Farm to furan derivatives

Fractionation of agricultural residues and transformation of sugars to chemical building blocks

Jonsdottir Glaser, Sara

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BIOTECHNOLOGY | FACULTY OF ENGINEERING | LUND UNIVERSITY

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

The transition of industrial production from petrochemistry to a more sustainable economy requires the use of carbon neutral, renewable feedstock, which does not compete with food and feed supplies – by-products of farming and agro-food industry offer this possibility. With a limited range of low-value applications today coupled with the need for maximal utilisation of resources, the by-products of farming can be repurposed as feedstock for biorefineries. The challenge, however, is to develop sustainable and scalable processes and systems whose benefits must outweigh the costs of implementation.

The research performed within the framework of this thesis involves investigations on fractionation of the complex agriculture residual biomass into different streams for further valorisation, and transformation of sugar and sugar derived furan to valuable building blocks for chemicals and materials. Sugar beet pulp and wheat bran are amongst the main agricultural residues in Sweden, generated as by-products of manufacture of sugar and wheat flour, respectively. Fractionation of beet pulp by a three-stage process comprising treatment with weak mineral acid, pectinases and cellulases, resulted in streams enriched in arabinose, galacturonic acid and glucose, respectively. A preliminary techno-economic assessment of the process together with conceptual valorisation of the sugars to arabitol, mucic acid and levulinic acid, respectively, revealed potential viability. In the case of wheat bran, a sequential fractionation involving starch- and protein hydrolysis, steam explosion for hemicellulose breakdown, hydrotropic extraction for release of lignin, and cellulose hydrolysis was tested to release separate streams originating from the different components of bran.

Levulinic acid (LA) is a five-carbon bifunctional chemical with ketone and carboxylic acid originating from C6 sugars in the biomass via 5-hydroxymethylfurfural (5-HMF). LA was directly produced with >70% yield from high concentrations of fructose and glucose, respectively, by heterogeneous catalytic dehydration using a strong cation exchange resin in the presence of salt. The study showed that the salts enhanced the rate of dehydration in the order of Cl->CO32->SO42-. Carboligation of 5-HMF, using whole cells of Escherichia coli bearing recombinant benzaldehyde lyase, led to a rapid formation of the C12 product, 5,5′-bis(hydroxymethyl)furoin (DHMF) at high yields. DHMF was further converted in situ by subsequent oxidation to 5,5′-bis(hydroxymethyl)furil (BHMF). The reaction performed in a fed-batch mode resulted in 53 g/L of DHMF in 5 hours by feeding 20 g/L 5-HMF every hour. The products were analysed for their potential as crosslinkers in coatings by reaction with adipic acid hydrazide to form hydrazones.

The thesis lays ground for further optimisation, scale up, techno-economic and life cycle assessments of biomass fractionation and production of building blocks for a low carbon economy.

Keywords
Biorefinery, sugar beet pulp, wheat bran, process design, bio-based platform chemicals, hmf

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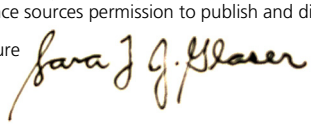
Signature  Date 2022-11-09

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To my children Markus, Jonathan and Maria

Abbreviations

BAL	benzaldehyde lyase
BHMF	5,5'-bis(hydroxymethyl)furil
DHMF	5,5'-bis(hydroxymethyl)furoin
DI water	deionised water
DMC	dimethylcarbonate
DS	de-starching/de-starched
DP	de-proteination/de-proteinated
DW	dry-weight
FTIR	Fourier transform infrared spectroscopy
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
HEX	hydrotropic extraction
HMF	5-(hydroxymethyl)furfural
HPLC	high performance liquid chromatography
LF	liquid fraction
MISR	metric for inspecting sales and reactants
NHC	N-heterocyclic carbene
NMR	nuclear magnetic resonance
NREL	National Renewable Energy Laboratory
Pf	*Pseudomonas fluorescence*
RI	refractive index
SBP	sugar beet pulp
SEC	size-exclusion chromatography
SF	solid fraction
STEX	steam explosion
SXS	sodium xylene sulphonate
ThDP	thiamine diphosphate
UV	ultraviolet
WB	wheat bran
WT	wild-type

Table of Contents

Abstract

The transition of industrial production from petrochemistry to a more sustainable economy requires the use of carbon neutral, renewable feedstock, which does not compete with food and feed supplies – by-products of farming and agro-food industry offer this possibility. With a limited range of low-value applications today coupled with the need for maximal utilisation of resources, the by-products of farming can be repurposed as feedstock for biorefineries. The challenge, however, is to develop sustainable and scalable processes and systems whose benefits must outweigh the costs of implementation.

The research performed within the framework of this thesis involves investigations on fractionation of the complex agriculture residual biomass into different streams for further valorisation, and transformation of sugar and sugar-derived furan to valuable building blocks for chemicals and materials. Sugar beet pulp and wheat bran are amongst the main agricultural residues in Sweden, generated as by-products of manufacture of sugar and wheat flour, respectively. Fractionation of beet pulp by a three-stage process comprising treatment with weak mineral acid, pectinases and cellulases, resulted in streams enriched in arabinose, galacturonic acid and glucose, respectively. A preliminary techno-economic assessment of the process together with conceptual valorisation of the sugars to arabitol, mucic acid and levulinic acid, respectively, revealed potential viability. In the case of wheat bran, a sequential fractionation involving starch- and protein hydrolysis, steam explosion for hemicellulose breakdown, hydrotropic extraction for release of lignin, and cellulose hydrolysis was tested to release separate streams originating from the different components of bran.

Levulinic acid (LA) is a five- carbon bifunctional chemical with ketone and carboxylic acid originating from C_6 sugars in the biomass via 5-hydroxymethylfurfural (5-HMF). LA was directly produced with >70% yield from high concentrations of fructose and glucose, respectively, by heterogeneous catalytic dehydration using a strong cation exchange resin in the presence of salt. The study showed that the salts enhanced the rate of dehydration in the order of $Cl^- > CO_3^{2-} > SO_4^{2-}$. Carboligation of 5-HMF, using whole cells of *Escherichia coli* bearing recombinant benzaldehyde lyase, led to a rapid formation of the C_{12} product, 5,5'-bis(hydroxymethyl)furoin (DHMF) at high yields. DHMF was further converted *in situ* by subsequent oxidation to 5,5'-bis(hydroxymethyl)furil (BHMF). The reaction performed in a fed-batch mode resulted in 53 g/L of DHMF in 5 hours by feeding 20 g/L 5-HMF every hour. The products were analysed for their potential as crosslinkers in coatings by reaction with adipic acid hydrazide to form hydrazones.

The thesis lays ground for further optimisation, scale up, techno-economic and life cycle assessments of biomass fractionation and production of building blocks for a low carbon economy.

Popular Abstract

The world is facing unprecedented challenges regarding the climate. The rate of global warming is rising fast, mostly as a result of industrialisation and widespread use of petroleum-based products. One chemistry-based solution for decreasing our dependence on fossil fuels is to develop biorefineries.

A biorefinery can provide the chemicals that a typical oil refinery produces, but instead of crude oil as the source for processing, waste streams from agriculture will be used. Not only can a biorefinery provide more sustainably-sourced chemicals, but it can also maximise the use of renewable resources, thus contributing to a more circular model of our economy.

This dissertation aimed to develop and evaluate processes that have the potential to increase the value of sugar beet pulp and wheat bran, major by-products of Swedish agricultural farms. A three-stage process was proposed for the fractionation of the three main components of sugar beet pulp, glucose, arabinose and galacturonic acid, in separate streams. For the wheat bran-based biorefinery, a five-stage strategy was investigated where the five main components were released in separate streams. Both strategies for sugar beet pulp and wheat bran aimed for further processing of each stream. Each of these streams is intended to be processed further to alternative chemicals that can be used instead of the oil-based products. These include plastics, fuels, plasticizers, foam, pharmaceuticals, food additives and many more.

Further use of the glucose stream was investigated through the production of furan-based platform chemicals 5-HMF and levulinic acid. These bio-based platform chemicals can be readily converted to more specialised compounds that may replace existing petrochemicals. Upgrading of 5-HMF into a longer, specialised compound has potential applications in the plastics industry. A complete process, all the way from the biotechnological preparation of the catalyst to the purification and characterisation of the product was included in the research work.

To test whether the laboratory results can be applied in real-life, an early-stage assessment was presented. Additional work in the direction of making a more complete feasibility evaluation of a biorefinery process is needed if we decide to replace our current oil refineries with biorefineries.

Populärvetenskaplig sammanfattning

Världen står inför oöverträffade utmaningar när det gäller klimatet. Takten av den globala uppvärmningen ökar snabbt, främst som ett resultat av industrialisering och utbredd användning av petroleumbaserade produkter. En kemibaserad lösning för att minska vårt beroende av fossila bränslen är att utveckla så kallade bioraffinaderier. Ett bioraffinaderi kommer att förse oss med de kemikalier som ett typiskt oljeraffinaderi producerar, men istället för råolja som källa för bearbetning kommer avfallsströmmar från jordbruket att användas.

Bioraffinaderier maximerar användningen av naturresurser och bidrar därmed till en mer cirkulär modell av vår ekonomi. Avhandlingen handlar om utveckling och utvärdering av processer som kan öka värdet av sockerbetsmassa och vetekli, biprodukter från det svenska lantbruket. En process i tre steg föreslås för fraktionering av de tre huvudsakliga monomerkomponenterna av sockerbetsmassa, glukos, arabinos och galakturonsyra, i separata strömmar. För det veteklibaserade bioraffinaderiet presenteras en femstegsstrategi där de fem huvudkomponenterna extraheras i separata strömmar. Båda strategierna för behandling av sockerbetsmassa och vetekli syftade till ytterligare bearbetning av varje komponentström.

Ytterligare användning av glukosströmmen undersöktes genom produktion av kemikalierna 5-HMF och levulinsyra. Dessa biobaserade plattformskemikalier omvandlas enkelt till mer specialiserade kemikalier som kan ersätta befintliga petrokemikalier. Uppgradering av 5-HMF till en specialiserad blandning har potentiell tillämpning inom plastindustrin. Upprättandet av en komplett process, hela vägen från den biotekniska beredningen av katalysatorn till rening och karakterisering av produkten, ingick i avhandlingsarbetet.

Slutligen, ett försök att tillämpa de experimentella resultaten i verkliga situationer sammanfattades i en preliminär genomförbarhetsanalys där en teknoekonomisk modell konstruerades. Ytterligare arbete i riktning mot att göra en mer komplett genomförbarhetsutvärdering av en bioraffinaderiprocess behövs om vi beslutar oss för att ersätta våra nuvarande oljeraffinaderier med bioraffinaderier.

Ágrip

Heimurinn stendur frammi fyrir áður óþekktum loftslagsáskorunum. Hlýnun jarðar eykst hratt, þá sérstaklega vegna iðnvæðingar og víðtækrar notkunar á olíuvörum. Efnafræðileg lausn til að minnka þörf okkar á jarðefnaeldsneyti er að þróa svokallaðar lífhreinsunarstöðvar.

Lífhreinsunarstöð mun útvega þau efni sem dæmigerð olíuhreinsunarstöð framleiðir, en í stað hráolíu sem uppsprettu til vinnslu verður úrgangur frá landbúnaði notaður. Lífhreinsunarstöðvar hámarka nýtingu náttúruauðlinda og stuðla þannig að hringrás í hagkerfi okkar.

Hér verður fjallað um þróun og mat á ferlum sem geta aukið verðmæti sykurrófumassa og hveitiklíð, aukaafurða frá sænskum landbúnaði. Hér verður lagt til þriggja þrepa ferli til að skipta þremur aðalhlutum sykurrófumassa, glúkósa, arabínósa og galaktúrónsýru, í aðskilda streymi. Lífhreinsunarstöðin sem byggir á hveitiklíð innihélt fimm þrepa feril þar sem fimm aðalhlutarnir voru slepptir út í aðskildum streymum. Báðir sykurrófumassa og hveitiklíðarferlarnir miða að frekari vinnslu hvert streymi. Frekari notkun glúkósastreymisins var könnuð með framleiðslu á fúran efnum 5-HMF og levúlínsýru. Þessum lífrænu efnum er auðveldlega breytt í sérhæfðari efni sem geta komið í stað núverandi jarðolíuefna.

Umbreyting 5-HMF í lengri, sérhæfða blöndu getur hugsanlega nýst í plastiðnaðinum. Allur ferill umbreytingu 5-HMF var kannaður, allt frá líftæknilegum undirbúningi hvata til hreinsunar og greiningar efnanna.

Niðurstöður rannsóknarinnar voru nýttar í bráðabirgðahagkvæmnigreiningu til að skoða áhrif þeirra á raunverulegar aðstæður. En þar var bæði tæknilegt og hagrænt líkan smíðað. Þörf er á frekari vinnu í átt að fullkomnari hagkvæmnimati á lífmassaferli ef ákveðið verður að skipta núverandi olíuhreinsunarstöðvum út fyrir lífhreinsunarstöðvar.

Buod

Matinding pagsubok ang hinaharap ng ating daigdig dulot ng pagbabago ng klima. Ang pag-init ng mundo ay mabilis na tumataas, na unti-unting naidulot ng simula ng industriyalisasyon at malawakang paggamit ng mga produktong galing sa langis. Kinakailangan nating bawasan ang ating paggamit ng langis upang hindi matuluyan ang sakuna dahil sa pagbabago ng klima. Isang paraan upang mabawasan ang ating pangangailangan ng langis ay ang paggawa ng tinatawag na mga biyoplanta, o biorefinery sa ingles, na sa halip na krudo, ay mga halaman ang pagmumulan ng produksyon ng mga kemikal.

Upang hindi mabawasan ang pinagkukunan ng pagkain ng mga tao, mga halaman na wala nang gamit, tulad ng labi mula sa pagsasaka, ang gagamiting pangunahing sangkap para sa isang biyoplanta. Sa ganitong paraang, mailulubos ng isang biyoplanta ang paggamit ng mga likas na yaman sa pamamagitan ng paggamit ng mga labi ng pagsasaka upang makagawa ng mga kemikal na kinakailangan ng lipunan.

Ang pananaliksik na ito ay tungkol sa pagbuo at pagsusuri ng mga proseso para pataasin ang halaga ng pulpa ng remolatsa at salbado ng trigo, mga labi ng pagsasaka sa Sweden. Nagmungkahi ako ng proseso na may tatlong hakbang para paghiwalayin ang tatlong pangunahing mga bahagi ng pulpa ng remolatsa: glukos, arabinos at galacturonic acid. Para sa biyoplanta gamit ang labi ng trigo, limang hakbang ang proseso upang mahiwalay ang limang pangunahing bahagi nitong materyal. Ang paghiwalay ng mga komponento ay paraan upang maiproseso pa ang bawat komponento upang makalikha ng kemikal na maaaring kalakalin.

Ang karagdagang paggamit ng glukos ay inimbestigahan sa pamamagitan ng paggawa ng mga kemikal na base sa furan tulad ng 5-HMF at levulinic acid. Itong mga simulaing kemikal na ito ay maaaring gawing espesyal na kemikal na maaaring pumalit sa mga umiiral na mga kemikal na galing sa langis. Ang pagtaas ng 5-HMF sa mas mahabang molekula ay may potensyal na gamit sa industriya ng plastik. Ang isang kumpletong proseso, mula sa paghahanda ng katalista hanggang sa paglilinis at paglalarawan ng produkto ay kasama sa aking ginawang pananaliksik.

Aking sinikapan na ilapat ang mga resultang eksperimental sa isang modelo kung saan maaaring makita ang posibilidad ng pagpapatupad ng ganitong proseso sa industriya. Naipakita sa modelong may batayan sa teknikalidad at ekonomiya na mayroong potensyal ang biyoplantang gamit ang pulpa ng remolatsa. Karagdagang gawain tungo sa isang mas kumpletong pagsusuri ng proseso ng biyoplanta ang kakailanganin upang tuluyan nang mapalitan ng mga biyoplanta ang kasalukuyang mga kemikal na binubuo galing sa langis.

List of papers

This doctoral thesis is based on the following research papers. The papers are appended at the end of the thesis. Published papers are reproduced with permission of their respective publishers.

Paper I: **Fractionation of sugar beet pulp polysaccharides into component sugars and pre-feasibility analysis for further valorisation**

Sara Jonsdottir Glaser, Omar Abdelaziz, Corentin Demoitie, Mats Galbe, Sang-Hyun Pyo, John P. Jensen, Rajni Hatti-Kaul. (2022)

Biomass Conversion and Biorefinery. (2022). doi.org/10.1007/s13399-022-02699-4

Paper II: **Wheat bran fractionation: Effect of steam explosion and hydrotropic extraction conditions on the recovery of sugars and lignin**

Sara Jonsdottir Glaser, Mats Galbe, Basel Al-Rudainy, Rajni Hatti-Kaul.

