. However, even though there are clear differences in reaction rates between the two viscosity categories, there are other factors present simultaneously – for instance, purity – which makes it hard to draw any certain conclusions from only the viscosity impact. The same elements are present in TJ as in CM, NFM and UFM but at a much lower concentration, as the purity of sucrose is much higher prior to the crystallisation process at the sugar mill. Different inhibitors present in the molasses can therefore not be ruled out.

Table 4.2. Viscosity measurements of PS, TJ, CM, NFM and UFM at 1000 s^{-1} in shear rate in room temperature. Data from *Paper III*.

	Low viscosity feeds		High viscosity feeds		
	PS	TJ	CM	UFM	NFM
Viscosity (mPa s)	2.58±0.01	3.45±0.01	5.02±0.01	6.29±0.04	8.25±0.06

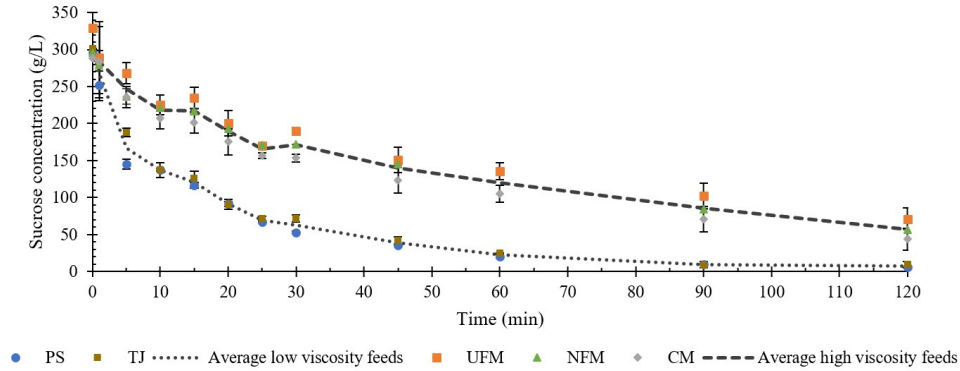


Figure 4.2. Hydrolysis inhibition results showing the effect of the two viscosity categories of feed solutions. Data from *Paper III*.

4.2.2 Ionic strength and salts

Furthermore, the impact of various salts present in the molasses was investigated in *Paper III*. The selected salts were individually assessed by addition to a pure sucrose solution, corresponding to the same concentration as in molasses. Their hydrolysis rates were then compared to PS and CM. The concentration of sucrose in all types of solutions for the hydrolysis experiments was around 300 g/L, in order to minimise the impact of substrate concentration. All hydrolysis experiments were performed at pH 4.5 and 55 ºC for 2 hours and by using *S. cerevisiae* as invertase source. The results showed that there was one element that slightly stood out from the others – potassium (Figure 4.3) – which became clearer when enhancing the effect by addition of four times the concentration of potassium to the sucrose solution. However, potassium is the most abundant inorganic element in CM, with a concentration of 20.3 g/L (at a sucrose concentration of 300 g/L). In a previous study by Takeshige and Ouchi (1995), this observation was confirmed at a similar concentration level [98]:

$$I = \frac{1}{2} \sum c_i z_i^2 \tag{4.5}$$

This leads to the question: Is it really potassium that inhibits the reaction, or is it just an effect of high ionic strength? Ionic strength is directly connected to the concentration of salts and their number of charges on the ion, as described in Equation 4.5 [99]. Another question is if this is only an issue when using whole cells of yeast? By addition of Na^+ at the same mass concentration as K^+ and by using pure isolated enzymes (I4504-1G, extracted from *S. cerevisiae*, Sigma-Aldrich Co., St. Louis, USA), this was investigated. The results, presented in Figure 4.5, lead to a couple of conclusions. First, the inhibitory effect is still present, whether or not

whole cells of baker's yeast or pure isolated enzymes are used. Second, it is not just potassium that inhibits the reaction rate, as also Na^+ at 80 g/L (four times the concentration dose, see Figure 4.4) inhibits the reaction rate of sucrose – concluding that it is the ionic strength, rather than potassium specifically, that causes the inhibition of invertase. In *Paper III*, this was confirmed through an *in-silico* simulation of the systems, where the area and cavity of the active site were compromised at high salt concentrations. These changes could affect the binding of substrate to the active site of the enzyme in a negative way.

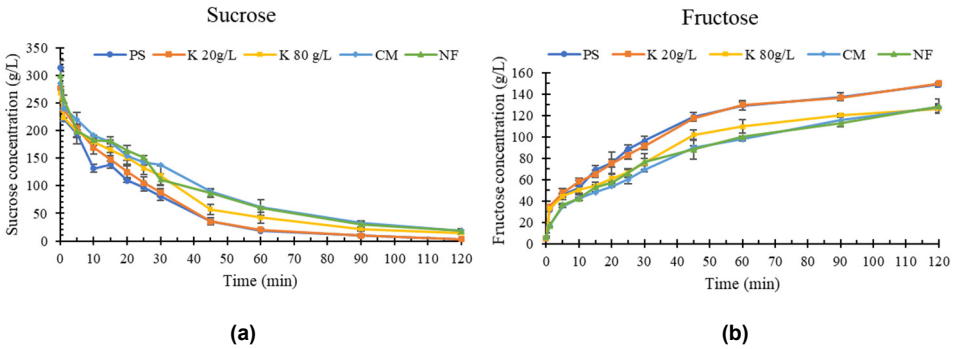


Figure 4.3. The impact of potassium on the enzymatic hydrolysis rate showing changes of **(a)** the sucrose concentration and **(b)** the fructose formation. Data from *Paper III*.

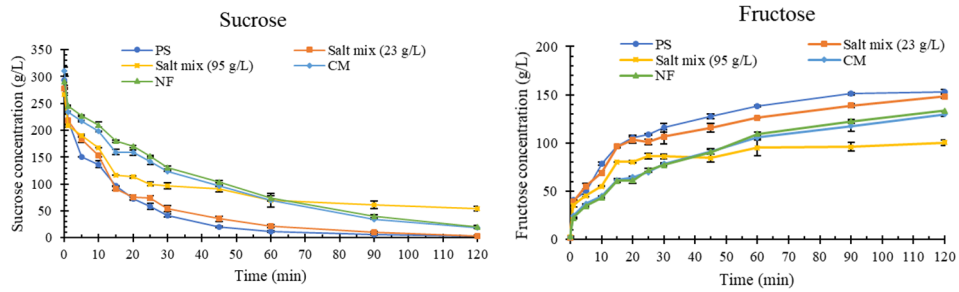
4.2.3 Synergy effect of salts

The ionic strength affects the hydrolysis rate. However, it is likely not the sole reason for the inhibition, as the concentration levels at which the inhibition becomes significant are higher than those present in the molasses.

