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Synergies and opportunities

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Integrated starch and lignocellulose based biorefineries: synergies and opportunities

Abstract

The transition from a reliance on fossil resources to the use of renewables for the production of energy, fuels and chemicals is essential for ensuring the sustainability of continued human development. Plant-based biomass is a renewable resource which can be transformed into all of these products. However, biomass is a heterogeneous material composed of several fractions with different chemical properties. Furthermore, the composition varies between species. In order to maximize the environmental and economic sustainability of biomass-based production, production systems that utilize all fractions of biomass to their fullest potential have to be developed. This is the goal of a biorefinery.

The work presented in this thesis mainly revolves around biorefineries that utilize feedstocks rich in starch and lignocellulose together to produce ethanol in an integrated process. The work is focused on comparing the performance of stand-alone and integrated biorefineries by investigating the impact that feedstock blending has on parameters important for the process economy, identifying potential synergies from integration and opportunities for improved material utilization.

It was found in this work, that the integration of starch- and lignocellulose-based feedstocks could result in improved ethanol productivity and yield during hydrolysis and fermentation compared to a stand-alone lignocellulose process without losing performance compared to a stand-alone starch-based process.

The prospects of introducing a sequential fractionation of the lignocellulosic biomass prior to integration was investigated. It was shown that this method could be used to produce separate fractions enriched in cellulose and lignin as well as improving the hydrolyzabilty of the cellulose fraction. This kind of fractionation could facility the utilization of all biomass fractions in both feedstocks by creating new byproduct streams as well as decreasing negative impacts on existing byproduct streams.

Key words

Process integration, fermentation, hydrolysis, lignocellulosic biomass, wheat grain, bioethanol

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Perfection is the enemy of progress

Winston Churchill

Abstract

The transition from a reliance on fossil resources to the use of renewables for the production of energy, fuels and chemicals is essential for ensuring the sustainability of continued human development. Plant-based biomass is a renewable resource, which can be transformed into all of these products. However, biomass is a heterogeneous material composed of several fractions with different chemical properties. Furthermore, the composition varies between species. In order to maximize the environmental and economic sustainability of biomass-based production, production systems that utilize all fractions of biomass to their fullest potential have to be developed. This is the goal of a biorefinery.

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Populärvetenskaplig sammanfattning

Mänskligheten är den art på jorden som har störst möjlighet att påverka sin miljö för att försäkra sig om sin fortsatta överlevnad och skapa det överflöd som har tillåtit den utveckling av teknik, kultur och samhälle som vi lever i idag. Från och med den industriella revolutionen har denna utveckling gått på högvarv och jordens befolkning har sedan 1800-talet hunnit öka från under en miljard till närmare åtta. Vi befinner oss nu i ett läge där de metoder vi har använt för uppnå den här utvecklingen kan rubba stabiliteten i de system som möjliggör vår fortlevnad på jorden. Om vi ska kunna säkerställa den fortsatta utvecklingen av mänskligheten och dess ideal, samt behålla möjligheten för varje individ att kunna leva drägliga liv även i framtiden, så krävs snabba men genomtänkta förändringar av dessa metoder.

Bland de system som påverkas av mänsklig aktivitet så är klimatet ett av de som mest akut kräver uppmärksamhet. Ökningen av globala temperaturer som kommit till följd av utsläpp av växthusgaser är nära den punkt där drastiska och oförutsägbara effekter kan uppstå till följd av självförstärkande system. Förbränning av fossila resurser är en av de ledande orsakerna till den här situationen, då det har lett till en snabb återinföring av kol från svunna tider in i vår atmosfär i form av växthusgasen koldioxid. Därför är all forskning och utveckling som kan sänka nytillförseln av kol eller minska den totala mängden kol i atmosfärens kretslopp av största vikt för klimatet.