(Manuscript)

Paper III: **Clean production of levulinic acid from fructose and glucose in salt water by heterogeneous catalytic dehydration**

Sang-Hyun Pyo, Sara Jonsdottir Glaser, Nicola Rehnberg, Rajni Hatti-Kaul.

ACS Omega. (2020), 5, 24, 14275–14282. doi.org/10.1021/acsomega.9b04406

Paper IV: **Carboligation of 5-(hydroxymethyl)furfural using whole cells of recombinant *Escherichia coli* expressing *Pseudomonas fluorescens* benzaldehyde lyase to form C12 furan derivatives and their use for hydrazone**

Sara Jonsdottir Glaser, Sang-Hyun Pyo, Nicola Rehnberg, Dörte Rother, Rajni Hatti-Kaul

(*Manuscript)*

My contributions to the studies

The work in the appended papers of this thesis was supervised by Prof. Rajni Hatti-Kaul, with co-supervision of Assoc. Prof. Sang-Hyun Pyo, and Adj. Prof. Nicola Rehnberg. My contributions to each study were as follows:

Paper 1 – I designed the study and performed the experiments. I analysed, processed and conceptualised the presentation of the data. I wrote the first draft of the manuscript. I revised the manuscript together with my co-authors.

Paper 2 – I designed the study with Assoc. Prof. Mats Galbe. I performed the experiments, analysed, processed and presented the data. I wrote the first draft of the manuscript. I revised the manuscript together with my co-authors.

Paper 3 – I analysed, processed and presented the data. I drafted the section of the manuscript regarding the analysis. I revised the manuscript together with my co-authors.

Paper 4 – I designed the study and performed the experiments. I developed the analytical method and processed the data. I wrote the first draft of the manuscript. I revised the manuscript together with my co-authors.

Conference presentations

Sara Jonsdottir Glaser. ”Bio-based platform chemical production from agricultural by-products.” Poster Presentation; “*LUBIRC Day*” 10 November 2021, Lund, Sweden

Sara Jonsdottir Glaser, Omar Abdelaziz, Corentin Demoitie, Mats Galbe, Sang-Hyun Pyo, John P. Jensen, Rajni Hatti-Kaul. ”Fractionation of sugar beet pulp polysaccharides into component sugars and pre-feasibility analysis for further valorisation.” Poster presentation; “*Renewable Resources and Biorefineries*” 1–3 June 2022, Bruges, Belgium

Sara Jonsdottir Glaser, Mats Galbe, Basel Al-Rudainy, Rajni Hatti-Kaul “Wheat bran-based biorefinery: fractionation of bran based on steam explosion and hydrotropic extraction*”* Oral presentation: “*Efnís Conference 2022: Chemistry and the environment*” 11–12 August 2022, Reykjavík, Iceland

Sara Jonsdottir Glaser, Sang-Hyun Pyo, Nicola Rehnberg, Dörte Rother, Rajni Hatti-Kaul. “Recombinant *E. coli* cells containing benzaldehyde lyase for upgrading 5-hydroxymethylfurfural into specialised C-12 platform compounds.” Poster and lightning talk presentation; “*BioCat 2022*” 28 August–1 September 2022, Hamburg, Germany

1 Introduction

The collective use of fossil fuels, natural gas and coal since the beginning of the Industrial Age in the 18th century until the present has been progressively taking its toll on our climate. This is primarily depicted in the increasing CO_2 emissions which in turn affects the global surface temperature. **Fig. 1.1** illustrates the near-linear correlation between the atmospheric CO_2 levels and the global surface temperature anomaly, defined as the difference in average temperature based on measurements since 1880, in the period between 1951–2021. The Intergovernmental Panel on Climate Change (IPCC) published a report in 2021 summarising the scientific basis of climate change and presented the overarching evidence of human influence on the unprecedented global warming in at least 2000 years[1].

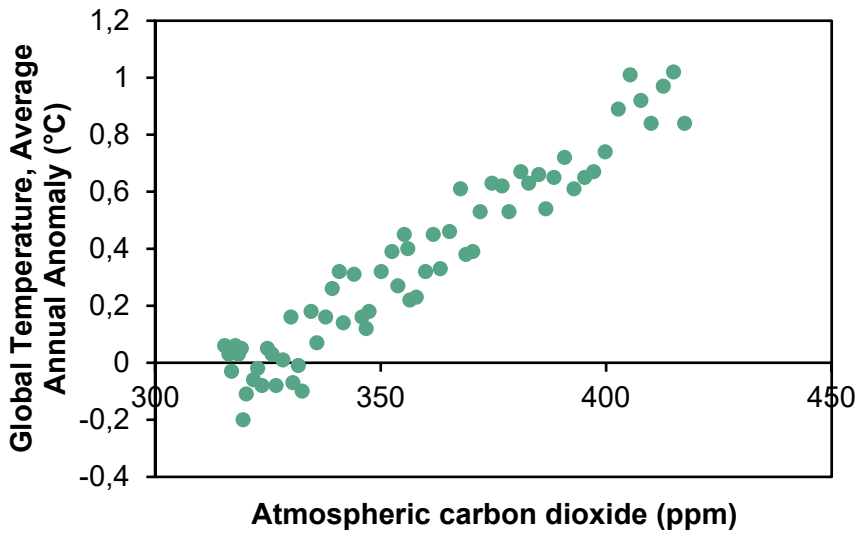


Fig. 1.1 Near-linear correlation between atmospheric carbon dioxide levels and annual global temperature anomaly, based on measurements since 1880. Original data from NASA [2]

Catastrophic consequences throughout the planet were outlined in the report if the global surface temperature were to increase beyond 2°C [1] and therefore it is crucial that every segment of the society coordinates the systemic decrease of the greenhouse gas emissions.

The United Nations addressed these concerns through the implementation of the Sustainable Development Goals (SDG) [3] with goals for sustainability to be achieved by 2030 (Agenda 2030). This culminated in the Paris agreement in 2015 where nations

pledged to decrease anthropogenic emissions, and thereby limiting the increase of global surface temperature to up to 1.5–2°C. The Glasgow Climate Pact that was agreed at the 2021 United Nations Climate Change Conference (26th Conference of the Parties: COP26) resolves to pursue the efforts to limit the temperature increase to 1.5°C as agreed upon at the Paris agreement. Almost 200 countries that were involved at COP26 pledged carbon neutrality by 2050 [4].

Sustainable chemistry and engineering plays an important role on realising climate and environment-related SDGs [5].

1.1 Biorefinery: an instrument for sustainability

Biorefineries are considered powerful solution frameworks for decreasing greenhouse gas emissions [6]. Substantially decreasing the reliance on fossil fuels and fossil-based products calls for another more sustainable feedstock to cover the global demand for these products. Biomass is a promising candidate as a feedstock replacement for further processing and refining – hence the term biorefinery, borrowing the concept of an oil refinery. Ideally, biomass that is not intended for food, such as agro-industrial waste streams, is proposed for this purpose. Sustainable practices include maximising the extraction of the resources and adding value, and minimising waste generation while avoiding environmental pollution and greenhouse gas emission [7]. Biorefineries exert great potential in the transition towards sustainability by expanding the value-chain of otherwise discarded waste or by-product streams. Hence, this concept allows waste to be recirculated into the bioeconomy.

Agricultural waste or by-product streams are regarded to be sustainable feedstocks as they pose no competition with the food supply. However, significant setbacks need to be overcome to make the bio-based production economically viable. Challenges include the diverse composition of this feedstock, perishability, and large volumes required for transportation [8]. Moreover, biomass conversion processes typically operate in aqueous systems, with low product concentrations, therefore downstream processing may prove challenging. A holistic approach for optimising biorefinery processes will be needed to determine the potential of a novel process for viability.

1.2 Scope and outline of this dissertation

The focus of this dissertation was to develop strategies and processes to valorise the residual biomass streams from agriculture and agro-food industry in South Sweden. This was done by fractionation of the biomass into separate component streams and

production of furan-based platform chemicals originating from the sugar stream. Two feedstocks were studied in this research work: sugar beet pulp and wheat bran, both being major by-products of agro-industrial farming in Sweden.

The thesis is based on 4 papers, two of which are published. *Paper I* presents a three-stage fractionation approach for sugar beet pulp releasing the three main monomer components, glucose, arabinose and galacturonic acid, in separate streams for further valorisation. Glucose extracted from agricultural residues offers a good substrate for conversion into furan and furan-based platform chemicals, such as 5-(hydroxymethyl)furfural (5-HMF) and levulinic acid. A multi-stage fractionation strategy was explored for wheat bran processing in *Paper II*, with the aim of releasing the main components cellulose, hemicellulose, lignin, starch and protein, in separate stages. *Paper III* reports on the production of levulinic acid, from fructose and glucose, using cation exchange catalysis. Finally, *Paper IV* offers a simple and potentially cost-effective means to upgrade 5-HMF into C_{12} products using whole *Escherichia coli* cells expressing recombinant benzaldehyde lyase. A potential application as cross-linkers was analysed for the products.

2 Agricultural side-streams as biorefinery feedstock

2.1 Agricultural residues in global and regional context

Agriculture is amongst the main pillars on which the very fabric of civilisation stands. The necessity of feeding growing populations relies on intensive agriculture, which was made possible through the combined developments in mechanisation, availability of industrial-grade nitrogen fertilizer, widespread pesticide use, hybrid or genetically enhanced crop varieties [9]. An annual global agricultural output of 9.8 billion tonnes required 14.5 million km^2 total area of land in 2020 [10]. The large areas of land occupied, and intensification of agriculture has sadly resulted in loss of biodiversity in several regions of the world, poor soil quality, and contributed to significant greenhouse gas emissions. These issues are not the subject of this thesis, which instead focuses on the residues generated from agriculture.

In 2020, the five biggest crops grown (sugar cane, wheat, maize, rice and oil palm fruit) constituted 50.6% of total agricultural output worldwide [10]. Meanwhile in the European Union (EU), the top five crops produced (wheat, sugar beet, maize, barley and potatoes) amount to 65.8% of total crop production in the region. In Sweden, 86.8% of national crop production is comprised of the top five - wheat, sugar beet, barley, potatoes and oats. (**Table 2.1**).

Table 2.1 Top 5 agricultural crop production worldwide, in the European Union and in Sweden

Crop	Annual total output - 2020 (Mt) [10]	Yield (kg/ha) [10]	Estimated Residue (Mt)
Global	***9819.5***		
Sugar cane	1869.7	70643.4	Ca. 200 [11]
Maize (corn)	1162.3	5754.7	Ca. 1000 [11]
Wheat	760.9	3474.4	Ca. 800 [11]
Rice	756.7	4608.9	Ca. 1000 [11]
Oil palm fruit	418.4	14561.4	>100 [12]
European Union	***603.2***		
Wheat	126.7	2287673.0	
Sugar beet	100.1	148891.0	
Maize (corn)	67.8	7252.4	
Barley	54.7	4960.6	
Potatoes	54.0	153641.0	
Sweden	***9.7***		
Wheat	3.2	7156.1	
Sugar beet	2.0	68137.8	
Barley	1.5	5171.5	
Potatoes	0.9	36443.7	
Oats	0.8	4534.0	

Currently, billions of tonnes of agricultural residues are under-utilised worldwide, with an estimated 3.7 billion tonnes (dry-matter) produced as waste stream or residues from global production of barley, maize, rice, soy bean, sugar cane and wheat [11]. The majority of agricultural side-streams still remain unexploited, with proper waste handling often lacking. Some residues are left on the ground for soil enrichment, some used for feeding cattle and as animal bedding, burnt for heating, and in some cases sent to landfills [13]. Currently, biogas production for energy purposes has become an invaluable outlet for the utilisation of residual biomass from agriculture [14]. Similarly, an increasing number of second generation biorefineries are being developed, such as those for ethanol production [15, 16]. The vast availability of these biomass sources gives a strong motivation for the development of bio-based economy in which they can be efficiently processed and transformed to value added chemicals and materials to replace those produced from fossil oil and gas. This would maximise resource utilisation, minimize waste and at the same time, decrease the reliance on the petrochemical industry.

Waste biomass utilisation should be carefully tailored to the local situation of each location. In Sweden, forestry is the largest and most important industry as more than half

of the country's land area (ca. 70%) [17] is covered with forests. The forestry industry in Sweden is concentrated in the middle part, Svealand, up to the Northern part, Norrland. The Southern part, Götaland, with its entirely different landscape compared to the majority of the country holds about 60% of arable land. The regions Västra Götaland and Scania (Skåne) dominate the national agricultural output, where the top 5 crops in Sweden (wheat, sugar beet barley, potatoes and oats) are primarily cultivated [18]. Around 9.7 million tonnes of these top crops were produced in Sweden in 2020 [10]. Depending on the crop, a significant portion of the plant is left over after the production of the food product, for instance, about 63,000 tonnes of sugar beet pulp (on a dry-weight basis) was produced fom the annual sugar beet production of 2.03 Mtonnes in 2019/2020 [10, 18]. Meanwhile, annual wheat production in Sweden amounted to 3.2 Mtonnes in 2020 [10, 18], generating between 480,000–640,000 tonnes wheat bran [19].

2.2 Lignocellulosic nature of the agricultural residues

Lignocellulosic biomass is the scientific term used for biomass including agricultural and forestry residues, whose main components consist of cellulose, hemicellulose and lignin. Minor components including starch, protein, ash, phenols, etc. are found in varied amounts with respect to each plant material [20]. **Table 2.2** shows various types of lignocellulosic biomass and their main structural components.

Table 2.2. Structural composition of various lignocellulosic biomass sources

Biomass	% Cellulose	%Hemicellulose	% Lignin	References
Agricultural residues				
Rice husks	49	19	21	[21]
Wheat straw	30	50	15	[21]
Wheat bran	11	30	5	[22]
Barley straw	31	23	17	[21]
Sugar beet pulp	20-25	25-36	1-3	[23]
Maize cob	36	34	17	[24]
Sugar cane bagasse	39.7	24.3	18.4	[25]
Coconut husks	21.3	17.3	43.4	[26]
Forestry residues				
Hardwood	38-50	25-29	18-25	[27]
Softwood	40-50	25-27	27-33	[27]
Others				
Grass	25-40	25-50	10-30	[28]

2.2.1 Cellulose

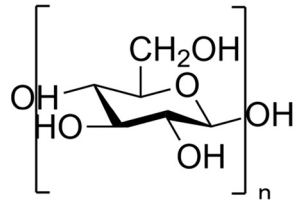


Fig. 2.1. Repeating glucose monomer unit of cellulose.

Cellulose is a major structural constituent of plant cell walls. It is a sturdy linear polymer comprised of 3000 or more glucose monomer units joined by β-(1,4)-glycodisic bonds [29]. It consists mainly of crystalline parts with some amorphous regions, which forms rod-like conformation, called microfibrils. These microfibrils in turn form larger groups making cellulose highly water-insoluble and difficult to break [27, 29]. Cellulose comprises about 50% of all the carbon found in vegetation making it the most abundant natural polymer on the globe [29].

2.2.2 Hemicellulose

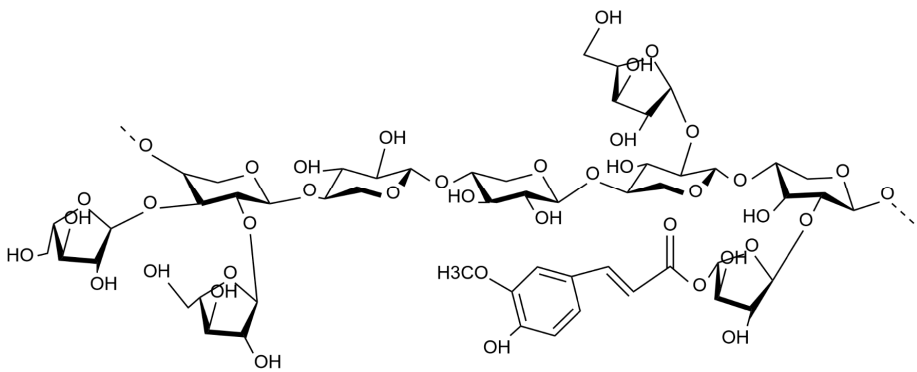


Fig. 2.2. Schematic structure of hemicellulose: arabinoxylan, containing primarily of arabinose units and xylose units, with one ferulic acid unit.