The synergy effect of the tested salts was therefore also evaluated, both by using baker's yeast (Figure 4.4) and pure isolated enzymes (Figure 4.5). As Figure 4.4 shows, the inhibitory effect was more profound when using the salt mix, with a $Q_{p/x,60min}$ of 67.5 g_p/g_{cells} h, compared to a $Q_{p/x,60min}$ of 76.2 g_p/g_{cells} h of potassium. At four times the concentration level of CM, the reaction inhibition was greater for the model solution with the salt mix than for CM, where the activity curve even began to level out, indicating an inactivation of the enzyme. When comparing the specific reaction rates and total specific yield to Na^+ and K^+ at the enhanced concentration levels (see Table 4.3), it can be concluded that there is in fact a synergy effect and not only the effect of ionic strength, since Na^+ of 80 g/L has a higher molarity (3.48 M) than the salt mix (2.64 M) but yet a faster reaction rate. Therefore, there must be a synergy effect between the ions in the salt mix solution affecting the enzymatic hydrolysis. This salt mix contains five different metal ions. Molasses contains many more types of salt ions (even though in low concentrations), which could explain why the synergistic effect is mainly significant at enhanced concentrations.

Table 4.3. Specific reaction rate after 1 hour hydrolysis and final specific yield after 2 hours, for enhanced concentrations of Na^+, K^+ and salt mix. PS and CM were used as references. Data from *Paper III*.

	PS	Na^+ (80 g/L)	K^+ (80 g/L)	Salt mix (95 g/L)	CM
$Q_{p/x,60min}$ (g_p/g_{cells} h)	5569±92	2509±81	3727±286	1577±161	3780±282
$Y_{p/x,120min}$ (g_p/g_{cells})	6415±107	3863±186	5459±106	1891±26	4997±300



(a) **(b)**

Figure 4.4. The impact of using a salt mix on the hydrolysis rate of sucrose (a) and the formation rate of fructose (b), at the same concentrations as in molasses and at four times the concentration (enchanced effect) using baker's yeast as catalyst. Data from *Paper III*.

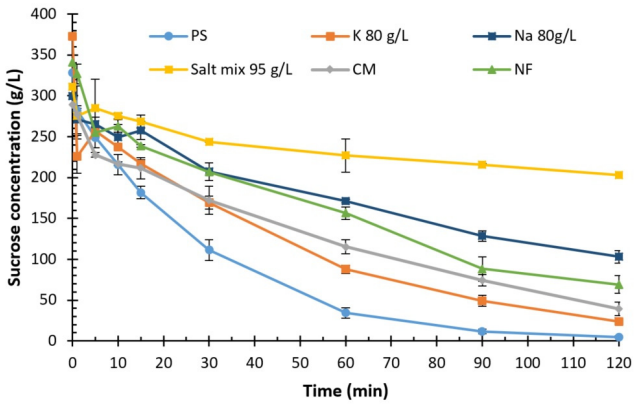


Figure 4.5. The impact of using the enhanced concentration of salt mix on the hydrolysis rate of sucrose and compared with 80 g/L of both K^+ and Na^+ ions, using isolated free enzymes as catalyst. Data from *Paper III*.

It becomes clear how complex the matrix of the molasses truly is. Its complexity makes it hard to pin-point out one specific cause for the inhibition problem. It is likely a combination of both limitations of transport processes (like viscosity), the impact of high ionic strength and a synergy effect of various salts that inhibits the enzyme.

5 Fructose dehydration

The dehydration of hexoses to HMF is a well-studied process, which can be performed in various ways (see Chapter 2). In this thesis, the selected process primarily focuses on the conversion of fructose to HMF, as it yields high product selectivity [56]. This is due to the tautomer configuration of fructose, enabling an easier dehydration reaction route to HMF [49, 56]. In water, both the cyclic furanose and pyranose forms of fructose exist, while for glucose, it mainly exists in the pyranose form [49]. Figure 5.1 presents the dehydration reaction mechanism from fructose to HMF.

The method to produce HMF from the obtained sugar solutions in this work, regardless of if it was hydrolysed molasses or a pure fructose solution, was in an acidic biphasic system using a volumetric water:DMC ratio of 1:3. The pH of the aqueous phase was decreased to 1.5-2, and the reaction was carried out in batch mode in a thermoshaker at 110 ºC for 2-3 h.

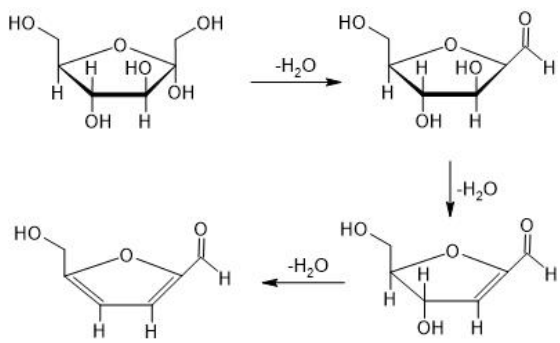


Figure 5.1. The reaction mechanism of the fructose dehydration (Adapted from Zhang et al., 2016 [100]).

5.1 Hydrolysed and purified molasses

The hydrolysed molasses, with and without membrane pretreatment, was assessed for the production of HMF in *Paper II*. Without pretreatment, only about half of the fructose in the hydrolysed CM was converted into HMF, compared to an almost complete fructose conversion in the hydrolysed PS (49 % and 92 % fructose conversion, respectively) but with a slightly better selectivity (Figure 5.2). The higher selectivity could be due to the lower conversion of glucose in hydrolysed CM compared with the hydrolysed PS. It can also be due to the presence of salts in molasses, which can support the mass transport of HMF into the organic phase (due to different partitioning coefficients) [56, 101, 102]. A more rapid mass transport of HMF to DMC limits the by-product formation, as both the rehydration and condensation reactions primarily occur in the aqueous phase [34, 36, 47, 103].

The use of UFM instead of CM for the dehydration reaction yielded a significantly higher fructose conversion (100 % fructose conversion), without jeopardising the HMF selectivity (Figure 5.2). This indicates that larger molecular complexes in the molasses, which were removed by the UF membrane upstream, somehow disturbed the dehydration reaction. About twice as high substrate conversion was reached, which means twice the amount of product was obtained (yield). For future scale-up of the process, UF of the molasses is a suitable pretreatment method to reach higher overall process yields of HMF.