Ett steg som skulle kunna tas i riktning ifrån vårt beroende av fossila resurser är en övergång till att använda biologiskt material, så kallad biomassa, för att producera bränslen och kemikalier. Tanken är gammal och mänskligheten har producerat olika produkter med råvaror från växtriket sedan urminnes tider, bryggning av alkohol från grödor eller utvinning av tjära från ved är bara några exempel. Fördelen med att använda växter som råvara är att de använder koldioxid från atmosfären som byggstenar för att växa. I teorin betyder detta att ett energisystem baserat på biomassa skulle kunna vara ett slutet kretslopp med avseende på kol, då den koldioxid som släpps ut vid förbränning av bränslet sedan skulle tas upp igen av de växter som odlas för att producera det. Till skillnad från fossila material som bildas under miljontals år skulle detta ske inom loppet av den tid det tar mellan plantering och skörd.

Produktion av biobaserade bränslen och kemikalier sker redan idag i en viss utsträckning. De flesta av dagens bioraffinaderier baseras på första generationens råvaror, detta är råvaror som även skulle kunna användas till matproduktion, så som vete, majs och sockerrör. Om biobaserade lösningar ska bidra till minskning av växthusgasutsläpp så måste denna produktion öka avsevärt. Att öka produktionen av bränslen och kemikalier baserade på första generationens råvaror kan dock föra med sig en rad problem. Det faktum att man konkurrerar

om samma råvaror som matproducenter kan potentiellt leda till ökade globala matpriser. Om jordbruket måste expandera för att tillgodose behovet råvaror så kan det till och med få negativa klimatkonsekvenser ifall ny åkermark skapas på bekostnad av urskogsskövling, då det kan leda till frigörandet av stora mängder kol som varit uppbunden i urskogen. Till råga på allt så är de negativa konsekvenserna på den biologiska mångfalden potentiellt förödande vid en sådan utveckling.

Ett sätt att undvika dessa problem och ändå öka produktionen av biobaserade bränslen och kemikalier är att övergå till processer som kan använda icke ätbar biomassa som råmaterial. Rester från jordbruk och skogsbruk så som halm, blast, flis och sågspån eller snabbväxande grödor som kan odlas på mark olämplig för vanligt jordbruk skulle kunna utgöra råvaran som sluter gapet till en klimatneutral framtid. Mycket forskning har ägnats åt att öka förståelsen för hur detta ska kunna genomföras. Vägen framåt ser lovande ut och på senare år har nya exempel på kommersiella projekt som utnyttjar den här typen av teknologi uppkommit. Vägen fram till en allmän storskalig övergång kantas dock fortfarande av olika tekniska, ekonomiska och logistiska utmaningar.

Att bygga integrerade bioetanolfabriker, som kombinerar första och andra generationens råvaror i en gemensam process, skulle kunna vara en lösning som medför de ekonomiska fördelar som krävs för kommersialisering av bioraffinaderier baserade på andra generationens råvaror. Men om det ska bli en verklighet så krävs en djupare förståelse för hur man ska kombinera två sådana processer samt vilka konsekvenser en sådan sammankoppling skulle kunna få. Temat i denna avhandling kretsar huvudsakligen kring just den här typen av bioraffinaderier, med ett specifikt fokus på råvaror tillgängliga i ett europeiskt sammanhang. Syftet med avhandlingen har varit att öka förståelsen för den här typen av processer genom att tackla följande frågeställningar:

- Vilka effekter uppstår på de centrala omvandlingsstegen i ett integrerat bioraffinaderi när man blandar materialströmmar baserade på råvarorna vete och vetehalm. Samt, vad ligger till grund för dessa effekter.
- Hur kombinerar man första och andra generations processer på ett sätt som utnyttjar egenskaperna i de respektive råvarorna för att uppnå gemensamma fördelar för båda processerna.
- Vilka strategier och teknologier kan utnyttjas för att hantera problem som uppstår när två processer integreras på det här viset.

List of publications

This thesis is based on the following publications, which will be referred to by their Roman numerals:

I. **Persson, M.**, Erdei, B., Galbe, M., Wallberg, O. (2017). Techno-Economic Aspects in the Evaluation of Biorefineries for Production of Second-Generation Bioethanol. In Hydrothermal Processing in Biorefineries (pp. 401-420). Springer, Cham.