Hemicelluloses are a group of polysaccharides in the primary and secondary cell walls of plants, second to cellulose in abundance in wood and cereal biomass [30]. Hemicellulose content in plants varies from 15–35% depending on the plant species. They are either derived from hexoses (including glucose, mannose and galactose in softwoods) or pentoses (including arabinose and xylose in hardwoods and crop residues) [27]. Unlike cellulose, hemicelluloses are characterised by non-crystalline

heteropolysaccharides and are more complex in composition and bonding. Hemicellulosic carbohydrates form hydrogen bonds with cellulose, covalent bonds with lignin and ester linkages with acetyl units and hydroxycinnamic acids [30]. Several types of bonds exist within hemicelluloses. In arabinoxylan, the xyloses in the main chain are linked by β-(1,4)-glycosidic bonds, while the branched arabinoses are attached with α-(1,2)-glycosidic or α-(1,3)-glycosidic bonds. In sugar beet pulp, for instance, linear galactan are β-(1,4)-linked while branched arabinan consists of α-(1,5)-linked backbone with α-(1,2)- and/or α-(1,3)-arabinofuranosyl substitutions [31, 32]. Hemicellulosic polymer chains are also typically shorter than the cellulosic chains, ranging from 40 to 200 degrees of polymerisation [29].

2.2.3 Lignin

Lignin is one of the major components of plant cell walls providing plants with mechanical strength and the structure for water and nutrient transport [33, 34]. Lignin structure is based on three main phenylpropanoid monomers, *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), derived from their respective precursor alcohols, *p*-coumaryl (H), coniferyl alcohol (G) and sinapyl alcohol (S) (**Fig. 2.3**). The three lignin groups form the amorphous three-dimensional polyphenolic structure of lignin via oxidative phenolic coupling reactions [29] forming β-*O*-4, α-*O*-4, β-5, β-1, 5-5, 4-*O*-5 and β-β linkages [35]. Individual plant species contain varying composition of the three monomer units, which in turn determines the heterogeneity of lignin. Cross-linking, size and available functional groups add to the complexity and variability of lignin structure across plant species [36]. Softwood lignin contains more than 95% of guaiacyl units. In contrast, hardwoods as well as dicotyl crops such as flax and hemp, has varying guaiacyl to syringyl ratio. Cereal straws and grasses normally contains higher *p*-hydroxyphenyl units than the wood species [36].

Other herbaceous plant species, including sugar beet pulp and flax fiber, have very low lignin content, below 5% [35, 37]. Instead, another complex polymer, pectin, contributes to the rigidity of the plant structure and holds other important roles in plant growth and development [38].

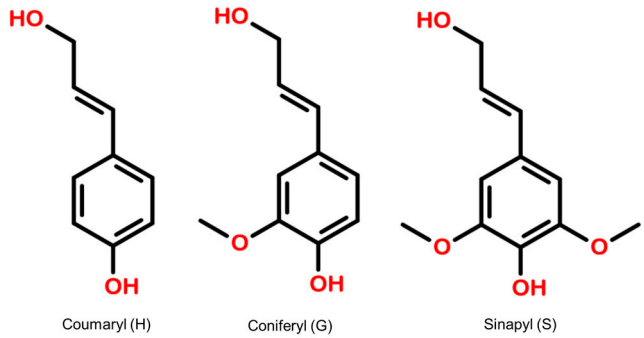


Fig. 2.3. Alcohol precursors of the phenylpropane monomers of lignin

2.2.4 Protein, starch, lipids and other components

Other components present in lignocellulosic biomass from agricultural by-products vary between plant species. Some have sizeable portions of protein such as in rapeseed, avocado residue, alfalfa leaves, wheat bran, etc [39-42]. Interesting phenolic antioxidants such as ferulic acid also occur naturally in plant cell walls, thus agricultural residues including wheat bran, sugar beet pulp, flax fibre, citrus peels, pineapple residues, etc, contain [43-45]. Still, others contain starch such as wheat bran that remains after wheat processing [46]. Moreover, considerable amounts of potentially interesting lipids, lipid derivatives and surfactant-like compounds can be found in agriculture-derived biomass including in wheat-germ, rapeseed cake [47, 48]. Structural compositional analysis of each biomass type is thus essential prior to designing an effective processing strategy with the goal of harnessing the value of each biomass component.

2.3 Agricultural residues used in the thesis

Sugar beet pulp obtained as side-product during sugar production from sugar beets (*Paper I*), and wheat bran obtained during processing of wheat for flour production (*Paper II*) were subjects of study in this research work.

2.3.1 Sugar Beet Pulp

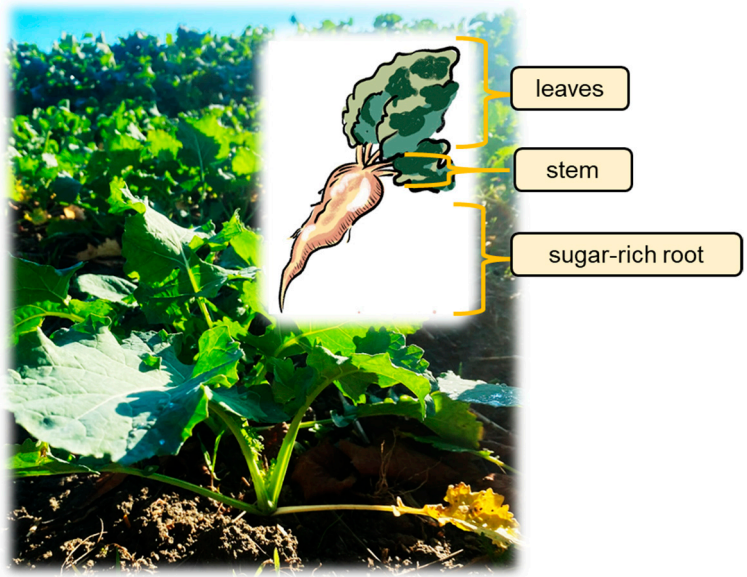


Fig. 2.4. Parts of the sugar beet plant. The root is harvested industrially due to its high sucrose content.

Sugar beet pulp (SBP) is a low-cost residual product of refined sugar manufacturing from sugar beets (*Beta vulgaris*). Globally, 253 Mtonnes of sugar beets were produced in 2020, wherein 39.6% of the total global production was produced in the EU, 13.4% in Russia, 12.1% in the United States and the rest of the production found across the globe [10]. **Fig. 2.4** shows the sugar beet plant. Sucrose is extracted from the sugar-rich sugar beet root through a multi-step hot water extraction process, generating a thick syrup, which is further refined and crystallised into the commercial white sugar, and the solids that remained after pressing is the sugar beet pulp. Another by-product of the processing is molasses, which is the remaining syrup that did not crystallise [49].

SBP (dry matter) accounts for ca. 19% of the total sugar beet production. In Sweden, sugar beets are primarily cultivated in the South where about 63,000 tonnes of SBP (dry-matter) was generated from 2.03 Mtonnes sugar beet production in the campaign of 2019/2020 [18]. Today, SBP is sold primarily as a low-cost animal feed [49] and in cultivation locations with no nearby livestock farming nor proper agricultural waste management, SBP is sent to landfills [13]. While its use as a nutritional additive to animal feed is a good application, the drying process is energy intensive, which can undermine the profitability [43]. Hence, from an economic perspective, it is highly

attractive to develop innovative ways to utilise sugar beet pulp as feedstock for a sugar based biorefinery for producing even non-food products.

2.3.2 Wheat Bran

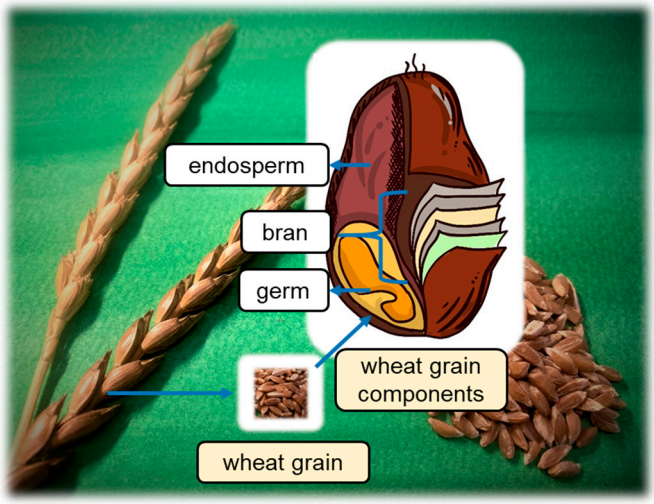


Fig. 2.5. Wheat plant and a schematic illustration of the wheat grain.

Wheat (the most common varieties belonging to *Triticum aestivum* L.) is ranked the third largest agricultural crop in the world with an annual production of 760.9 Mtonnes in 2020 [10, 50]. The European Union generated 126.7 Mtonnes of wheat, and in Sweden, 3.2 Mtonnes was produced in 2020 (**Table 2.1**) [10]. Vast amounts of by-product streams arise from wheat processing: wheat straw generated during harvesting, accounting for more than 529 annually worldwide [51], while processing by-products, mainly composed of wheat bran, comprises 15–20% of the grain weight amounting to 187 Mtonnes annually [19]. There are current applications of wheat bran in livestock feed [52], as well as a minor percentage of bran is used in commercial food applications [19, 53], as ingredient in breakfast cereals, bread, muesli bars and baby food [53], however the vast majority of the wheat bran is not efficiently utilised.

2.4 Pretreatment and fractionation of biomass

The complex structure of lignocellulosic biomass carries both the value and the challenges for utilisation as feedstock for industry. *Section 2.2* dealt with the individual components of lignocellulosic biomass. In reality, these potentially valuable components are embedded in a recalcitrant matrix linked via covalent bonds and strong

intra- and interchain hydrogen bonding [16]. **Fig. 2.6** illustrates the schematics of releasing the entrapped components from the biomass.

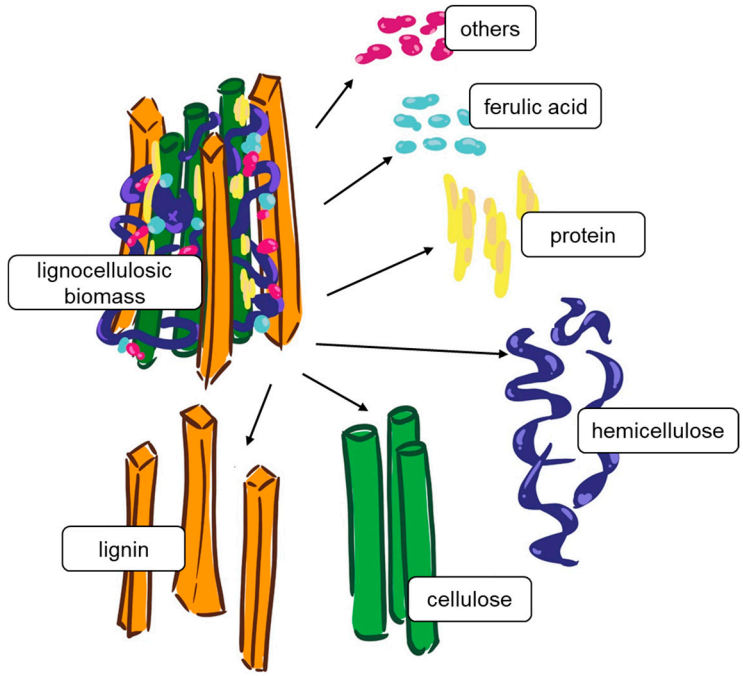


Fig. 2.6. Schematic illustration of the dissolution of individual components of lignocellulosic biomass.

To reap the benefits associated with lignocellulosic biomass utilisation, each component must be separated from the biomass matrix for further valorisation processes. The last few decades have seen huge number of studies dedicated to biomass pretreatment strategies with the aim of extracting value from the biomass that does not compete with food availability [54-56].

During many years of research and development dedicated to biomass utilisation starting in the early 90s, the main focus was on the production of alternative bioenergy and biofuels owing to the global need for vast amounts of energy and fuels that are primarily fossil-based. Thermochemical routes for biomass processing include syngas production through gasification, which can then be further upgraded catalytically to methanol, ethanol or dimethyl ether. On the other hand, fermentation routes involve utilising the carbohydrates in the biomass for ethanol, butanol and biogas production through the use of microorganisms [16]. Studies around the fermentation routes had generated a number of innovative methodologies to pre-treat the biomass in order for

the fermenting microorganisms to access sugars, mainly glucose from the cellulose component, which is deeply enmeshed in the lignocellulosic matrix.

Glucose continues to be the primary raw material for producing more valuable bio-based platform chemicals and building blocks for polymers with industrial potential, including lactic acid, succinic acid, 5-(hydroxymethyl)furfural (5-HMF), glucaric acid, sorbitol, etc. [57].

It was however clear that the other components present in the lignocellulosic biomass also have industrial potential, such as the carbohydrates found in the hemicellulosic constituent, including arabinose and xylose. Lignin, first thought to be a cheap energy source and a nuisance in obtaining the carbohydrates, has in fact great industrial potential owing to its functional aromatic structure. Also, proteins found in residual biomass provides a potential alternative protein source – a promising alternative for increasing food self-sufficiency of countries that have relatively low domestic agriculture [58]. Ferulic acid and other phenolic compounds also provide commercial potential, since higher-value commercial products, such as in antioxidant and flavouring markets, can be obtained from these constituents [59, 60].

Some of the classical and more innovative methods to treat and fractionate lignocellulosic biomass are described in the following pages.

Table 2.3. Overview of different biomass treatment methodologies

Treatment strategy	Target recovery	Advantages	Limitations	Reference
Acid treatment	Random reduction of polymer length, for pretreatment; Arabinose	Strong pretreatment effect, simple process scheme	Recycling difficulty, toxicity and corrosiveness of acid	[16]; *Paper I*
Alkaline treatment	Arabinoxylans, protein, ferulic acid	Strong pretreatment effect; simple process scheme	Recycling difficulty, high pollution and chemical recovery cost	[16, 61]; *Papers I* and *II*
Steam explosion	Hemicelluloses: arabinoxylan, galactomannan	Rapid and easily scalable; tailored hemicellulose recovery	High energy demand	[16, 62]; *Paper II*
Organosolv treatment	Lignin, cellulose, hemicellulose, oil and protein	Fractionating ability, solvent reusability and recovery	Potential inhibitory effects of the solvents on further processing	[41, 63]
Deep eutectic solvents	Cellulose, lignin, protein, phenolic compounds	Low cost, easy preparation, low toxicity	Difficult recovery, recyclability of the solvent; low solvent stability	[64]
Hydrotropic treatment	Lignin	Low cost, easy preparation	Product recovery	[65, 66]; *Paper II*
Enzymatic treatment	Monosaccharides	Low energy demand, mild and eco-friendly	Highly diluted, low biomass loading	[67-69]; *Papers* I and *II*

2.4.1 Acid treatment

Acid hydrolysis relies on severing the polymeric chains of the complex biomass through inducing hydrolysis of the glycosidic bonds connecting the monomer units and disrupting the network of intra- and interchain hydrogen bonds between the components within the biomass [70]. Hydrolysis of biomass using concentrated hydrochloric acid at elevated temperatures has been used to solubilise both hemicelluloses and celluloses releasing the carbohydrates [71]. However, hydrochloric acid is highly corrosive, hence the difficulty of upscaling such a process. Other more lenient applications of acid hydrolysis involve dilute acid hydrolysis using less corrosive acids such as sulphuric acid followed by further biomass treatment stages such as enzymatic treatment. A milder acid hydrolysis induces disruption of the inter-connecting hydrogen bonds within the lignocellulosic matrix and some degree of hydrolysis of the ester linkages, and thereby making the biomass more susceptible for the following treatment [16].

2.4.2 Alkaline treatment

Alkaline treatment for biomass has been shown to be a noteworthy strategy in a biorefinery perspective due to the fact that depending on the alkaline conditions, selective solubilisation of the biomass components can be achieved [61]. Strong alkali treatment has been used for efficient extraction of arabinoxylans from oat hulls [72], as well as ferulic acid recovery from maize bran [45]. In addition, protein has been recovered from wheat bran under mild alkali treatment [39].