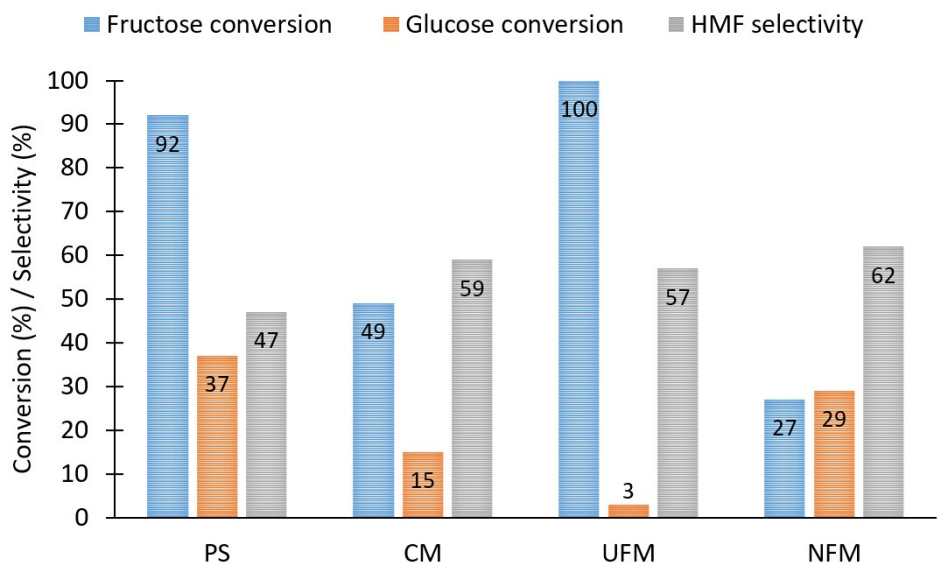


Figure 5.2. The fructose conversion and HMF selectivity of hydrolysed PS, CM, UFM and NFM in *Paper II*.

5.2 Regenerated DMC

One important factor to make this process sustainable, both environmentally and financially, is the reuse of solvent. In a biphasic system, it would be very expensive if new DMC would be needed for each reaction cycle and if the used DMC would be just discarded. Regeneration of DMC is essential on a larger scale, and therefore, evaluating the regenerated DMC is crucial. The use of recirculated DMC was tested and assessed in *Paper IV*.

A pure fructose solution was used for this assessment. The regenerated DMC was obtained through the recovery process of the produced HMF, which is then recovered as condensate after evaporation. The evaporation condensate of two types of solutions were obtained – DMC both with and without pretreatment of granular activated carbon (GAC) (explained in Chapter 6 and *Paper IV*). The two regenerated DMC solutions were compared with a new unused DMC solution and assessed for the dehydration of pure fructose at pH 1.5 and 110 ºC for 2 h, with a water:DMC ratio of 1:3.

The results (Figure 5.3) show that there is no major difference between the two types of regenerated DMC and new unused DMC product. Moreover, there was no difference between the two types of regenerated DMC. Thus, the adsorption process did not impact the solvent quality. Overall, the HMF selectivity (35-46 %) was lower than previously seen (even for the new unused DMC solution), which again could be explained by a low partitioning coefficient of extraction (as the pure fructose solution lacks salts, compared to molasses). However, very high conversion rates of fructose were achieved for all three different types of DMC solvents (97-99 %). This means that reuse of regenerated DMC works similarly to a new unused commercial product and that the regeneration process was successful.

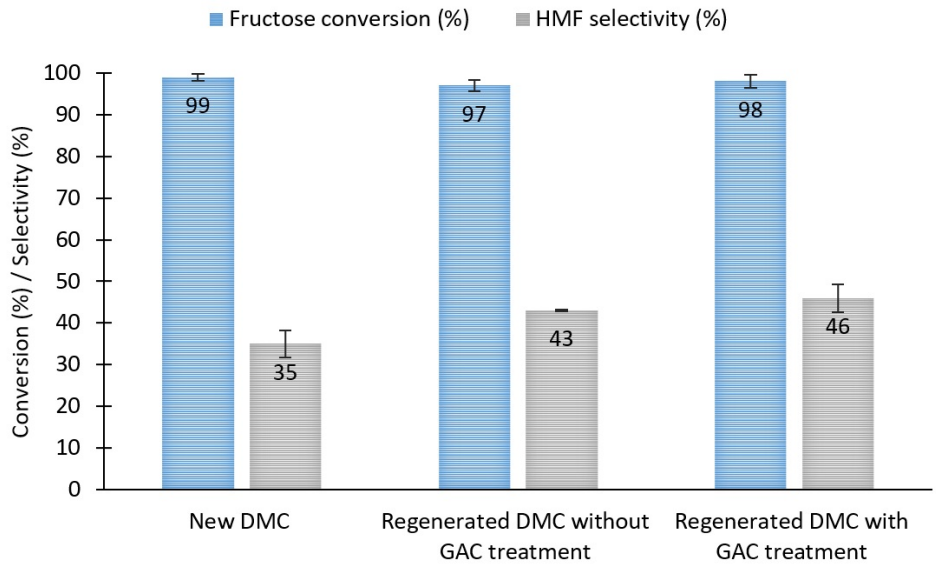


Figure 5.3. The fructose conversion and HMF selectivity during dehydration of a pure fructose solution and by using three different types of DMC – new DMC and regenerated DMC both with/without an upstream adsorption process. Data from *Paper IV*.

6 HMF recovery and purification

Once the HMF is produced and extracted to the organic phase, it needs to be recovered to be usable for various processes – preferably as solid crystals. Moreover, the HMF also needs to be further purified to be valuable for downstream processing. As presented in *Paper II*, the selectivity of HMF is around 60 % in the dehydration reaction of molasses, due to by-product formation. The most likely by-products in the organic phase are different kinds of humins, which are various polymerised complexes of HMF and sugars formed through condensation reactions [36, 104]. They can occur as soluble low-MW compounds, as agglomerated oligomer species and as large macromolecular complexes [52]. Humins do not only lower the product yield but they can also cause clogging of the system, substrate/product absorption and limit the dehydration efficiency [53]. There are several reaction pathways to form humins, which depend on the substrate and the reaction conditions [53]. While it has been postulated that the humin formation primarily occurs through condensation between HMF and its derivatives, the suggested mechanism concerning the formation of humins from HMF-derived intermediates might not be applicable to humins derived from saccharides [105-107]. Furthermore, the rehydration reaction of HMF to levulinic acid and formic acid is possible, but the access to water molecules in DMC is somewhat limited and therefore less likely to occur [47]. Rosenfeld et al. (2020) investigated the challenges regarding scaling up of an HMF production process and found that the separation and purification of the HMF is one of the main bottlenecks for future process development [36].