II. **Persson, M.**, Galbe, M., Wallberg, O. (2020). A strategy for synergistic ethanol yield and improved production predictability through blending feedstocks. Biotechnology for biofuels, 13(1), 1-11.

III. **Persson, M**., Galbe, M. & Wallberg, O. Mitigation of pretreatment-derived inhibitors during lignocellulosic ethanol fermentation using spent grain as a nitrogen source. Biomass Conv. Bioref. (2021). https://doi.org/10.1007/s13399-021-01454-5

IV. **Persson, M.**, Galbe, M., Wallberg, O. Integrated wheat grain and wheat straw based ethanol production: A comparison between SSF and SHF. (Manuscript in preparation)

V. Olsson, J., **Persson, M**., Galbe, M. Wallberg, O., Jönsson, AS. An extensive parameter study of hydrotropic extraction of steam-pretreated birch. Biomass Conv. Bioref. (2021). https://doi.org/10.1007/s13399-021-01425-w

VI. **Persson, M.**, Galbe, M., Wallberg, O. Simultaneous saccharification and fermentation of sequentially fractionated wheat straw: A case study. (Manuscript in preparation)

My contributions to the publications

I. I performed the literature review that formed the basis for the publication. I wrote the initial draft and critically reviewed the manuscript before submission.

II. I planned and designed the study in collaboration with my co-authors. I performed all experimental and analytical work. I wrote the article, which was critically reviewed and commented by all co-authors.

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IV. I planned and designed the study in collaboration with my co-authors. I performed all experimental and analytical work. I wrote the article, which was critically reviewed and commented by all co-authors.

V. I participated in the conception and planning of the study. I participated in the interpretation of the results. I wrote the parts of the article relating to experimental design and collaborated with the main author on the final revisions of the manuscript.

VI. I planned and designed the study. I performed the experimental work and analytical work related to fermentation and hydrolysis. I wrote the article, which was critically reviewed and commented by all co-authors.

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Abbreviations

DGS	Distillers grains with solubles
HEX	Hydrotropic extraction
LEB	Lignocellulose-based ethanol biorefinery
PDI	Pretreatment derived inhibitor
SEB	Starch-based ethanol biorefinery
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
STEX	Steam explosion
WIS	Water-insoluble solids
WPH	Wheat protein hydrolysate
YE	Yeast extract

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1 Background and aim

The current standard for human industrial activity is impacting Earth on a global scale. As proposed by Rockström et al. [1] there are a set of earth system processes that could have a crucial impact on the prospects of future human development on planet Earth. Nine different earth system processes are listed, among which climate change, ocean acidification and stratospheric ozone depletion are three examples. These earth system processes are affected by human activity and in the paper a set of planetary boundaries defining the safety limits for human development are presented. These planetary boundaries are thresholds for control variables specific for each earth system process, which if crossed could lead to drastic and catastrophic non-linear changes to the environment on a continental or planetary scale [1]. Climate change has been singled out as one of two core boundaries, together with biosphere integrity, which if transgressed on their own can shift the Earth system from the relatively stable state of the Holocene (the present geological epoch) into a new state where the possibility of human society as we know it today is uncertain [2]. While many factors contribute to changing climate, such as emissions of methane, nitrogen-oxides and halocarbons, it is anthropogenic emissions of carbon-dioxide that contribute the most to global warming [3]. Decreasing anthropogenic carbon-dioxide emissions is therefore of great importance if we are to reach the goals of the Paris Agreement (2015), which set out to hold the increase in global average temperature below 2°C. The use of fossil carbon for the production of fuel and energy is the main cause of anthropogenic CO_2 emissions [4]. Therefore, finding alternative pathways for the production of fuels and energy, which would reduce carbon dioxide emissions, is necessary for reaching these goals. One such pathway is the transformation of renewable materials into energy and chemical products in biorefineries [5,6].