2.4.3 Enzymatic treatment

Saccharification of polysaccharides is a widely-studied reaction which is commonly catalysed by glycoside hydrolases (GH), a huge family of enzymes whose main mode of activity is the cleaving of the glycosidic bonds present in complex sugars. Cellulases, consisting of endoglucanase, exoglucanase (cellobiohydrolase) and β-glucosidase, act in synergy for cellulose breakdown [68]. Xylanases and arabinases are other important enzymes which hydrolyse the hemicellulosic fraction of the biomass [67, 68]. For the degradation of pectin, which primarily consists of galacturonic acid, pectinases can be used, consisting of three sub-classes: pectin depolymerase, pectin lyase and pectin esterases. Pectin depolymerase is also a glycoside hydrolase that cleave the α-(1,4)-glycosidic linkage in polygalacturonic acid, whereas pectin lyase, which belongs to another enzyme family type lyase, catalyses the elimination of polygalacturonic acid into galacturonide. Pectin esterase, on the other hand, belongs to the esterase family,

performs de-esterification of the sugar units removing the methyl and acetyl groups in polygalacturonic acid [73].

2.4.4 Steam explosion

Steam explosion treatment (STEX) has been studied extensively both as a fractionation and a pretreatment method [16, 62] that relies on treating the biomass with a high-pressure steam for a relatively short time, between 6 – 20 min, followed by abrupt decrease of pressure causing the underlying biomass structures to implode [16, 74]. Most common steam explosion treatment involves acid impregnation, such as with sulphuric acid, to facilitate the acid hydrolysis of the hemicelluloses [16]. Other steam explosion treatments involve alkaline impregnation, which has been shown to solubilise longer oligosaccharide chains [75]. Depending on the application, the conditions of steam explosion treatment can be tailored to the desired fractionation or solubilisation of the biomass components.

2.4.5 Organosolv treatment

The organosolv method typically involves the use of methanol, ethanol, acetone, glycol or phenol for biomass treatment [16]. This is advantageous due to the fractionating possibilities of the biomass into cellulose, lignin and hemicellulose with high purity. The relatively easy recovery and reusability of the solvent also adds to the benefits of this treatment [63].

2.4.6 Deep eutectic solvent treatment

Deep eutectic solvents generally consist of two components: hydrogen bond donor and hydrogen bond acceptor. The strong intramolecular bonds between the moieties of deep eutectic solvents enhances the breakage of the hydrogen bonds within biomass [64]; thereby with proper tuning of the solvent composition, selective solubilisation of biomass components can be possible [76].

2.4.7 Hydrotropic extraction

Hydrotropes are characterised by their ampiphilic structure, i.e. hydrophilic head and hydrophobic tail, that resemble surfactants but with shorter tails [77]. These are common compounds with wide applications in soap, cleaning agents and beauty products. Hydrotropes have seen applications in solubilising difficult compounds due

to their unique structure and hence have been investigated for lignin extraction [66]. The exact reaction mechanism is yet to be elucidated, however there are indications that the mechanism of hydrotrope solubilisation may be comparable to a surfactant's microemulsion system which results in micelle formation. Hydrotropes do not form micellar structures, although at specific threshold concentrations, the solubility of organic molecules does increase [65, 78]. This property makes hydrotropes ideal for solubilising difficult organic molecules, such as lignin, because through simple dilution of the hydrotropic solution, the previously solubilised organic molecule can be precipitated. Different classes of molecules can be classified as hydrotropes, including urea, nicotinamide and sodium-salts of alkylbenzene sulphonate [77]. **Fig. 2.7** shows some of the common hydrotropes.

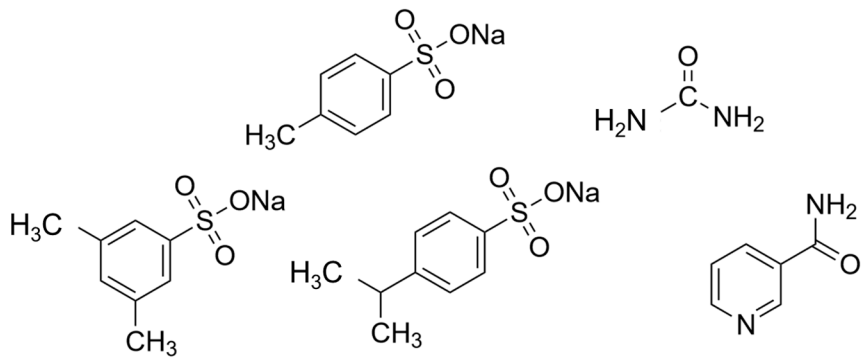


Fig. 2.7. Common hydrotropic compounds.

Sodium xylene sulphonate (SXS) has been used to extract lignin from hardwood chips, with up to 50% of lignin being successfully fractionated after steam explosion treatment of the wood chips [66].

3 Furans – an important group of biobased chemicals

Furans are heterocyclic organic compounds having a five-membered aromatic ring with four carbons and one oxygen atom. Furans are present in heat-treated commercial foods and are formed as a result of thermal degradation of the food components. Biomass-derived furan platform chemicals include 5-(hydroxymethyl)furfural (5-HMF), dehydration product of C_6 sugars formed by the removal of three molecules of water, and furfural, the product of dehydration of C_5 sugars. Characterised by the furan rings, aldehyde functional groups, and also a hydroxyl group for 5-HMF (**Fig. 3.1**), the main advantage of these furan platform chemicals is their reactivity and hence the ability to produce an array of diverse specialised compounds.

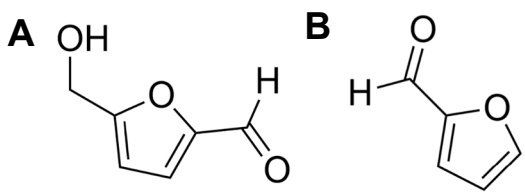


Fig. 3.1. Furan platform chemicals: (A) 5-hydroxymethyl furfural, with furan ring, hydroxyl and aldehyde groups (B) furfural, with furan ring and an aldehyde group

Another important advantage of biomass-derived furan compounds is that the furan component is more functionalised than hydrocarbons obtained from crude oil, but less functionalised than carbohydrates, making these compounds ideal as industrial reagents [79]. 5-HMF readily gets rehydrated into levulinic acid (LA) which is also a versatile furan-derived building block. *Paper III* investigated LA production from fructose and glucose.

Over the past few decades, numerous studies have been done on the production of platform chemicals from sugars which can be extracted from biomass [79, 80]. **Table 3.1** shows a minute fraction of the recent published data on the yields of both 5-HMF and LA produced from carbohydrates.

Table 3.1. Comparative results from literature for 5-HMF and levulinic acid production from carbohydrates

Platform chemical	Source	Catalyst, solvent system	Yield	References
5-HMF	Fructose	HCl, DMC/water	87.2%	[81]
5-HMF	Fructose	HCl, acetone/water	95%	[82]
5-HMF	Glucose	alumninium-based catalyst, MIBK/water	52%	[83]
5-HMF	Cellulose	Iron-based catalyst, butanol/water	49.1wt%	[84]
LA	Fructose	cation exchange resin, salt aqueous system	74.6%	*Paper III*
LA	Glucose	cation exchange resin, salt aqueous system	70.7%	*Paper III*
LA	Cellulose	Carbon-based acid catalyst, metal salt aqueous system	¨40 wt%	[85]

3.1 HMF – as platform chemical

5-(Hydroxymethyl)furfural (5-HMF) has been studied extensively due to its versatility attributed to its characteristic structure. The unique combination of furan ring, aldehyde and hydroxyl functional groups makes 5-HMF an attractive molecule for upgrading to a wide variety of industrially relevant compounds and energy carriers, as presented graphically in **Fig. 3.2**.

The top-value added chemicals from biomass published by the U.S. Department of Energy in 2004 include two compounds that can be directly formed from 5-HMF: levulinic acid and 2,5-furan dicarboxylic acid (FDCA). Other potentially industrially-relevant 5-HMF derivatives are adipic acid which is widely used as a monomer in nylon and biodegradable polyester polybutylene adipate terephthalate (PBAT) [86]; caprolactone, an already established molecule used to make biodegradable polycaprolactone; 2,5-dimethylfuran, a bio-based fuel additive, tetrahydrofuran and gamma-valerolactone, bio-based organic solvents; 2,5-bis(hydroxymethyl)furan, a potential bio-based resin compound. The applications of 5-HMF derivatives span several relevant industries (polymers, resins, solvents, precursors and fuel additives) with sizeable established markets making 5-HMF a highly attractive platform chemical [87].

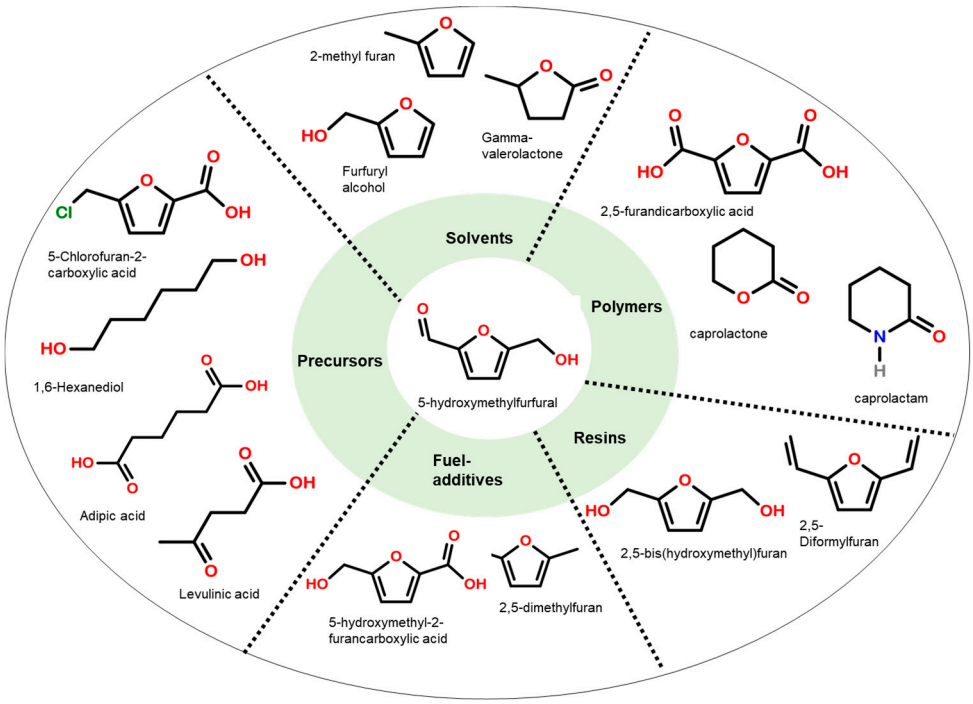


Fig. 3.2. 5-HMF as bio-based platform chemical. Adapted from Thoma *et al.* (2020) [87]

3.1.1 5-HMF Production

5-HMF can be produced through hydrothermal dehydration of hexoses, either glucose or fructose, the latter being the more favoured sugar as raw material. It is widely accepted the sugar dehydration to 5-HMF undergoes a cyclic mechanism involving a furanose intermediate. Since both cyclic furanose and pyranose tautomers of fructose co-exist in water while glucose exists predominantly in its pyranose form, fructose more readily undergoes dehydration [88]. Essentially, glucose is first isomerised to fructose before being dehydrated to 5-HMF. It is also commonly known that the dehydration process is Brønsted acid-catalysed [89]. Mineral acids, including sulphuric acid and hydrochloric acid have been shown to catalyse 5-HMF production generating 5-HMF yields of up to 93% from fructose [88, 90]. However, the use of strong acids is often discouraged due to the challenges it entails during product recovery and process-upscaling, including environmental risks, high cost of separation process and equipment corrosion [91]. Alternatively, acid-based heterogeneous catalysts, including tin-based zeolites (Sn-β) [82], acidic ion-exchange (H-form) [92], alumina-based catalysts [83, 91] have been investigated widely with varying 5-HMF yields from both fructose (up to 85%) [92] and from glucose (up to 63.1%) [91]. Acid catalysis in

aqueous medium at high temperatures invariably leads to the formation of humins as side products, leading to lower product yields.

Several different solvents have been used to decrease the water amount for the reaction. It has been found that biphasic systems have higher product yields as 5-HMF is extracted into the solvent phase and humin formation is reduced. Also, a continuous process has been found to be better than a batch process since the reaction is not continuously exposed to the high temperature [81, 92].

3.2 Levulinic acid – a 5-HMF derivative and also a platform chemical

Levulinic acid (LA; 4-oxo-pentaenoic acid), is a bi-functional compound, characterised by the carboxylic acid and ketone groups. These functional groups are beneficial for the production of various chemicals, hence levulinic acid finds broad applications in industries such as in flavour and fragrance, polymer resin, plasticiser, textile, animal feed, solvents, cosmetic and pharmaceutical industries [80, 93, 94]. **Fig. 3.3** shows various industrially-relevant compounds that can be derived from levulinic acid, with applications ranging from polymer resins, flavor/fragrance compounds, fuel additives, antimicrobial agents, herbicides, etc [80, 95].

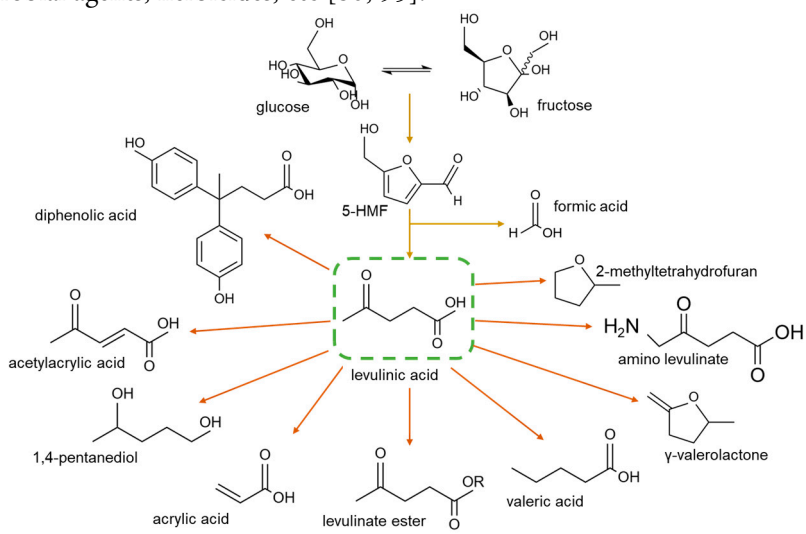


Fig. 3.3. Levulinic acid as bio-based platform chemical. Adapted from Morone *et al.*[96].

3.2.1 Production of levulinic acid

Extensive studies have been done on levulinic acid production from carbohydrates (mono- and polysaccharides), from lignocellulosic biomass, and even from algal biomass [96, 97]. Levulinic acid production is commonly achieved through homogenous or heterogeneous catalysis [94, 97]. Mineral acids, such as HCl and H_2SO_4, are often employed in homogeneous catalysis, which had generated high LA yields from both fructose (up to 74 mol%)[98] and glucose (up to 51%)[99]. However, the corrosiveness, toxicity and poor recyclability of these compounds remain a serious setback for upscaling such a process [96]. Heterogeneous catalysis, including zeolites, Amberlyst resins or Dowex, offers a better alternative for LA production since catalyst recovery is more feasible.

Fructose is recognised as the preferred substrate for levulinic acid and 5-HMF production. Aside from the furanose tautomer form of fructose which is supposed to be preferred for dehydration [88], the high ratio of Bronsted acid sites in catalysts could also potentially inhibit the glucose-fructose isomerisation [100, 101]. A combination of Lewis acid catalyst, promoting glucose to fructose isomerisation, and Bronsted acid catalyst is thus a probable option for enhancing the reaction. In one study, 46% LA yield was obtained from glucose in one-pot reaction involving Lewis acid $CrCl_3$ and HCl [102]. Other reaction parameters, including solvent addition and inclusion of metal halides have been shown to improve the catalysis [103, 104].

3.3 C_{12} platform chemicals from 5-HMF carboligation

Section 3.1 described the various 5-HMF derivatives and their applications in several areas of industry. The aldehyde group of 5-HMF has been subjected to both oxidation and reduction reactions that yield other building blocks useful for polymer synthesis. Yet another interesting reaction of 5-HMF is carboligation in which the aldehyde groups on two 5-HMF molecules react to generate a carbon–carbon bond which would elongate the carbon chain of the resulting compound. C_{12} platform chemical production is one of the potential 5-HMF upgrading. From the perspective of alternative gasoline production, extending the molecule prior to subsequent catalytic conversion will yield high-density range (C_9–C_{16}) compounds, hence expanding potential applications of 5-HMF into the diesel and jet fuel sectors [105, 106]. This type of 5-HMF upgrading by the coupling of two 5-HMF molecules, also finds specialised applications in the polymer industry [107]. Polymers with healing abilities [73], shape memory [108], and recyclability [109] are few examples in which furan-derived polyurethanes can find niche applications.