In this chapter, the purification of HMF through adsorption of humins on GAC and product recovery through evaporation of DMC is presented and evaluated.

6.1 Adsorption using granular activated carbon

Adsorption is a separation process where compounds in a liquid or gas are deposited onto a solid surface [108]. Adsorption has a broad range of applications, ranging from flue gas purification to water/wastewater treatment. In this study, it was used to remove impurities from the produced HMF in the DMC solution. In adsorption, the compounds that are desired to be removed are called adsorbates, and the solid surface to which they are attached is called adsorbent. How strongly bound an adsorbate is to the adsorbent depends on many factors, such as temperature, concentration, charges, polarity, size, etc. Adsorption can be categorised into two main types of phenomena: physisorption (which is based on weak van der Waal's bonds or hydrophobic/hydrophilic interactions) and chemisorption (where the adsorbate is chemically covalently bond to the adsorbent). The capacity of an adsorption process at equilibrium (mass balance based) can be expressed according to Equation 6.1, where q_E describes the adsorbed amount of adsorbate to the surface per weight of adsorbent:

$$q_E = q_0 + \frac{(c_0 - c_E)\,V}{m} \tag{6.1}$$

The equilibrium between adsorbate and adsorbent at a fixed temperature is called an adsorption isotherm. There are many different types of isotherms, but depending on how strong the interaction between adsorbate and adsorbent at equilibrium is at different concentrations, they can be categorised into favourable, linear and unfavourable isotherms [109]. Common models used for the non-linear isotherms are the Langmuir isotherm [110], the Freundlich isotherm [111], the Sips isotherm [112], and a modified Brunauer-Emmett-Teller (BET) isotherm [113]. HMF has previously been identified to follow a Freundlich isotherm [114], but that does not have to be the case when using organic solvents, as utilised in this study. Furthermore, in this process, it is not the HMF that is the target for adsorption but the impurities and by-products in the solution.

However, not all processes operate at the isotherm's equilibrium, as the time it takes to reach absolute equilibrium makes the process inefficient. Therefore, the adsorption kinetics play a valuable role in the design of full-scale adsorption processes. Common kinetic models to describe the adsorption rate are pseudo-first-order, pseudo-second-order and Elovich [115, 116].

Both the isotherm modelling and the kinetic modelling of GAC adsorption behaviour of humins and other impurities were investigated in *Paper IV*. These models were calculated from experimental data produced in batch experiments, which are described in the following sections.

6.1.1 GAC type screening

Eight different GAC products (listed in Table 6.1) were assessed in screening experiments, where 4 g of prewashed GAC was added to 10 mL of HMF/DMC solution and continuously stirred in an incubator at room temperature for 48 h. The analysis of the humins was difficult to perform, due to being comprised of multiple different compounds. Therefore, a method using the visual spectrum (VIS) was developed. The reduction of impurities was analysed using a UV-VIS spectrophotometer. The absorbance at all wavelengths in the visual spectrum (400-800 nm) was measured, and the integral of the curve was calculated and compared with the feed solution (control without GAC treatment) to calculate the reduction efficiency. Since absorbance is directly proportional to concentration (according to Beer-Lambert's law), the integral of concentrations should correspond to the total amount in the given spectrum [117, 118]. No absolute concentrations could be obtained using this method, but relative numbers of reduction could be used. The HMF concentrations were measured using high-performance liquid chromatography (HPLC).

Table 6.1. List of the 8 different GAC evaluated, including some properties of interest provided by the manufacturer.

GAC	Manufacturer	Specific surface (BET) (m^2/g)	Mean particle diameter (mm)	Activation
Cyclecarb 401	Chemviron Carbon (Feluy, Belgium)	n.s.*	1.1	steam
Organosorb 10-CO	Desotec (Roeselere, Belgium)	1050	n.s.*	steam
Norit GCN 1240	Cabot, Norit Americas inc. (Marshall, USA)	1150	n.s.*	steam
Norit GAC 400	Cabot Norit Americas inc. (Marshall, USA)	n.s.*	n.s*	steam
Aquasorb 6300	Jacobi, Osaka Gas Chemicals CO., Ltd. (Kalmar, Sweden)	1000	1.4	n.s.*
Aquasorb 2000	Jacobi, Osaka Gas Chemicals CO., Ltd. (Kalmar, Sweden)	950	1.4	n.s.*
Norit GAC 1240W	Cabot Norit Americas inc. (Marshall, USA)	1100	n.s.*	steam
Organosorb 20-AA	Desotec (Roeselere, Belgium)	830	n.s.*	n.s.*

* Not specified

Interestingly, the results showed that the GAC with the lowest specific surface (BET surface) was also the most efficient for removing humins (Organosorb 20-AA). However, information regarding the pore size distributions of the different GACs has not been obtained, making conclusions regarding the size of impurities and diffusion limitations uncertain (even though more micropores often means larger BET surface and more macropores means less BET surface) [119].

The results showed that adsorption using Organosorb 20-AA (Figure 6.1) was able to reach 97.6 % removal of impurities but with a loss of 36.4 % of the HMF. In addition, it was visually confirmed (Figure 6.2) that Organosorb 20-AA was able to remove most humins, as it yielded the brightest colour but required a very high GAC dose. To avoid this high level of product loss, a dose optimisation experiment was conducted.