1.1 Transition to a sustainable economic system

The transition from a dependence on fossil resources into a system that relies on biorefining has several advantages. In contrast to fossil resources, the waste that is generated in a production system based on biomass contributes to the formation of new biomass. The CO_2 generated at the end of the product lifecycle is also a

necessary component for plant growth and compost or digestate from anaerobic digestion can be used as soil conditioner [7]. This means that an ideally implemented biorefinery system has the potential to be carbon neutral. Furthermore, since fossil resources are finite, developing production systems based on renewable resources is necessary to assure security of supply of energy [8] and provide a sustainable feedstock for chemical production [9]. However, the potential of a biorefinery to achieve these goals is affected by the choice of biomass feedstock.

Biorefineries can be classified according to the type of biomass used as feedstock. 1st generation biorefineries use food or feed crops as feedstock while 2nd generation biorefineries utilize biomass from non-edible crops or waste [5]. There are more types of categories, but biorefineries that utilize these types of feedstocks are the most common [10]. In terms of production volumes and commercial scale production facilities, 1st generation biorefinery systems dominate the market. However, 2nd generation biorefineries are generally considered to be a more sustainable alternative due to superior performance with regard to direct and indirect land use change [11] as well as potentially avoiding the issues raised by the food vs fuel debate [12]. However, the commercialization of 2nd generation biorefineries has been slow. This has mainly been attributed to the cost competitiveness of 2nd generation biorefineries products being limited by factors related to the process economy such as high capital investment costs and operational costs [13,14].

There are many ways of addressing the issue of cost competitiveness. The contribution of economists and policy makers might be to investigate and implement regulations as well as systems of taxation and subsidization to encourage or discourage specific economic activities. The environmental scientist might contribute by broadening understanding of the issues and spreading public awareness, thus encouraging individuals to make informed and responsible decisions with regard to their consumption. Engineers and engineering scientists on the other hand have a different approach to addressing this issue. They focus their attention on the development and optimization of new technology as a method of increasing the inherent attractiveness of the transition and removing technical and economic obstacles standing in the way of its implementation.

In the hands of the engineer the general question is broken down into questions like: what materials, tools and technologies are available to accomplish the goals and how are they utilized most efficiently without transgressing the overarching constraints of the problem. The engineering scientist answers the question by developing and deepening the understanding of these materials, tools and technologies, thus giving the engineer more ways of solving the problem.

Even though the border between these categories is often fluid and people often find themselves wearing their engineering hat one day to suddenly find themselves with a policy maker hat the next, my goal with this thesis has been to focus on the role of the engineering scientist in the context of this question. Specifically, by investigating the impact of integrating 1st and 2nd generation processes on factors affecting the process economy.

1.2 Aim and outline

The aim of my work was to evaluate the impact on different parameters affecting the performance of ethanol biorefineries and to highlight potential opportunities for synergistic interactions when designing a biorefinery treating integrated substrate streams of starch, used in 1st generation biorefineries, and lignocellulosic feedstocks, used in 2nd generation biorefineries.

The biorefinery concept in general, as well as concepts more specific to biorefinery systems that are the focus of this work, are introduced in Chapter 2. Process-engineering parameters of general importance in a lignocellulose-based ethanol biorefinery, the main issues related to them and the potential impact that integration with a starch-based ethanol biorefinery could have are highlighted in Chapter 3. In Chapter 4, the results from my work regarding process integration of starch- and lignocellulose-based biorefineries are presented (Paper I-IV) and in Chapter 5 results on the maximization of utilization of two of the major biomass components, cellulose and lignin, are discussed (Paper V, VI). In Chapter 6 the main conclusions from my work are summarized and future prospects for research on this topic are presented.