Fig. 3.4 shows the C_{12} chemical formed by carboligation of 5-HMF: 5,5'-bis(hydroxymethyl)furoin (DHMF). DHMF is an interesting compound due to possibility of dehydration/hydrogenation into liquid alkanes of the enolisable C_{12} organic molecule, which can then be used to produce bio-based kerosene/jet fuels [110]. In addition to fuel applications, C–C bond formation has been a key topic of interest in pharmaceutical research [111]. Both chemical and biological catalysts have been found to catalyse C–C bond formation [111-113].

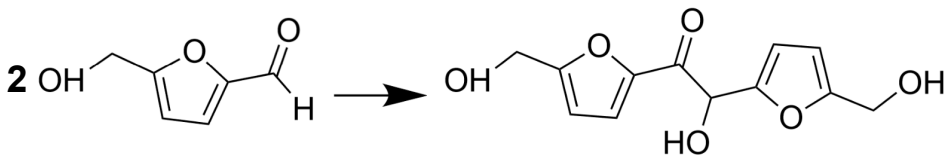


Fig. 3.4. Carboligation reaction between two 5-HMF molecules forming a C_{12} molecule DHMF

The oxidised form of DHMF, 5,5'-bis(hydroxymethyl)furil (BHMF), contains a diketone, where the middle hydroxyl group in DHMF has been oxidised into a ketone group. Both DHMF and BHMF find potential specialised applications in making polyurethanes, polyesters and jet fuels [105, 114, 115]

3.3.1 Carboligation methods

Reaction between the aldehyde groups on the 5-HMF molecules using a suitable catalyst, results in a C–C bond formation or carboligation with a ketone attached to one carbon atom, and a hydroxyl group attached to the other carbon atom [112]. The reaction mechanism of the C–C bond formation in 5-HMF has been compared to an umpolung or aldol self-condensation reaction mechanism [115]. Generally, aldol self-condensation requires α-H atoms that will readily react with each other to form a C–C bond. However, 5-HMF does not have α-H atoms [110]. Even so, recent developments have shown that reaction between the carbonyl groups in 5-HMF is possible through a mechanism similar to aldol condensation, via N-heterocyclic carbene catalysis [115].

3.3.2 Carbene catalysts

Liu *et al.* reported that 5-HMF undergoes carboligation enabled by *N*-heterocyclic carbene catalysis (NHC). They observed rapid degradation of 5-HMF using acetate-based room-temperature ionic liquid 1-ethyl-3-methyl imidazolium acetate,

(EMIM)OAc. Further investigation revealed that (EMIM)OAc contained low-levels of NHC with the basic acetate ion [116], enabling the formation of a furoin from 5-HMF [115]. Subsequent studies using a discrete NHC, the Enders triazolylidene carbene, TPT (1,3,4-triphenyl-4,5-dihydro1*H*-1,2,4-triazol-5-ylidene) showed furoin synthesis from 5-HMF with yields up to 98% [115].

3.3.3 Enzyme-based carboligation

Carboligation has been observed using the enzymes decarboxylases and lyases since the 1930s. A special group of enzymes called thiamine-diphosphate (ThDP)-dependent enzymes are versatile biocatalysts that are capable of both making and breaking bonds between carbon and hydrogen, oxygen, sulphur or nitrogen, and between two carbon atoms [117]. The carboligation activity of ThDP-dependent enzymes has direct coupling to the co-enzyme thiamine, or Vitamin B_1, which is a natural N-heterocyclic carbene catalyst owing to the structure of catalytically active species [118]. One of the first commercialised biotechnological processes involved a process catalysed by ThDP-dependent pyruvate decarboxylase in yeast which allowed the formation of (*R*)-phenylacetylcarbinol, a precursor of (1*R*,2S)-(–)-ephedrine from pyruvate and benzaldehyde (**Fig. 3.5**) [119, 120].

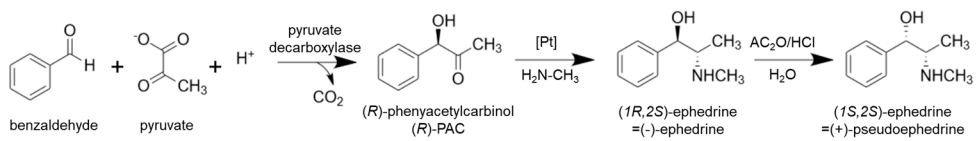


Fig. 3.5 Carboligation of pyruvate and benzaldehyde producing (*R*)-phenylacetylcarbinol, which can then be transaminated to (1*R*,2*S*)-(–)-ephedrine. Adapted from [121]

Aside from pyruvate decarboxylase, other ThDP-dependent enzymes such as benzoylformate decarboxylase, 2-ketoacid decarboxylase, benzaldehyde lyase have been investigated for carboligation reactions via umpolung condensation-like mechanism [117, 122].

The main advantage of enzyme catalysis is the inherent reaction specificity and selectivity. In some cases, an enzyme could also take another substrate with a sufficiently similar structure as its native substrate. This was the premise for the preliminary investigation involving three different ThDP-dependent enzymes whether they could accept 5-HMF as the substrate for carboligation. Pyruvate decarboxylase (*Ap*PDC) from *Acetobacter pasteurianus*, benzoylformate decarboxylase (*Pp*BFD) from *Pseudomonas putida*, and benzaldehyde lyase (*Pf*BAL) from *Pseudomonas fluorescens*,

were tested for activity against 5-HMF. Only *Pf*BAL showed activity for 5-HMF, which is in line with an earlier report by Donnelly *et al* [123]. *Pf*BAL was further investigated in *Paper IV* for 5-HMF carboligation.

3.3.4 Whole-cell catalysis

A more cost-effective strategy for enzymatic catalysis is the use of whole cells instead of the purified enzyme for the process [124]. The use of whole cells as catalysts cuts the cost for cell lysis and enzyme purification, as well as the need for the expensive co-factors, since the cells have inherent co-factor production [124]. *Paper IV* delved into the whole-cell catalysed 5-HMF carboligation into 5,5'-bis(hydroxymethyl)furoin (DHMF) which then gets spontaneously oxidised into 5,5'-bis(hydroxymethyl)furil (BHMF).

4 Development of biomass fractionation strategies (*Papers I* and *II*)

Several methods for biomass treatment are found in the literature. The main challenge is the selection of appropriate treatment methods for any specific feedstock. The choice of treatment relies on the composition of the material. Suitable treatments were investigated in this PhD work, for sugar beet pulp and wheat bran. SBP is the remaining solid pulp after the pressing of sugar-rich thick juice from the sugar beet crop, without the leaves. The sugar beet pulp material investigated in *Paper I* was received from Nordic Sugar A/S (Copenhagen, Denmark). Wheat bran is obtained as a side-product of wheat grain processing, and was a gift from Lantmännen AB, Sweden, in 2020. The outcome of the treatment procedures was evaluated using various analytical procedures.

This led to sequential combinations of treatments that were aimed for efficient fractionation of the feedstock. This chapter shows the work of the development of fractionation strategies starting with the analytical methods involved for the evaluation of each treatment, compositional analysis of the feedstock and the design of fractionation approaches.

4.1 Analytical procedures

4.1.1 Analysis of the solid fractions – extractives, structural carbohydrates, ash and lignin

Biomass analysis protocol provided by the National Renewable Energy Laboratory (NREL) was used for the compositional analysis of sugar beet pulp and wheat bran including extractives [125], structural carbohydrates and lignin [126]. A two-stage extraction, water followed by ethanol, was performed to remove the non-structural components (extractives) of the biomass. The dried extracted biomass was then subjected to the labour-intensive analyses involving gravimetric analysis for ash and lignin determination, and total acid hydrolysis for the determination of structural

carbohydrates. The latter protocol [126] was applied for the treated biomass, the washed solid fractions after separation from the liquid fractions, or hydrolysate.

4.1.2 Analysis of the liquid fractions - carbohydrate analysis

Monosaccharide analysis is a well-established method which is most often performed by HPLC or HPAEC systems equipped with either ion-exclusion or ion-exchange columns. In case of both sugar beet pulp and wheat bran, ion-exclusion column (H^+) was used for the quantification of glucose, arabinose and galacturonic acid. This was complemented by the use of ion-exchange CarboPac 20 for the remaining monosaccharides. For the analysis of wheat bran samples, ion-exclusion column (Pb^{2+}) was used for monosaccharide quantification.

Although the main objective is to release individual monomers from the biomass, incomplete hydrolysis does occur resulting in the presence of soluble oligosaccharides. For the purpose of quantification of these shorter carbohydrate chains, a complementary analysis employing both size-exclusion chromatography (SEC) and a modified total acid hydrolysis method based on the NREL protocol [126] was performed. **Fig. 4.1** shows an example of the three-fold carbohydrate analysis of the acid hydrolysates of sugar beet pulp.

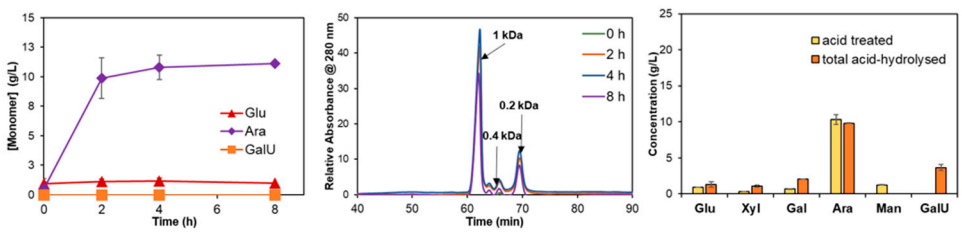


Fig. 4.1. (a)Monosaccharide analysis of acid hydrolysate from SBP by high-Performance Liquid Chromatography (HPLC) equipped with ion-exclusion column Aminex (H^+); (b) qualitative oligosaccharide analysis via size-exclusion chromatography (SEC) equipped with Sephadex columns; (c) monosaccharide analysis of total acid hydrolysed SBP acid hydrolysate by HPLC. Adapted from *Paper I*.

4.1.3 Protein analysis

Determination of protein is generally performed through the Kjeldahl method [127] or Dumas, the combustion method [128], both based on the total nitrogen content of the samples. More recent protein analysis techniques feature colorimetric techniques involving UV absorbance: the Bradford method [129] or the bicinchonic acid assay [130] which typically involve a standard calibration using bovine serum albumin. The Bradford method is based on the binding of Coomassie Brilliant Blue G-250 protein

to the protein in the sample. The change of UV absorbance of the protein is used for quantification. Similarly, bicinchonic acid assay is based on the coupled reaction arising from the reaction of the Cu^{2+} ions in the copper (II) sulfate solution and the peptide bonds in the protein, that results in the reduction to Cu^{1+} which chelates with bicinchonic acid producing an intense purple colour, with maximum absorbance at 562 nm. These assays are both rapid, sensitive, and easily accessible in laboratories [129, 130]. In this work, protein determination was performed using both Bradford and bicinchonic acid assay.

4.1.4 Lignin analysis of the liquid fraction

Lignin analysis is included in the NREL protocol [126], where it is determined through gravimetric analysis as the difference in weight between the dry-weight of the sample, obtained at 105°C, and the lost weight after sample incineration at 575°C. However, this method became unreliable when performed on the hydrotropically extracted lignin from the treated wheat bran biomass, due to the varying SXS content which remained after incineration. Hence, a precipitation-based analytical method for lignin content was performed [131]. Due to the characteristic of the hydrotrope SXS that was used to solubilise lignin, reducing SXS concentration, by diluting with DI water, releases the dissolved lignin in SXS, leading to lignin precipitation. The percentage of the solubilised lignin can be determined by measuring the amount of precipitated lignin (on dry-weight) with respect to the initial dry-weight of the hydrotropically treated sample.

4.1.5 Other analytical methods

In this work, ^{1}H and ^{13}C Nuclear magnetic resonance (NMR) analysis was used for structure elucidation and confirmation. Fourier Transform Infrared Spectroscopy (FTIR) was used for preliminary analysis for target product formation. Silica-gel column chromatography (SGC) was used for initial product purifications. Liquid Chromatography – Mass Spectroscopy (LC-MS) and Gas chromatography (GC) were used during the exploration for method development. Reversed-phase (C18) chromatography column was used for the separation and analysis of DHMF and BHMF in *Paper IV.*

4.2 Structure and composition of the biomass materials

4.2.1 Sugar beet pulp composition

Sugar beet pulp remains after pressing the sugar-rich root of the sugar beet plant (**Fig. 4.2A**) for sucrose extraction. The dry-matter content of the SBP material given by Nordic Sugar A/S was 25% (w/w). Compositional analysis on dry-weight basis is presented in **Fig. 4.2B**. The main monomer constituents of SBP are glucose (23%), arabinose (23%) and galacturonic acid (18%) arising from the polysaccharide chains of cellulose, arabinan and pectin, respectively. Protein content in SBP ranges between 8-15%. Unlike most lignocellulosic biomass from agricultural residues, lignin content is low (<1%) in SBP [23, 31, 132]. Additionally, another interesting value-added component of SBP which was not included in the study is ferulic acid, normally present at 1% of total biomass weight [133, 134].

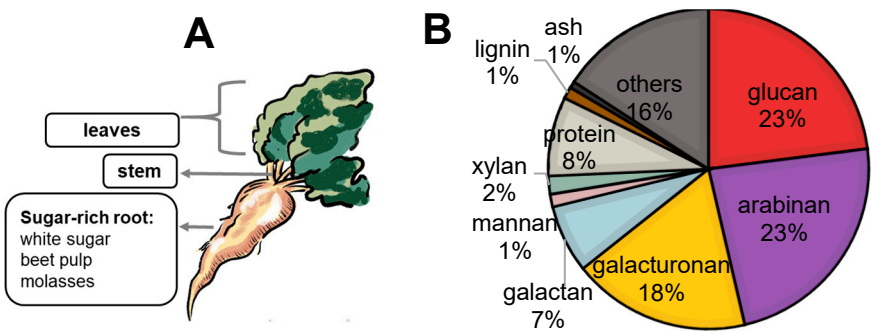


Fig. 4.2 (A) Schematic illustration of the parts of a sugar beet plant. (B) Structural composition of sugar beet pulp from campaign 2016/2017 given by Nordic Sugar A/S. Adapted from *Paper I*.

4.2.2 Wheat bran composition

Wheat bran comprises the outer layers of the wheat grain (aleurone and hyaline layers, testa and the inner and outer pericarp) and the remnants of starch after wheat extraction [135] (**Fig. 4.3A**). Structurally, wheat bran consists of 14–25% starch, ca. 11% cellulose, 11–26% hemicelluloses, 13–18% protein and ca. 5% lignin [46]. Precise compositional values of the bran vary depending on the time and location of wheat cultivation as well as the wheat processing methods. **Fig. 4.3B** presents the composition of the batch of wheat bran received from Lantmännen AB (Sweden) in 2020.

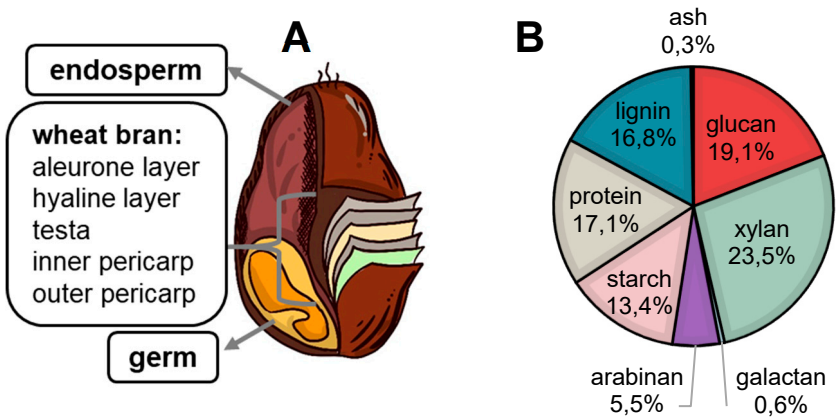


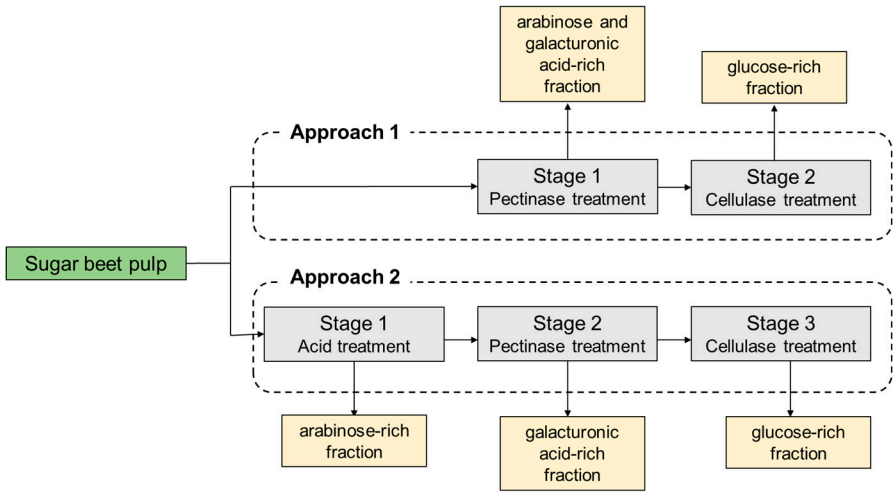
Fig. 4.3 (A) Schematic illustration of the components of a wheat grain. (B) Composition of wheat bran given by Lantmännen. Adapted from *Paper II*.