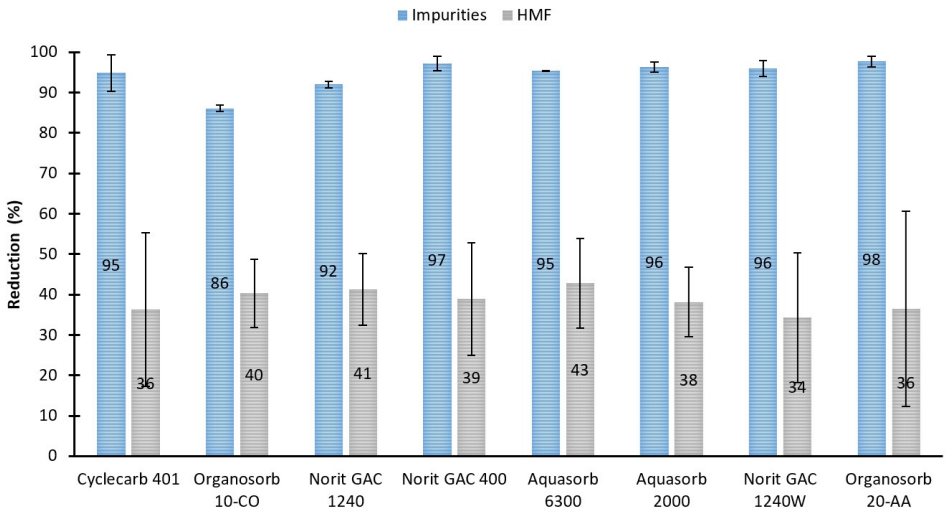


Figure 6.1. The reduction of impurities and HMF by adsorption using eight different types of GAC at a high adsorbent dose. Data from *Paper IV*.

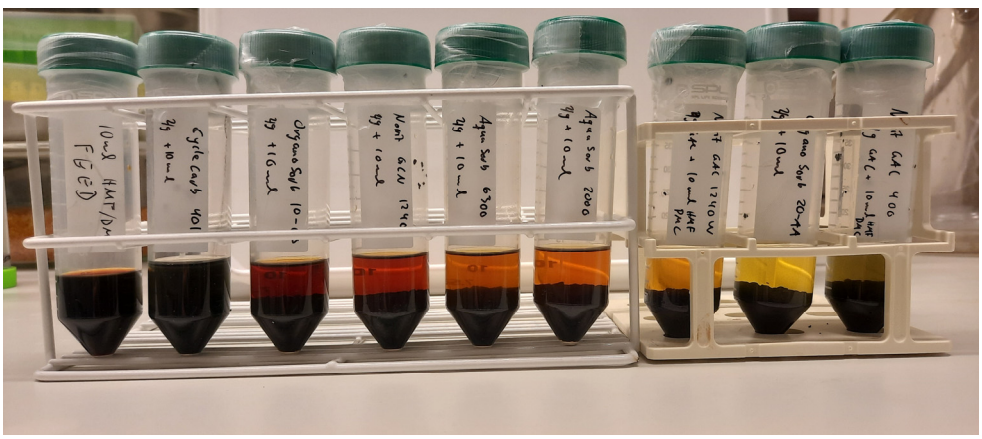


Figure 6.2. A picture of the visual effect by using different types of active carbon types at equal dosing. Here, it can also be seen some carbon detachments in the solution, from two of the GAC types (Norit GAC 400 and Cyclecarb 401).

6.1.2 GAC dose optimisation

The optimal dose of GAC was determined by testing the addition of various amounts of Organosorb 20-AA to a 20 mL solution of HMF/DMC, in order to minimise the product loss of HMF but still maintain a removal efficiency of humins at 80 %. The results (Table 6.2 and Figure 6.3) show that doses between 2-4 g of GAC to 20 mL HMF/DMC solution would be able to remove about 80 % of the impurities and yet only lose about 20 % of the HMF product. These experiments were carried out for 48 h, and prior to trying a larger batch experiment, the time factor was also optimised.

Table 6.2. Dose optimisation of Organososrb 20-AA during 48 h of adsorption at 25 ºC, evaluated with regards to humin removal and HMF loss. Data from *Paper IV*.

GAC dose	Impurities removal (%)	HMF loss (%)
8 g	96.5±0.0	48.4±21.6
6 g	94.8±0.6	39.0±11.8
4 g	90.4±1.8	26.8±1.0
2 g	75.7±0.6	19.4±2.3
1 g	55±2.5	17.6±14.9
0.5 g	41.3±13.7	5.7±1.8
0.1 g	23.5±10.7	5.7±0.1

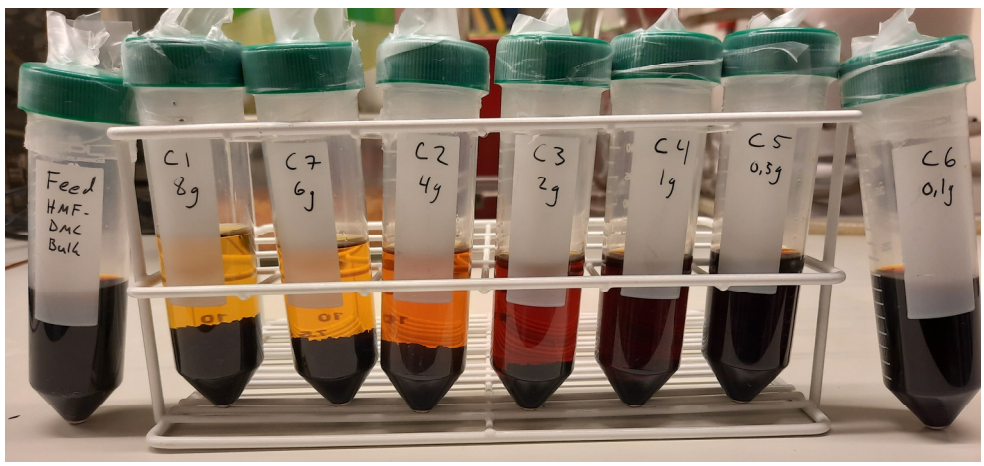


Figure 6.3. A picture of the visual effect by using different doses (g) of Organosorb 20-AA to 20 mL of HMF/DMC solution.

6.1.3 GAC time optimisation

A long adsorption time results in large unit operation equipment, which in turn means large capital expenditures. To minimise the cost of process equipment and yet to maintain good adsorption performance, a time optimisation experiment was conducted using two different doses of Organosorb 20-AA: 5 g and 10 g to 50 mL HMF/DMC solution. The results (Figure 6.4) show a rapid adsorption rate of HMF, while the impurities (humins) required longer time. In order to reach 80 % reduction of impurities, 24 h of adsorption time and using a GAC dose of 10 g / 50 mL were required. After 24 h, the HMF loss was roughly 20 %. An indication of how the solution gradually becomes more and more pure over time is also visually displayed in Figure 6.5).