2 Biorefineries

While there have been many different proposed definitions of the biorefinery concept [15], one of the most general is the following: “the sustainable processing of biomass into a spectrum of marketable products and energy” as proposed by IEA task 42 [16]. The concept of a biorefinery can in many ways be said to be analogous to that of an oil refinery, and has the potential to supplant the oil refinery as a source of materials and energy [5]. However, the heterogeneous nature of biomass as a feedstock compared to crude oil presents both opportunities and challenges during processing. On one hand it makes it possible for biorefineries to produce more classes of products [5], on the other hand a more diverse range of technologies are needed to produce them [5,17]. Additionally, many of the technologies most commonly employed have not yet reached technological maturity [14,18,17]. These factors have led to a multitude of proposed biorefinery systems. In order to facilitate discussion, generalizations and the comparison of different biorefinery systems it is useful to use a system of classification. Cherubini et al. [19] proposed a general system of classifying biorefineries based on the following four main features of a biorefinery: **feedstock**, **platform**, **process** and **product**.

In principle any source of biomass could act as **feedstock** for biorefineries. However, in general, the commonly considered feedstocks for biorefineries can be divided into two categories, these are dedicated crops and residues [19]. Dedicated crops are crops that are expressly farmed for use in a biorefinery, these include food crops that produce sugar (e.g. sugar cane [20], sugar beet [21]), starch (e.g. maize [22], wheat [23]) and oils/fats (e.g. rapeseed [24], palm oil [25]) as well as non-edible crops such as lignocellulosics (e.g. wood [26], switchgrass [27]) and aquatic biomass (e.g. microalgea [28], macroalgea [29]). In addition to the dedicated crops, various sources of residues and waste have been considered for use as biorefinery feedstocks. For example, agricultural and forestry residues (e.g. straw [30], bagasse [31], sawdust [32]) as well as municipal waste [33]. These examples are in no way exhaustive but are meant to illustrate the multitude of potential feedstock sources for biorefineries as well as the diverse set of compositions and chemistries that these feedstocks offer.

The biorefinery **platform** refers to an intermediate that links the feedstock to the final product [19]. The concept of platforms is useful as a tool for generalization when comparing different potential biorefinery concepts. The same platform

intermediate can be derived from different feedstocks, used to synthesize many different products and all of this can be achieved using a wide variety of different processes. The most important platform intermediates are hexose sugars, pentose sugars, oils, biogas, syngas, hydrogen, organic juice, pyrolytic liquid, lignin and lastly electricity and heat [19]. All of these can be made from various types of biomass and established processes exist to convert these into products.

The **processes** in a biorefinery refer to the specific technological solutions used in the biorefinery to achieve specific chemical or biological conversions and the separation of feedstock constituents and product streams. There are a wide variety of available biorefinery processes. The choice of process for fractionation/preprocessing, separation and conversion depends on the feedstock and the product.

Many different **products** can be produced in biorefineries. These can generally be divided into energy products and material products [19]. The energy products include biofuels and other energy carriers. The production of bioethanol, biodiesel and HVO dominates the biofuel sector with the combined global production exceeding 160 billion L in 2019 [34]. Biobased energy products also include pellets and biomethane which can be used for heat and electricity production. Material products made in biorefineries can include everything from food or feed to chemicals and polymers.

While there is a staggering amount of possible combinations for how to configure biorefineries with all of these features in mind, this thesis is mainly focused on hexose sugar platform biorefineries for the production of ethanol through a biochemical conversion process combining starch and lignocellulose feedstocks. The implications and trade-offs with respect to performance parameters and product diversity from adding both pentose and lignin platform processes are also addressed.

2.1 Biomass

In this work, biomass feedstocks relevant in a European context were studied. The majority of all biomass produced in Europe comes from the agricultural and forestry sectors. It has been estimated that the total annual European biomass production from agriculture is 956 Mt and forestry is 510 Mt [35]. Cereal crops, e.g. wheat and maize, represent the largest segment of agricultural biomass production, with 258 Mt of primary crop, i.e. grains, and 329 Mt of residues being produced annually [35]. Out of 803 identified biorefineries in Europe, 216 use sugar or starch-based feedstocks, 76 use agricultural residues as feedstock and 77 use wood as feedstock [10]. The feedstocks studied in this thesis were wheat grain, wheat straw and birch.