The cellulose component, represented by glucan, comprised 19.1% of wheat bran (on dry-weight basis), 29.6% of the bran was hemicellulose, represented by xylan, arabinan and galactan, 16.8% was composed by lignin, 17.1% was protein, and the last major component was starch which constituted 13.4% of the bran. Minor constituents of bran include ferulic acid and lipids [22, 46].

4.3 Fractionation of the treated biomass

4.3.1 Fractionation of sugar beet pulp

Preliminary studies consisting of both enzymatic and acid treatment of SBP culminated in the investigation of two fractionation approaches (**Fig. 4.4**) which aimed to fractionate the main sugar constituents in separate stages of fractionation. Approach 1 is a two-stage enzymatic treatment consisting of pectinase followed by cellulase treatment. Approach 2 is a three-stage process starting with acid treatment, followed by pectinase then cellulase treatments.



Fig, 4.4 Sugar beet pulp fractionation approaches: Approach 1: two-stage fractionation–pectinase treatment and cellulase treatment; Approach 2–three-stage fractionation: acid treatment, pectinase treatment, cellulase treatment.

Each stage of treatment generated two fractions: solid (SF) and liquid (LF) fractions which were then subjected to compositional analysis. The liquid fractions after each treatment stage contained the targeted solubilised monomer constituents. More detailed compositional analysis results are published in *Paper I.*

The results revealed that the three-stage treatment (Approach 2) was more ideal, since the liquid fractions after each stage contained majority of each target monomer constituent. The results of the analyses of samples from the treated biomass according to second fractionation strategy Approach 2, are summarised in **Table 4.1**. Acid treatment (Stage 1) solubilised 71.3 % of the initial arabinose content, pectinase treatment (Stage 2) solubilised 68.5% of the initial galacturonic acid content, and finally cellulase treatment (Stage 3) released 81.9% of initial glucose content. The resulting hydrolysate streams were not pure but the relatively selective solubilisation of the main SBP components in separate streams gives a fairly good start for valorisation.

Table 4.1. Compositional analysis of SBP and the process streams from each stage of fractionation Approach 2. SF=solid fraction, LF=liquid fraction

Sample	SBP	Acid-treated		Pectinase treated		Cellulase treated	
Carbohydrates,	(wt%, on dry weight basis)	SF (%)	LF (g/L)	SF (%)	LF (g/L)	SF (%)	LF (g/L)
Glucan	23.0	11.4	0.9	21.6	0.0	11.6	24.9
Arabinan	23.4	6.4	10.4	0.4	0.6	9.8	n.d.
Galacturonan	17.9	9.3	n.d.	n.d.	20.6	5.0	3.0
Galactan	6.9	1.5	2.5	n.d.	0.6	7.8	0.0
Mannan	1.3	0.5	2.3	n.d.	1.2	n.d.	1.0
Xylan	1.8	0.6	0.5	2.3	n.d	1.7	1.7
Protein	8.1	2.6	2.0	16.0	2.5	28.5	0.6
Lignin	1.2	2.8		19.8		49.7	
Ash	0.7	3.3				0.5	

4.3.2 Fractionation of wheat bran

The fractionation strategy for the wheat bran feedstock initially aimed to separate the five major components (starch, protein, hemicelluloses, lignin, and cellulose) into different streams. **Fig. 4.5**-Approach 1 shows the four-stage fractionation of wheat bran, which intended to solubilise each component of the bran through each processing stage: de-starching for starch extraction, de-proteination, for protein extraction, steam explosion for the solubilisation of hemicelluloses, and hydrotropic treatment for lignin extraction. In comparison, Approach 2 comprised of steam explosion followed by hydrotropic extraction, while Approach 3 consisted of only hydrotropic extraction. At the end all of the sequentially treated wheat bran as well as the raw material was subjected to cellulase treatment.

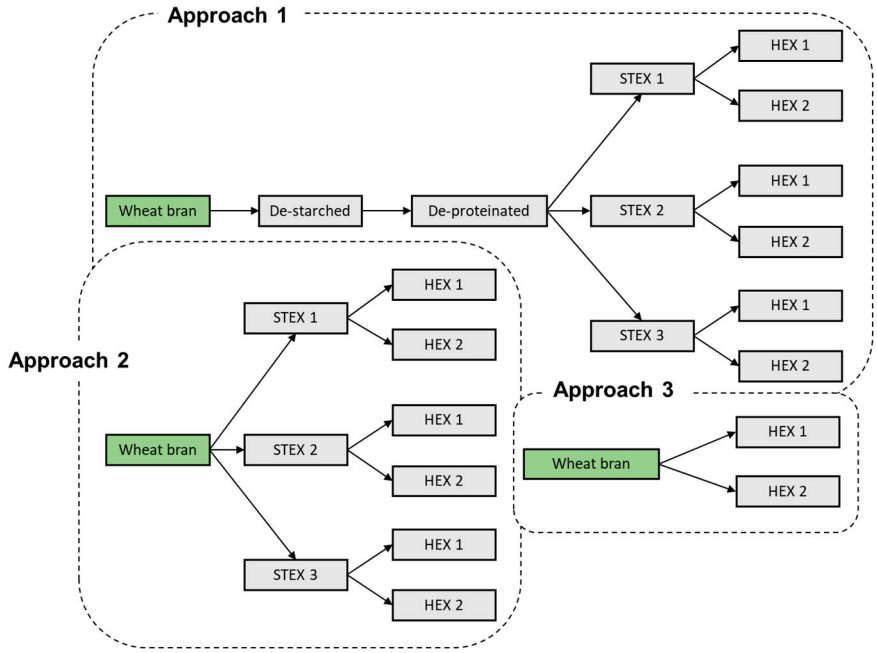


Fig. 4.5. Investigated fractionation approaches for wheat bran. Approach 1: de-starching, de-proteination, steam explosion (STEX) and hydrotropic extraction (HEX); Approach 2: steam explosion, hydrotropic extraction; Approach 3: hydrotropic extraction.

Table 4.2 summarises the results from the first fractionation strategy for wheat bran, which included de-starching, de-proteination, steam explosion (condition #1: 218°C and 11 min) and hydrotropic extraction (condition #1: 170°C, 1 h). Further details of the results as well as the specifications of each experiment are available in *Paper II*.

It is evident that target components were released as intended after each processing stage. For instance, only 0.1% starch was left in the solid fraction after de-starching, while 25 g/L glucose stemming most likely from starch was quantified in the liquid fraction. The protein content, although did not completely disappear, was down to 9.7% after de-proteination. The hemicelluloses, mostly xylan and arabinan, were down to 2.2% and 0.7%, respectively after steam explosion. After hydrotropic extraction, 37.1% of the solid fraction still accounted for lignin, but as observed here, the hydrotropic solution contained 16.1 g/L lignin indicating its solubilisation.

Table 4.2. Compositional analysis of WB and the process streams after each stage of treatment using fractionation Approach 1, including de-starching, de-proteination, steam explosion condition #1 (218°C, 11 min), hydrotropic extraction condition #1 (170°C, 1h). SF=solid fraction; LF=liquid fraction

Sample	WB	De-starched		De-proteinated		Steam exploded		Hydrotropically-treated	
Carbohyrates,	(wt%)	SF (%)	LF (g/L)	SF (%)	LF (g/L)	SF (%)	LF (g/L)	SF (%)	LF (g/L)
Glucan	22.9	23.2	25.0	25.0	0.4	21.0	0.9	34.5	0.1
Xylan	23.6	28.2	1.4	30.8	0.1	2.2	5.1	2.3	0.2
Galactan	0.5	1.3	0.5	1.5	0.03	0.2	0.5	0.3	0.1
Arabinan	8.1	5.5	0.7	13.9	0.1	0.7	6.1	0.5	0.2
Mannan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Starch	13.9	0.1		0.6		n.d.		n.d.	
Protein	17.7	16.4		9.7		10.3		13.0	
Lignin	17.4	18.1		17.2		52.9		37.1	16.1
Ash	0.3	0.3				n.d.			

5 Valorisation of the glucose stream via 5-HMF (*Papers III* and *IV*)

C_6 sugars arising from lignocellulosic biomass are primarily composed of glucose, and minor ones including galactose, and mannose. The release of monomeric glucose units is amongst the main objectives of fractionating the lignocellulosic biomass. Chapter 5 deals with the studies performed during the PhD work on the valorisation of glucose and its isomer fructose via 5-HMF into levulinic acid (*Paper III*) and upgrading into C_{12} furan building blocks (*Paper IV*), as illustrated in **Fig. 5.1**.

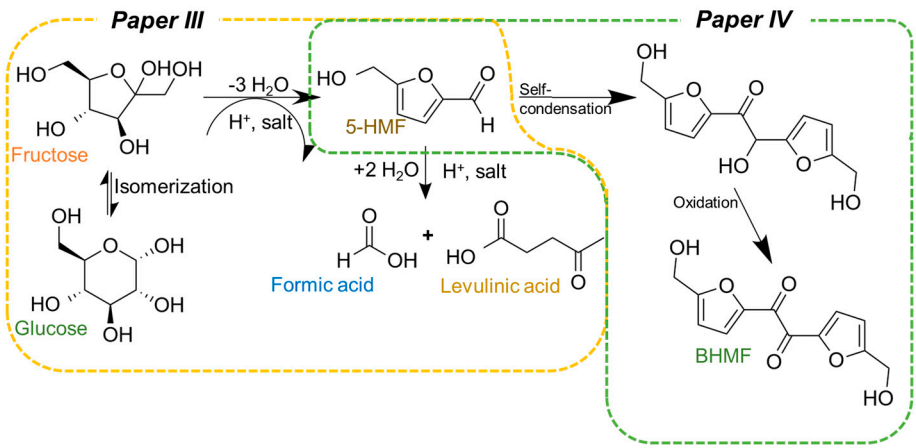


Fig. 5.1. Overview of the valorisation work from glucose/fructose to levulinic acid via 5-HMF pathway (*Paper III*) and carboligation of 5-HMF into C_{12} compounds 5,5′-bis(hydroxymethyl furoin (DHMF) and 5,5′-bis(hydroxymethyl)furil (BHMF) (*Paper IV*)

5.1 Levulinic acid production from fructose and glucose

Fructose is a preferred starting material for the production of 5-HMF and its derivatives like levulinic acid [88]. Fructose is a component of sucrose (beet or cane sugar), which is a disaccharide of glucose and fructose. Inversion of sucrose using the enzyme invertase

or an ion-exchange resin results in an equimolar mixture of the monomers [136, 137]. Such a mixture is also obtained by isomerisation of glucose via glucose isomerase, a well-known process being performed industrially [136, 138]. Separation of fructose and glucose is possible by ion-exchange chromatography on an Ca^{2+} charged ion exchange resin to yield two streams enriched (to 90%) in the monomers [139].

In *Paper III*, production of LA from glucose and fructose, respectively, was investigated using a heterogeneous catalyst heterogeneous catalyst, acidic ion-exchange resin (Dowex DR-2030). This resin has earlier been used in our laboratory for the dehydration of fructose to 5-HMF in DMSO [92]. Our understanding is that DR-2030 catalyses the isomerisation of glucose into fructose via an enediol-ion exchange complex, as observed with other heterogeneous catalysts [140]. The fructose is then dehydrated to 5-HMF and subsequently rehydrated in an aqueous system to form levulinic acid and formic acid (**Fig. 5.1**).

The results from the study indicated that levulinic acid production was enhanced by the inclusion of salt in the reaction medium. Reaction with fructose (100 mg/mL) in the presence of 200 mg/mL NaCl at 110 °C and catalyst concentration of 30 g/L resulted in complete substrate conversion with 75% yield of levulinic acid after 12 h. Reaction with glucose required longer time (24 hours) and higher temperature (145 °C) for complete sugar conversion and LA yield of 71%. Similar results have been observed for xylose degradation to furfural being enhanced by chloride salts [104, 141].

The effect of the salt was related to the anions in the order of $Cl^{-}>CO_3^{2-}>SO_4^{2-}$. The chloride ions may have had stabilising effects of the cationic (monovalent C^{+}) intermediates in the dehydration reaction [104]. Additionally, pH reduction was observed with the addition of the salts, with most decrease seen by adding 10% w/v NaCl, from pH 2.98 to 0.78. This indicates that the presence of chloride ions promoted the release of H^{+} from the ion-exchanger catalyst, which in turn enhanced the dehydration of the sugars to levulinic acid.

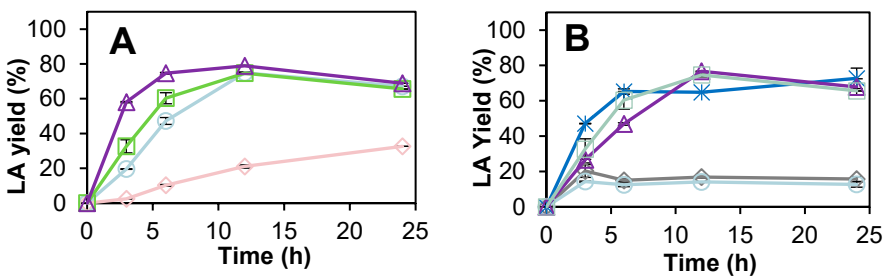


Fig. 5.2. Effects of salts on the heterogeneous catalysis of fructose to levulinic acid. (A) Levulinic acid yield as a function of time at different salt concentrations: 0(◇), 50 (○), 100 (□), 200 mg/mL NaCl (△). (B) Levulinic acid yield as function of time with inclusion of different types of salt: NaCl (□), Na_2CO_3 (◇), Na_2SO_4 (○), $CaCl_2$ (*) and KCl (△). Adapted from *Paper III*.

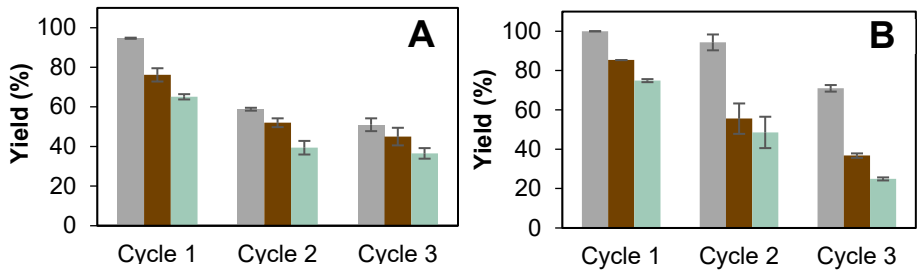


Fig. 5.3. Reusability of the ion-exchange catalyst for three successive reaction period (0.3 w/w to sugars in 1 w/w NaCl to sugars ratio for dehydration reactions) in (A) 1.1 mmol fructose at 110°C, and (B) 1.1 mmol glucose at 145°C. FA=formic acid; LA=levulinic acid. Adapted from *Paper III*.

The stability and reusability of catalysts is crucial for process feasibility and scale-up potential. The stability of the ion-exchanger catalyst was thus investigated by reusing the catalyst 3 times for the dehydration of fructose (**Fig. 5.3A**) and glucose (**Fig. 5.3B**). The trend was clear that after each reaction cycle, product yield as well as substrate conversion decreased. Although the catalyst was still stable even after the three consecutive cycles, partial deactivation lowered the substrate conversion and product yield, most likely as a result of the adsorption of the side-products of the reactions, such as humins. An elemental analysis of a similar strong cation sulfonated copolymer resin revealed an increase in C and H and decrease in S, suggesting that products adsorbed to the resins during previous reactions were stable enough to withstand washing [142]. This prompts further development of methods for increasing stability and reusability of ion-exchange resins.