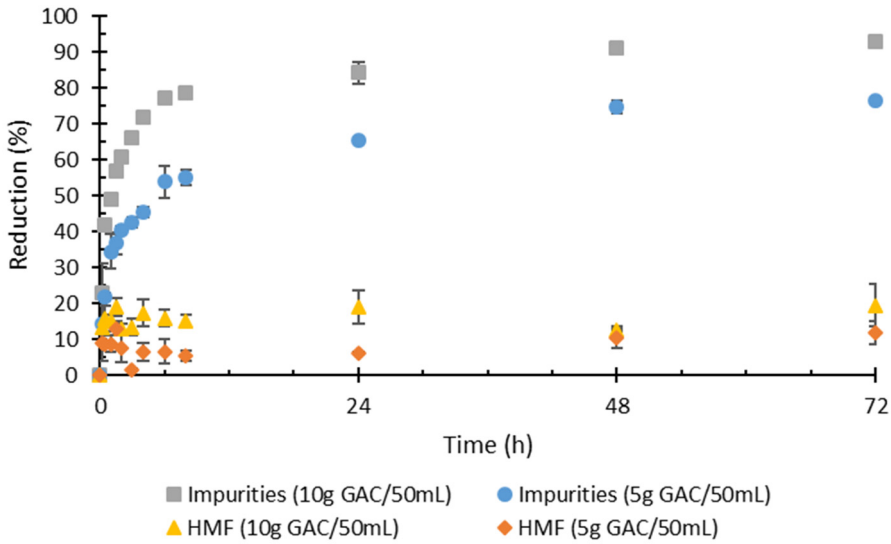


Figure 6.4. The changes in reduction of impurities and HMF over time, at two different doses of Organosorb 20-AA. Data from *Paper IV*.

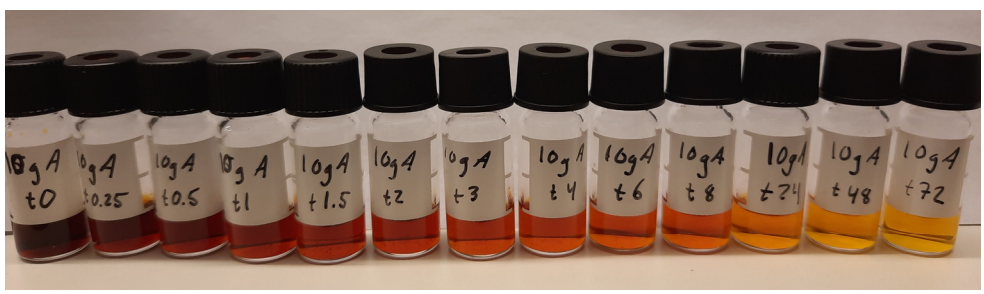


Figure 6.5. The effect of adsorption time, visually displayed.

6.1.4 Desorption possibilities

Even though there are some losses of HMF under the given optimal conditions in the previous sections, it is important to know the possibilities regarding HMF recovery through desorption. Spent GAC from 48 h of adsorption and using a dose of 10 g /50 mL was used in combination of both water and DMC, at four different doses, respectively. The desorption occurred for 48 h, and the results are presented in Figure 6.6. HMF desorbs more easily in DMC, and humins desorb more easily in water. This provides the opportunity to apply a two-stage sequence of the desorption process to recover more HMF: first by applying water to the adsorbent for removal of humins, followed by a DMC addition to recover HMF. However, this hypothetical sequential process has not been tested, thereby leaving the potential purity of such a recovered HMF unknown.

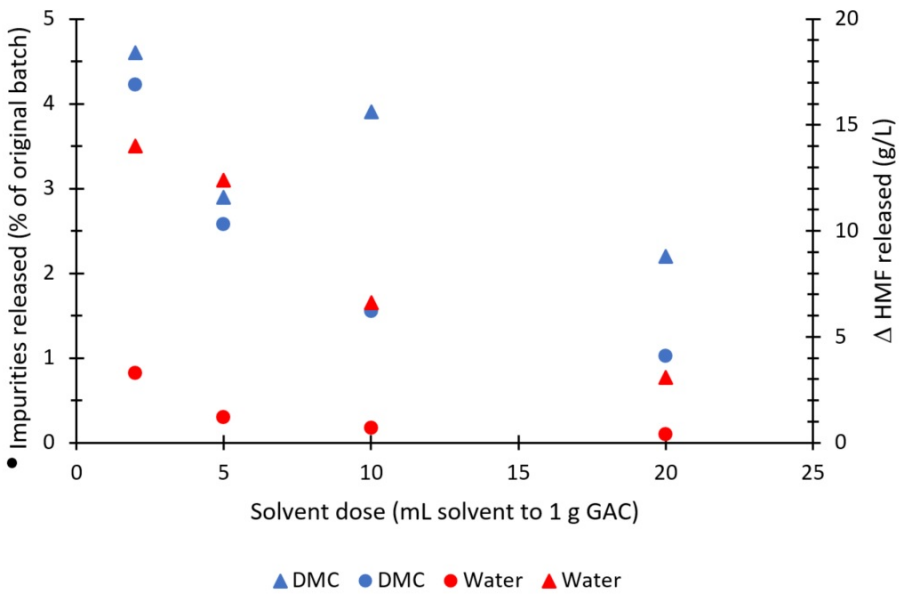


Figure 6.6. The potential of utilising desorption of two different solvents, DMC (blue) and water (red), at various doses for the release of humins (•) and HMF (Δ). Data from *Paper IV*.

6.2 Evaporation for HMF recovery

The optimal conditions obtained in Section 6.1 were considered in order to produce a larger batch of 250 mL. Thereby, 50 g of Organosorb 20-AA was added to 250 mL of HMF/DMC solution for impurity adsorption during 24 h. Thereafter, the GAC was separated from the purified solution through decantation after sedimentation. The impurities decreased by 85.6 %, but unfortunately, they were accompanied by a 19.2 % loss of the HMF. Figure 6.7 illustrates the purified HMF/DMC solution.

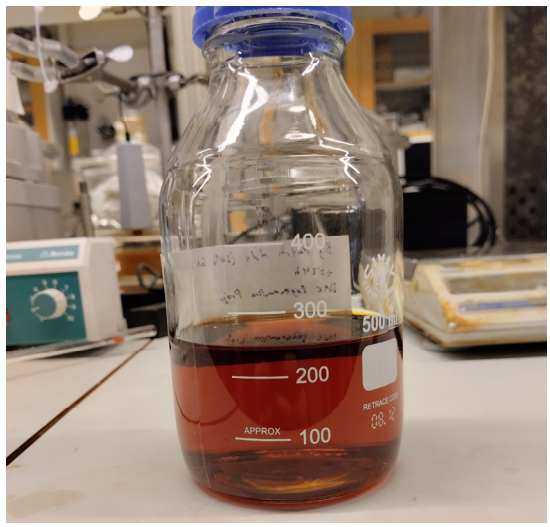


Figure 6.7. The 250 mL purified HMF batch from the adsorption process, to be evaporated.

The evaporation was then performed in a Rotavapor at 50 ºC and under vacuum in order to minimise generation of humins formed by heat. The solution started to boil at 200 mbar pressure, and it was then gradually reduced during the experiment due to boiling point increase as the concentration increased and reached 35 mbar when almost all DMC had evaporated from the solution. Out of the 250 mL solution, 9 g concentrate was obtained. According to HPLC analysis, the HMF purity was 78 %. This was then compared to the untreated (without GAC) HMF concentrate, which only reached an HMF purity of 58 %. Very small amounts of DMC remained in the concentrates, corresponding to 0.52 mg/mL and 0.64 mg/mL for the samples treated with and without GAC adsorption, respectively. Evaporation was therefore considered as an efficient and suitable separation process for the solvent removal. Figure 6.8 shows the two different evaporator concentrates, after freezing. Interestingly, the purified HMF concentrate was pure enough to form crystals, which was not possible utilising the untreated HMF concentrate.

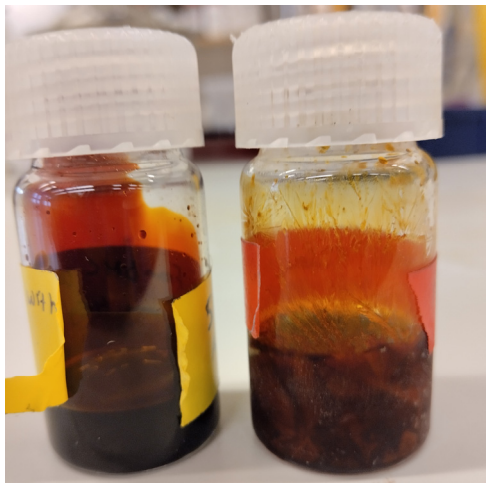


Figure 6.8. The evaporator concentrate, after freezing. The vial to the left is without GAC purification, and the vial to the right is purified using GAC adsorption. The purified HMF to the right can clearly crystallise when decreasing the temperature, which was not possible in the untreated sample to the left.

The HMF concentrates were further analysed using Fourier-transform infrared spectroscopy (FTIR). The purified and untreated HMF concentrates were compared with pure commercially available HMF, in order to see differences between the samples – i.e., what types of impurities were removed through the GAC adsorption.

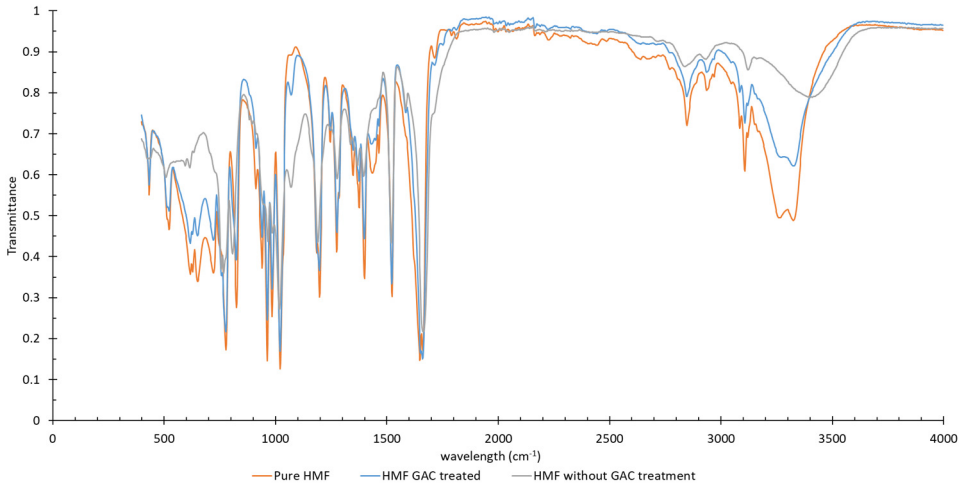


Figure 6.9. The FTIR spectra of a pure HMF sample (reference, organge), the untreated HMF concentrate (grey) and the purified HMF concentrate (blue). Data from *Paper IV*.

Figure 6.9 shows the FTIR spectra of the pure HMF sample, the untreated HMF concentrate and the purified HMF concentrate. In this case, the peak around 1200 cm^{-1} corresponds to the typical =C-O-C= bond of the furan ring, a ring deformation occurs at 1020 cm^{-1}, the peak around 1700 cm^{-1} corresponds to the aldehyde group, the region of 1320-1520 cm^{-1} corresponds to C=C stretches and 770-820 cm^{-1} is C-H out-of-plane formation [120].

More interesting are the differences in the spectra at the regions of 600-700 cm^{-1}, 1050-1200 cm^{-1}, 1400-1500 cm^{-1} and 3200-3400 cm^{-1}. The region of 1050-1200 cm^{-1} could correspond to ether bonds, having a stronger signal for the untreated HMF sample than for the treated and pure HMF [121]. This indicates a polymerisation of the HMF (humin formation) and that the adsorption process can remove these compounds efficiently. In the other end of the spectra, 3200-3400 cm^{-1}, signal differences in this region could correlate to OH groups, where the untreated HMF product has a lower signal compared to the purer HMF samples [122, 123]. This also supports the theory regarding etherification between HMF, as two hydroxide groups can form an ether bond plus a water molecule. The spectral range between 1400-1500 cm^{-1} reveals a reduced occurrence of C=C stretches in the untreated HMF sample, in comparison to the commercial product. This observation suggests alterations in the C=C bonds within the furan ring structure [120]. The 600-700 cm^{-1} region is hard to draw any conclusions from in general, even though there obviously are some differences between the samples.

6.3 Liquid-liquid extraction

In the remaining aqueous phase from the dehydration reaction, there is still some HMF dissolved (33 g/L). In order to increase the overall process yield, a liquid-liquid extraction (LLE) of the aqueous solution with DMC could be suitable. This was investigated in *Paper IV* using both the regenerated DMC from the evaporation step (evaporator condensate), with/without GAC pretreatment, and compared with a new DMC solution. The results (Figure 6.10) show that it is possible to further recover more than 70 % of the HMF in the aqueous phase by using LLE with DMC. Furthermore, there is no significant difference between the regenerated DMCs and a new commercial product. Also here, the recovered DMC from the evaporator step could be also utilised to increase the overall process yield further. From a process point of view, this LLE step could ideally be placed between the evaporator and the dehydration reaction. The recycled DMC would then already contain some HMF when it enters the dehydration reactor, which might decrease the diffusion driving force to the organic phase of some of the newly formed HMF from fructose.