5.2 Upgrading of 5-HMF to C_{12} compounds

The first report by Liu *et al.* on the use of ionic liquid 1-ethyl-3-methylimidazolium acetate which is also a N-heterocyclic carbene (NHC) for the selective conversion of 5-HMF into 5,5-bis(hydroxymethyl)furoin (DHMF) prompted further studies on the use of other NHCs for DHMF production [114, 115]. Thiamine (Vit. B_1), a natural NHC [118], together with the thiamine diphosphate (ThDP)-dependent enzyme benzaldehyde lyase (BAL) has been shown to catalyse 5-HMF carboligation into DHMF, with an added observation that 5,5'-bis(hydroxymethyl)furil (BHMF) was also produced [123].

5.2.1 Whole cell catalysis for carboligation of 5-HMF

The biological approach was chosen in *Paper IV* for the upgrading of 5-HMF employing ThDP-dependent enzyme BAL. Recombinant *Escherichia coli* cells expressing the enzyme BAL were used to catalyse the conversion of 5-HMF into DHMF, which was allowed to spontaneously oxidise to BHMF.

Several parameters for the whole-cell catalysis of 5-HMF, including bio-based co-solvents, substrate and cell concentration, and stability agents were investigated in *Paper IV*. The most optimal condition achieved (10% dimethyl carbonate as co-solvent, 5 g/L initial 5-HMF, 2 g_{cdw}/L cell catalyst) generated a reaction profile (**Fig. 5.4**) in which DHMF was formed maximally within 1 h of reaction which then subsided gradually and converted into BHMF until 72 h of reaction.

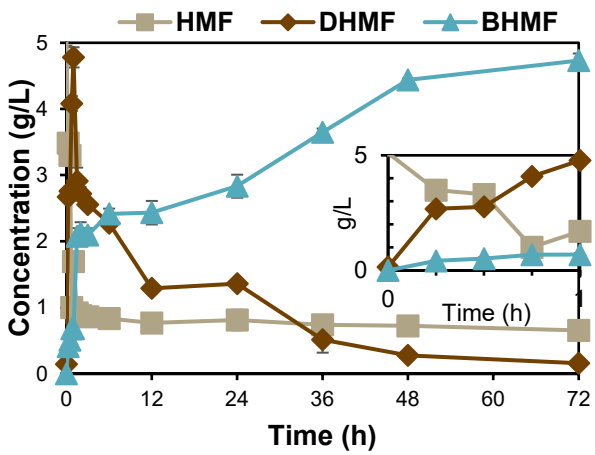


Fig. 5.4. Standard reaction profile of whole-cell catalysed carboligation of 5-5-HMF into DHMF, followed by slow but spontaneous oxidation to BHMF. Adapted from *Paper IV*

The reaction was performed in a fed-batch mode in which the equal amounts of the substrate were added after 1 hour of reaction. A maximum DHMF titre of 52 g/L was achieved after feeding five times of 20 g/L 5-HMF (total of 100 g/L) (**Fig. 5.5**). It was clear that the highest 5-HMF conversion and DHMF formation occurred during the first batch and declined progressively during subsequent feeds, indicating some form of inhibition.

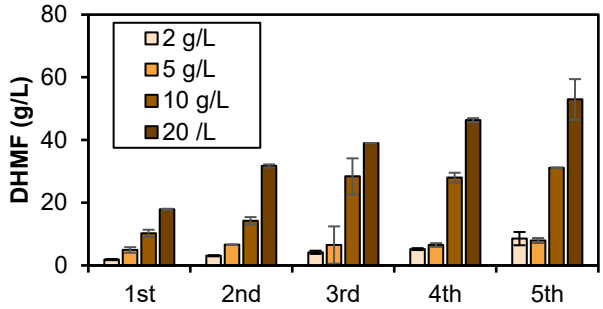


Fig. 5.5. Fed-batch experiments for the carboligation of 5-HMF using 2, 5, 10 and 20 g/L initial 5-HMF concentration 2 g_{cdw}/L BAL, 30°C, 50 mM KH_2PO_4/K_2HPO_4 buffer pH 8.0, 2.5 mM $MgSO_4$, 0.1 mM ThDP, 10% dimethylcarbonate. Adapted from *Paper IV*.

Substrate or product inhibition was suspected after the reduction of DHMF yields in the cell recycling experiments (**Fig. 5.6A**). A single cycle constituted 1 h of reaction, thereafter the cells were separated from the medium and re-used on a fresh 5-HMF medium. The trials observed more decrease in DHMF yield on the second and third cycles of the cell catalysts at higher substrate concentration (10 g/L) than in 5 g/L). The BHMF yields however remained low even in the subsequent cycles (**Fig. 5.6B**) which is attractive when the focus is maximal production of DHMF, as the furoin appears quickly.

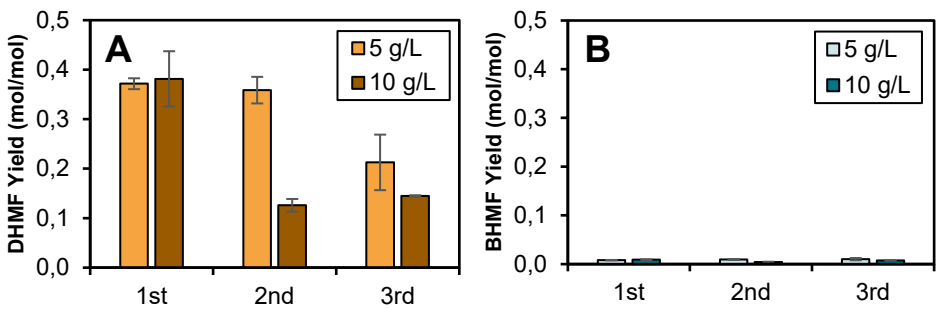


Fig. 5.6. Cell catalyst recycling for three consecutive batch reactions. (A) DHMF yield (mol/mol) at initial 5-HMF concentrations 5 g/L and 10 g/L. (B) BHMF yield (mol/mol) at initial 5-HMF concentrations 5 g/L and 10 g/L

When the purpose is to produce the furil BHMF, an optimisation involving enhanced air or oxygen sparging may be required as it has been observed in the research work that air availability had an effect on the speed of the oxidation of DHMF to BHMF.

5.2.2 Purification of the C_{12} furan products

A simple precipitation-based purification was developed for the two dimer products. This involved repeated liquid-liquid extraction into ethyl acetate, pooling the organic phase and evaporating it until a brownish viscous liquid was left. A mixture of ethyl acetate and heptane (65:35) was then added to the viscous liquid until a white precipitate (DHMF) was formed. Meanwhile, BHMF was left in the ethyl acetate-heptane mixture, which was evaporated, leaving a bright yellow powder of BHMF. H^1 NMR analysis of the purified DHMF and BHMF showed 96.7% and 98.1% purity, respectively.

5.2.3 Hydrazone formation

A potentially novel application for both DHMF and BHMF was demonstrated through their ability to form hydrazones. The reactivity of the keto groups present in these C_{12} compounds can be functionalised through cross-link generation. A direct application of this capability would be enhanced performance of commercial products, e.g. polyurethane coatings and specialised polymers [73, 107-109].

Adipic acid dihydrazide (ADH) was used to determine hydrazone-forming capability of the C_{12} compounds. **Fig. 5.7** shows the structure of ADH characterised by the $-CONHNH_2$ groups. The $-NH_2$ function of the hydrazide group reacts with the ketone functional group of either DHMF or BHMF in a condensation resulting in a hydrazone molecule.

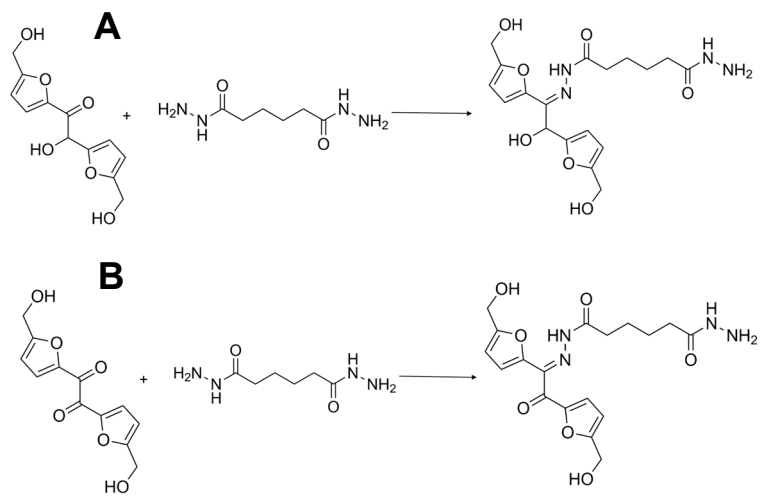


Fig. 5.7. Hydrazone formation, (A) DHMF and adipic acid dihydyrazide forming DHMF-based hydrazone, (B) BHMF and adipic acid dihydyrazide forming BHMF-based hydrazone

pH-sensitivity which is characteristic of hydrazone compounds may see potential applications in the pharmaceutical industry. One study reports on the use of hydrazones as biodegradable carriers for intracellular drug delivery [107]. Another promising feature of hydrazones is the ability to coordinate metal ions [143]. Metal-containing polyurethanes have previously been shown to exhibit antimicrobial activity, such was the case with Schiff base incorporated with transition metals [144]. Enhanced antimicrobial activity and flame retardancy was observed when a polyurethane coating was mixed with o-methoxybenzaldehyde benzoylhydrazone ligand and its metal complexes [143]. The potential of hydrazones for providing antimicrobial activity and flame-retardancy in a paint formulation without using the classical organotin compounds, mostly tributylin, that are harmful to marine eco-systems, would be useful [145].

Hydrazone formation was demonstrated by reacting the pure DHMF and BHMF with adipic acid dihydrazide. The preliminary analysis using Fourier Transform Infrared Spectroscopy (FTIR) showed some variation on the target functional groups =O before and after reacting DHMF and BHMF with adipic acid dihydrazide. The hydrazones formed were then verified through H^1 NMR (*Paper IV*).

5.3 Other potential sugar valorisation routes

Paper I suggested three valorisation routes, levulinic acid produced from glucose, arabitol produced from arabinose and mucic acid produced from galacturonic acid. *Section 3.2* discussed the relevance of levulinic acid. Arabitol, like levulinic acid, is also included in DoEs top value bio-based chemicals due to the industrial potential of the compound and its derivatives [57]. Yeast fermentation was suggested for the valorisation of arabinose. With the advantage of selectivity to the substrate and the possibility of acid tolerance, subsequent purification of arabinose from the acid hydrolysate generated by the acid treatment of SBP may potentially be circumvented [146].

Mucic acid, or galactaric acid, was suggested to be valorised from the galacturonic acid stream. In a short parallel study, mucic acid was produced from the galacturonic acid-enriched stream generated from the pectinase treatment of acid-hydrolysed SBP, using a procedure described previously by Vastano *et al.* based on a laccase-TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) system [147].

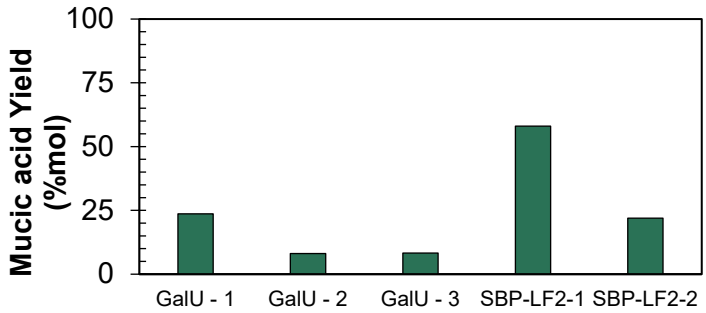


Fig. 5.8. Mucic acid yield from galacturonic acid via laccase-TEMPO system as described by Vastano et al. [147]. GalU-1 = 100 mmol galacturonic acid, citrate buffer, 0.02 mmol TEMPO, 4 g/L laccase; GalU-2 = 100 mmol galacturonic acid, acetate buffer, 0.02 mmol TEMPO, 4 g/L laccase; GalU-3 = 100 mmol galacturonic acid, citric acid buffer, 0.01 mmol TEMPO, 4 g/L laccase; SBP-LF2-1=liquid fraction after the pectinase treatment preceded by acid treatment (*Paper I*) with 100 mmol GA, 0.02 mmol TEMPO, 4 g/L laccase; SBP-LF2-2=liquid fraction after the pectinase treatment preceded by acid treatment (*Paper I*) with 100 mmol GA, 0.01 mmol TEMPO, 4 g/L laccase

6 Feasibility evaluation of the integrated biorefinery design

The scientific literature provides extensive list of promising bio-based production strategies utilising waste biomass streams for production of bulk/fine/specialty chemicals. However, as of date, there is no widespread implementation and commercialisation of bio-based processes which seriously competes with the petrochemicals. This chapter aims to shed light on how by-product-based biorefineries could be driven further into real-life applications.

6.1 Technical assessment

Technical evaluation of novel processes is generally required prior to upscaling. *Paper I* presented a proposed block-flow diagram of the sugar beet pulp-based biorefinery (**Fig. 6.1**). The experimental part of the study was grouped in the Hydrolysis system block, while the valorisation of the three resulting streams was grouped in the Valorisation system block. For commercialising the proposed products, levulinic acid, arabitol and mucic acid, their purification to a marketable form is needed as depicted in **Fig. 6.1**. The solid residue left after the extraction of the three main monomer constituents, glucose, arabinose and galacturonic acid, can be treated in an in-house anaerobic digestion system where biogas can potentially be produced. Since biotechnological processes are generally water-intensive, it is crucial to have a wastewater treatment system where the process water is reused. Finally, it is paramount that the utilisation of energy in the biorefinery is optimised, as depicted in the combined heat and power plant system, where heat exchanger networks could be designed for cross-utilisation of heat within the unit operations of the biorefinery network.

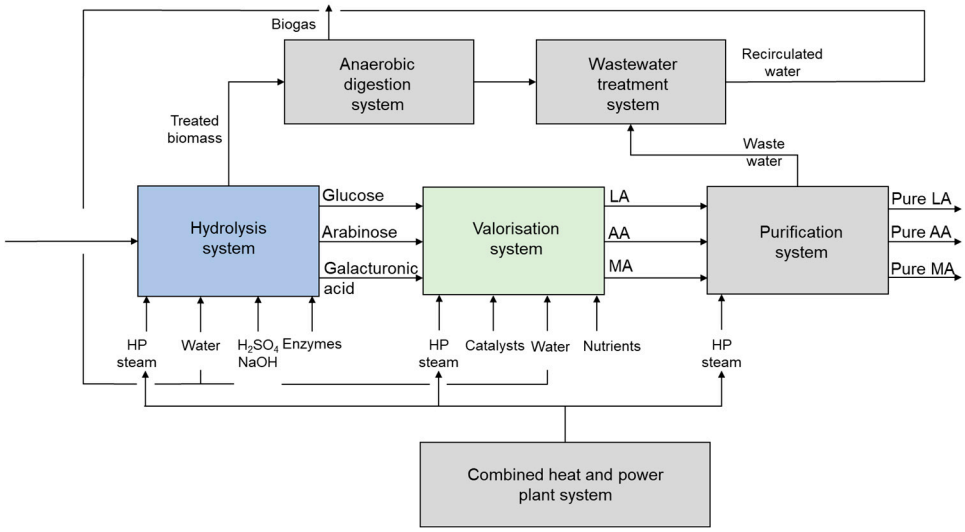


Fig. 6.1 Box-flow diagram of the proposed sugar beet pulp-based biorefinery via valorisation of the three main monomer SBP components; LA=levulinic acid, AA=arabitol, MA=mucic acid. Adapted from *Paper I*.

6.2 Aspen simulation

Aspen Plus ® V10 software (Aspen Technology, Bedford, MA, USA) is one of the leading process simulation softwares used by chemical industries worldwide. The program was used to simulate the experimental process proposed for the hydrolysis and valorisation systems of the SBP-based biorefinery. **Fig. 6.2** shows the Aspen flowsheet for the hydrolysis system containing the acid hydrolysis unit operation (AH) generating arabinose-rich stream, pectinase treatment (EH1) releasing the galacturonic acid-rich stream, and finally cellulase treatment (EH2) where glucose-rich stream is produced. Further processing of the monomer-rich streams is performed in the valorisation system where arabinose undergoes yeast fermentation (YF) to produce arabitol [146], galacturonic acid undergoes laccase-based oxidation (OC) into mucic acid [148], and glucose undergoes heterogeneous catalysis (HC) into levulinic acid (*Paper I*).