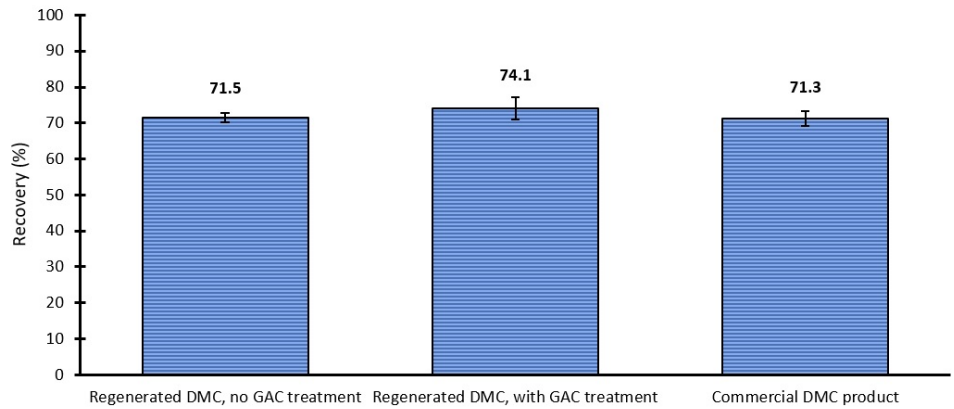


Figure 6.10. The additional recovery of HMF from the aqueous phase, using DMC from three different sources. Data from *Paper IV*.

7 Conclusions and future work

This thesis shows the opportunities and challenges using molasses as a feedstock for the production of HMF. The core of today's research for the production of HMF is performed on model solutions and does not focus on real feedstocks from industries that require more processing – a cost factor that is often neglected.

UF is one such additional processing step. By using UFM, the conversion of fructose in the dehydration step was higher than that of CM, at similar HMF selectivity. It appears that high MW compounds have a negative impact on the biphasic acidic dehydration reaction. However, the dilution factor of the molasses prior to filtration can significantly affect the retention of various products (sucrose included). It is desired to minimise the use of water to reduce energy demand and costs in the following evaporation step but still have an efficient membrane filtration process. Initially (*Paper II*), NF also demonstrated promising results in the hydrolysis step where higher reaction rates could be achieved. This was identified in *Paper III* as an effect of the lower salt content of the NF molasses and thereby lower buffering capacity to pH changes. It is both easier and cheaper to pH-adjust the molasses to more optimal pH ranges for the enzymatic hydrolysis than implement an NF process upstream.

The hydrolysis step was comprehensively investigated in *Paper III*, where the observed inhibition of the enzymatic reaction rate was in focus. A high salt content was proven to affect the hydrolysis rate. However, for the salts individually investigated, it was required to apply higher doses than what is present in the molasses to see a significant effect. Although, when applying the salts together as a mix, the inhibition effect was more profound. This indicates that there is some synergistic effect of the various salts to the enzyme and not only an effect of ionic strength.

It was possible to improve the batch-wise dehydration process by using UFM, with regards to a higher conversion of fructose and at a similar HMF selectivity. Moreover, and maybe even more importantly, it was shown in *Paper IV* that recycling of the regenerated DMC is possible and works just as well as a new DMC product for the extraction of the HMF. This enables this biphasic process to be cost-efficient, which is of fundamental importance for future scale-ups. The regenerated DMC also worked well to extract more HMF out from the aqueous phase.

The solvent regeneration process is also linked to the recovery of HMF, through evaporation. However, the purity of the recovered HMF could be increased by using adsorption with GAC, where both carbon type, dose applied and residence time for the batch-based purification process were optimised. Humins and other impurities could be removed to a large extent, with a relatively low product loss. How this process would operate in continuous mode, however, is yet to be determined.

Overall, what makes this thesis unique is the usage of a real industrial process side stream serving as feedstock for the established biphasic process with DMC for the production of HMF, with the process development and engineering solutions to make the molasses to be used as a feedstock feasible. The process development is crucial for a future scale-up of the HMF production from this type of feedstock.

7.1 Future prospects

Much has been done, but much is yet to be determined before a full-scale installation of this process setup can be established.

Primarily, evaluating how this process would function in continuous mode is essential for a more efficient process. Some assessments of continuous setups have been conducted but by using model solutions of pure fructose [56]. Combining this continuous process with molasses as feedstock would assess if further process modifications are required in order to obtain a productive HMF process.

Assessments of the hydrolysis and dehydration reactions using molasses in continuous mode instead of batches need to be performed. Also, a continuous adsorption in a packed column instead of batch setup needs to be evaluated, for future scale-ups. This could preferably even be performed at pilot scale.

Next, the optimal concentration of molasses needs to be determined. Balancing the membrane process efficiency versus how much water needs to be evaporated prior to the sucrose hydrolysis is an important energy optimisation to perform. Optimally, perhaps the evaporation step can be completely avoided. This is also correlated to the aggregation phenomenon, depending on molasses concentration, which could be further investigated. In addition, if, and when, pH adjustments should be performed needs to be further enlightened. The dehydration reaction requires pH 1.5-2, and the hydrolysis process is optimal around pH 4.5. However, if this pH adjustment should be performed already before the ultrafiltration step, it is necessary to assess how that would affect the membrane's retention, filtration capacity (flux) and even membrane fouling and cleaning strategies.

Furthermore, this process results in more than just HMF. There is also an aqueous phase that needs to be taken care of. Since this is rich in glucose (and other aqueous soluble compounds from the molasses), it is highly recommended to utilise this flow

as a resource instead of seeing it as a wastewater stream. One possible process route that could be utilised and evaluated is the isomerisation of glucose to fructose, which can be recycled to the dehydration step to increase the overall HMF productivity, as more fructose would be available (as in Figure 2.2).

Finally, a techno-economic analysis of the system also needs to be performed. If this process is to be constructed, it is required to know the minimum production cost of the HMF and if such a plant would be economically profitable with the prices of HMF we have on the market today (and in the future). Which process step has the highest capital expenditure, and which operating cost is the bottleneck that needs to be in focus and negotiated on? Such questions need to be addressed before a profitable full-scale installation can be in place and for HMF from molasses to be on the market.

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