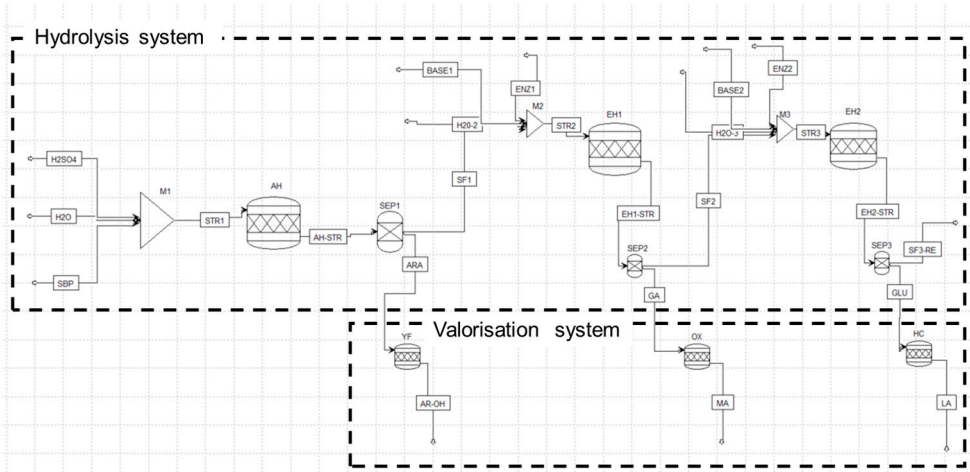


Fig. 6.2. Aspen Plus® flowsheet for the hydrolysis and valorisation systems of the sugar beet pulp-based biorefinery. Adapted from *Paper I*.

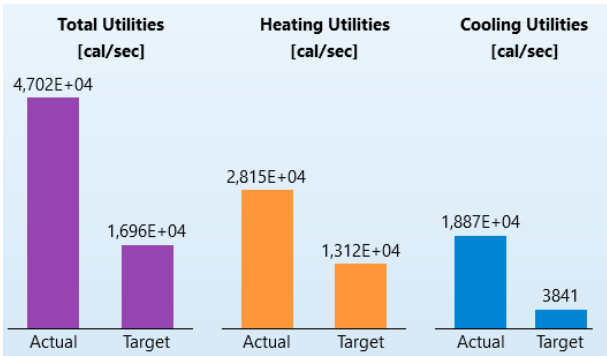
The specifications for the simulation for the most important unit operations are summarised in **Table 6.1.** It should be noted here that the yields and reaction specifications for the hydrolysis system were based on experimental data from *Paper I,* whereas the information used for the valorisation system were obtained from published literature for the arabitol yield from arabinose using *Candida auringiensis* [146] and levulinic acid yield from as described in *Paper III.* The mucic acid yield value was obtained from an experimental data (**Fig. 5.8**) using the galacturonic acid-enriched stream from *Paper I* based on a laccase-TEMPO system, a method described previously [147].

Table 6.1. Specifications for the unit operations used in the process simulation in Aspen Plus ®

Stage	Abbrev.	Unit model	Specs.	Reactions: fractional conversion
Hydrolysis system				
Acid treatment	AH	RStoic	80 ° C, 1 atm	0.71 arabinan to arabinose
Pectinase treatment	EH1	RStoic	50 °C, 1 atm	0.69 galacturonan to galacturonic acid
Cellulase treatment	EH2	RStoic	50 °C, 1 atm	0.82 cellulose to glucose
Valorisation System				
Yeast fermentation	YF	RStoic	25 °C, 1 atm	0.73 arabinose to arabitol [146]
Laccase treatment	OX	RStoic	30 °C, 1 atm	0.63 galacturonic acid to mucic acid (**Fig. 5.8**)
Ion-exchange catalysis	HC	RStoic	145 °C, 1 atm	0.71 glucose to levulinic acid (*Paper I*)

An important indicator that was generated from the Aspen simulation was the estimation of the energy needed for upscaling the process at 1000 kg/h SBP (dry-basis) feed. According to the preliminary simulation of the conceptual biorefinery, a total of 6432 MJ/h is needed for processing. It should be noted here that lab-scale data was used as basis for the simulation therefore the energy value is over-estimated. In comparison, a complete process simulation of 1000 kg/h wheat straw valorisation to ethanol required 2160 MJ/h [149].

Fig. 6.3 presents the possibilities for optimising energy utilisation for the two systems of the SBP-based biorefinery. This is possible through optimisation of the heat exchanger networks in the system via energy redistribution between heating and cooling utilities. Very early estimates of the process analysis showed about 64% decrease of total energy input by energy optimisation using Aspen Plus Energy Analyzer ®. It should be noted here that these are preliminary estimated values. A complete energy optimisation analysis involving optimal heat exchanger networks in a more complete technical simulation with detailed unit operations is needed for further evaluation.



Summary Table

	Actual	Target	Available Savings	% of Actual
Total Utilities [cal/sec]	4,702E+04	1,696E+04	3,006E4	63,93
Heating Utilities [cal/sec]	2,815E+04	1,312E+04	1,503E+04	53,39
Cooling Utilities [cal/sec]	1,887E+04	3841	1,503E+04	79,64

Fig. 6.3. Potential for energy optimisation of the process through Aspen Plus Energy Analyzer ®.

Several indicators can be obtained from a technical assessment at an early stage of a novel process. A preliminary analysis was performed based on the material input/output values of the experimental data and literature values of the conceptual SBP-based biorefinery.

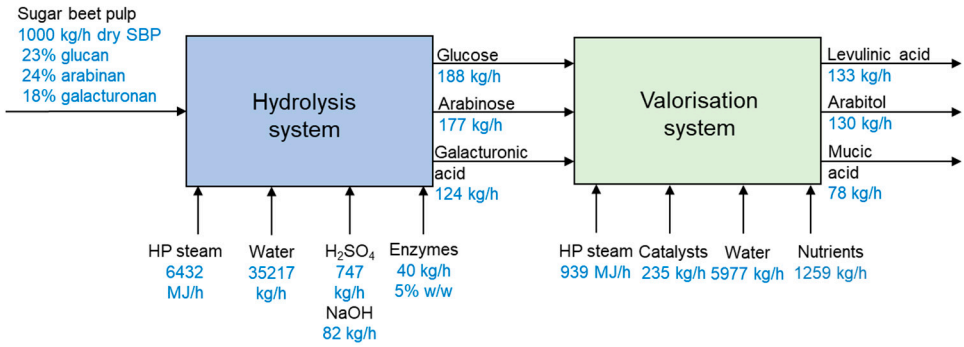


Fig. 6.4. Mass and energy flows for the hydrolysis and valorisation system in the proposed sugar beet pulp-based biorefinery with an input capacity of 1000 kg/h dry SBP. Adapted from *Paper I*.

Mass and energy indicators provide some general information on a process. For the proposed sugar beet pulp-based biorefinery, **Fig. 6.4** presents the material flows in the system with 1000 kg/h dry SBP input capacity. Mass and energy indicators as summarised in **Table 6.2** obtained from the values for the flow of materials.

Table 6.2. Mass and energy indicators for sugar beet pulp-based biorefinery, calculated per tonne dry SBP (*Paper I*)

Indicator	Value	Unit
Monomer Yields		
Glucose	188.6	kg/t SBP
Arabinose	177.5	kg/t SBP
Galacturonic acid	123.5	kg/t SBP
Valorisation Products		
Arabitol	129.5	kg/t SBP
Mucic acid	77.8	kg/t SBP
Levulinic acid	133.9	kg/t SBP
Total Product Yield	341.3	kg/t SBP
Total Material Consumption	47 218	kg/h
Mass Intensity	139.4	kg input/ kg product
Specific Energy Consumption	7.4	MJ/kg SBP
E-factor	127.9	kg waste/kg product
E-factor (with water recirculation and enzyme/catalyst immobilisation	6.4	kg waste/kg product

The mass intensity indicator value of 139.4 kg input per kg product is an important indicator indicating the magnitude of material going into the process to produce 1 kg of product. In this case, up to 87% of the input material is water. Water-intense

processing is characteristic of biotechnological-based processes, and in this case amplified by the high-water content of the beet pulp. It is therefore crucial to have an efficient water recirculation for this process to be feasible. This was reflected in the E-factor, a recognised green chemistry indicator which gives insight on waste generation per kg product [150]. Without water recirculation of process materials the E-factor is 127.9, which is already higher than the range of E-factors for the pharmaceutical industry (**Table 6.3**). However, with water recirculation as well as enzyme/catalyst immobilisation, the E-factor for the conceptual SBP-based biorefinery decreased to 6.4 – which is at the lower end of the E-factor range for the fine chemical industry [150].

Table 6.3. E-factors in the chemical industry [150]

Chemical industry type	Volume/tonnes Per annum	E-factor (kg waste per kg product)
Bulk chemicals	$10^4 – 10^6$	< 1–5
Fine chemicals	$10^2 – 10^4$	5 ≥ 50
Pharmaceuticals	$10 – 10^3$	25 ≥ 100

6.3 Preliminary economic assessment

Benchmarking tools provide valuable strategies for evaluating and screening novel processes prior to performing more cumbersome full techno-economic assessment [151]. At a very early stage such as the proposed thousand-kilogram scale biorefinery process based on studies performed at lab-scale, early economic indicators can help justify funding for further evaluation.

One such tool is a so-called stoichiometric-economic indicator that couples the price of the products and reactants of a process. Used for early decision-making when limited data is available, an early economic indicator could shed light whether a proposed process has potential for economic viability. The metric for inspecting sales and reactants (MISR) as shown in Eq. 1, is an example of this early economic indicator [151].

$$MISR = \frac{\sum_{p=1}^{N_{product}} Annual\ production\ rate\ of\ product\ p * Selling\ price\ of\ product\ p}{\sum_{r=1}^{N_{reactant}} Annual\ feed\ rate\ of\ reactant\ r * Purchased\ price\ of\ reactant\ r} \qquad \text{Eq.1}$$

The assessed process may be considered for further evaluation at MISR>1, with minimum value of 1. Alternatively, for MISR values ≤1 the process is not economically viable. The higher the MISR value, the more desirable is the process [151].

Fig. 6.5 shows the MISR assessment plot for the proposed sugar beet pulp-based biorefinery. The dotted line denotes MISR=1 and thereby the threshold for the economic potential of the process. Since the purification was not included in the technical assessment for the process and the fact that purification generally accounts for a major chunk of production costs in chemical processes [152], a range of 10–50% of the total selling costs was allotted for the purification stage. At 10% allotted cost of purification, the proposed process becomes unfeasible at SBP cost over 2276 US$/t, whereas at the maximum of 50% purification allotment, the process loses its potential for viability at SBP cost over 1140 US$/t.

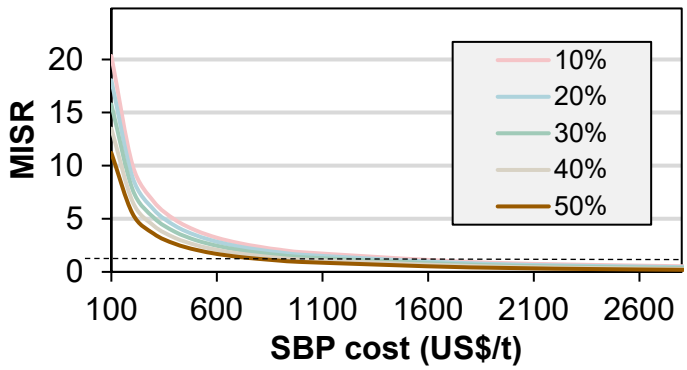


Fig. 6.5. Metric for inspecting sales and reactants (MISR) for screening economic potential of the proposed SBP biorefinery. Adapted from *Paper I*.

This is a satisfactory margin given that SBP is sold as animal feed in Sweden between 150-200 US$/t [153]. The satisfactory result of the preliminary economic assessment through MISR therefore warrants further techno-economic evaluation for confirmation of process viability and a prospect on profitability.

In addition to techno-economic analyses and profitability predictions, an evaluation of the sustainability performance of proposed novel processes should be included. Life-cycle assessment (LCA) provides the toolkit for the quantification of overall environmental impact indicator, thereby strengthening the case of process sustainability [154, 155]. The tools for overall process evaluation as described here will be valuable for encouraging the growth of biorefineries in the chemical industry.

7 Conclusion

This thesis has demonstrated possible technological solutions for a resource-efficient and sustainable valorisation of biomass feedstocks from Swedish agricultural farms, which fulfils the original aim of the research.

While the main focus of the thesis resided on the glucose fraction of the biomass for the production of furan derivatives, the fractionation of biomass residues results in other streams with promising prospects for upgrading. This was presented in *Paper I*, where the three main monomer constituents of the biomass polysaccharides, glucose, arabinose and galacturonic acid, were almost selectively released in separate streams, which essentially created three possible commercial products. Levulinic acid, arabitol and mucic acid were suggested in the paper, however other useful products can also be produced from these monomer-enriched streams. Although this approach is in its infancy, an attempt to simulate the process with limited amount of data was presented in the preliminary feasibility study. The early-stage economical benchmarking tool used showed potential viability for the process and thus a more elaborate techno-economic assessment is recommended.

Since the composition of agricultural residues vary from one plant species to the other, each feedstock needs tailored processing strategy. This was evident in how wheat bran was fractionated compared to sugar beet pulp. After the attempt of releasing three components from sugar beet pulp, the fractionation approach for wheat bran sought to fractionate even more, and therefore the strategy was designed so that five of the components (starch, protein, hemicelluloses, lignin and cellulose) could be released separately. In *Paper II*, the focus was mostly on the sequential steam explosion and hydrotropic extraction, releasing hemicelluloses and lignin, respectively, as starch hydrolysis and protein extraction have already been extensively studied. It was clear that each component is released at the intended stages, almost selectively, especially the hemicelluloses being almost solubilised after steam explosion. Lignin solubilisation and recovery remain a challenge for the fractionation, although up to 43% of the initial lignin was recovered using this method, under the current best condition. Other hydrotropes should be investigated further for this purpose with the same ease of lignin recovery as SXS. Since there are potentially several streams to be valorised in this five-

stage fractionation strategy, a techno-economical assessment would be worthwhile for evaluating whether producing variety of potentially high-value products would offset possible losses in lower yields of solubilisation.

Moving on to the valorisation of the glucose stream via 5-HMF, *Paper III* showed enhancement of levulinic acid production from fructose and glucose using a heterogeneous cation exchange catalyst in the present of salt. Under optimal conditions, over 70% levulinic acid was obtained from glucose, and over 74% from fructose. While heterogeneous catalysts provide better upscaling prospects for sugar dehydration, compared to the corrosive mineral acids, further work on increasing the stability and recyclability of the catalysts is needed. For instance, humin fouling on catalyst surface should be addressed, as this contributes to reduced reactivity of the catalyst. Eventually, using such a catalyst for levulinic acid production directly from cellulose would be interesting to study.

Yet another case of valorisation was demonstrated in *Paper IV*, where the novel C_{12} compounds building blocks, DHMF and BHMF, were produced, offering promising potential for specialised applications in polyurethane, polyester and jet fuel industries. *Paper IV* proposed a mild and cost-effective approach for upgrading the 5-HMF into these C_{12} compounds via whole cell catalysis. Without the need for enzyme purification from the cells and no prior 5-HMF purification makes this process potentially cost-effective and could offer noteworthy possibility for integration with biomass processing.

Future prospects and outlook

High capital cost is typically the bottleneck for novel processes entering the market, especially biotechnological processes, which are generally water-intensive with low substrate loadings. Therefore, it is important that when designing sustainable chemical production routes from carbon-neutral feedstocks, a process design with upscaling perspectives and cost competitiveness should be prioritised.

An exploration of other possible commercial products from the biomass, such as the protein fraction of the feedstocks as alternative protein source for humans [58]. Extraction of ferulic acid from sugar beet pulp and wheat bran also merits further study as this compound offers broad range of applications such as in food and pharmaceutical industries [59, 60]. The cellulose fraction after the fractionation of other lignocellulosic components of the biomass can be exploited for its valuable derivatives including cellulose-based biopolymers [156]. Together with the potentially commercial products that were presented in this thesis, a diverse portfolio of high-value compounds can be

put together in a smart biorefinery design. For this purpose, continued and expanded synergy between academia and industry is the way forward to promoting biorefineries as driving tools of innovation and transition to a bio-based economy.

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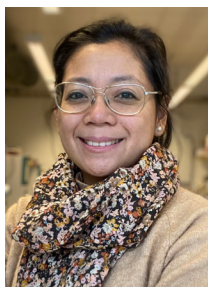

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