2.1.1 Lignocellulose

Lignocellulose is a term for biomass that is mainly composed of the following three components: cellulose, hemicelluloses and lignin [36]. These components are the main constituents of plant cell walls [37]. In the cell wall, cellulose, hemicelluloses and lignin are connected through various types of covalent and noncovalent bonds to make up the structure of the lignocellulosic matrix [38]. The interconnections of these three components are part of what gives plants their rigidity, tensile strength and resilience to the natural elements as well as attack from biological factors. Additionally, lignocellulosic biomass contains nonstructural components generally referred to as extractives [39] and ash [38]. The composition of lignocellulosic feedstocks varies depending on factors such as plant species, growth conditions and age of the plant [36]. Examples of compositions for the lignocellulosic feedstocks used in my work are given in Table 1.

Table 1. Composition of different lignocellulose feedstocks [40].

Compound	Material	
	Wheat straw (%)	Hardwood (%)
Cellulose	30	43-47
Hemicelluloses	50	25-35
Lignin	20	16-24
Extractives	5	2-8

Cellulose is a linear polysaccharide composed of glucose subunits [38]. Hemicelluloses are branched polymer carbohydrates. Compared to cellulose the composition is more heterogeneous. Hemicelluloses can be made up of many different carbohydrate subunits, both hexoses such as glucose, mannose and galactose and pentoses such as xylose and arabinose [41]. The specific composition of the hemicellulose fraction in lignocellulose depends on the specific plant species from which the lignocellulose originates [42]. Lignin is a polymer or macromolecule made up of three main precursors: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol [43]. These subunits are mainly interlinked by carbon-carbon or ether bonds [43]. The irregular nature of this interlinking, as well as crosslinking with carbohydrates makes characterization of lignin difficult and the exact structure of lignin is still unknown [44]. Extractives in lignocellulose are soluble nonstructural components in biomass such as waxes [45]. Ash represents the inorganic non-combustible material in biomass, and is dominated by various types of mineral elements such as silicon and magnesium [38].

2.1.2 Wheat grain

In Europe, wheat is the cereal crop produced in the largest quantities [35]. Wheat is grown for its hard seeds, the wheat grain. Wheat grain is composed of starch, dietary fiber, protein, lipids, minerals and soluble sugars. A typical composition of wheat grain from common wheat is given in Table 2.

Table 2. Average composition of wheat grain from common wheat, numbers represent % of dry matter [46].

Compound	Content
Starch	66.7
Dietary fiber	15.2
Protein	12.1
Lipids	2.1
Minerals	1.9
Sugars	0.7

In starch-based biorefineries, starch, which is converted to ethanol, and protein, which is marketed as animal feed, are the components of main interest. Starch is a polymeric carbohydrate composed of two different types of polysaccharides, the linear polysaccharide amylose composed of glucose subunits connected by α-1,4 linkages and the branched amylopectin which is composed of glucose subunits connected by both α-1,4 and α-1,6 linkages [47]. Dietary fiber is composed of non-starch carbohydrates such as hemicelluloses and cellulose as well as lignin [46]. The protein fraction in wheat can be divided into gluten proteins, which make up 80-85% of the total wheat protein, and non-gluten proteins, which make up 15-20% of the total wheat protein. Non-gluten proteins are mostly made up of water soluble monomeric proteins [48].

2.2 Bioethanol processes

In this work, the synergistic interactions in a biorefinery treating integrated substrate streams of starch and lignocellulosic feedstocks were studied. The general process layouts for transforming either starch or lignocellulosic feedstocks into ethanol are broadly similar. Regardless of the feedstock, ethanol biorefineries can be divided into four different stages; preprocessing for increasing the availability of the raw material, liquefaction/saccharification where the glucose containing polymers are broken down to release fermentable substrate, fermentation where the substrate is transformed into ethanol by a microorganism and downstream processing where the ethanol is recovered. A schematic illustration of a general bioethanol process is presented in Figure 1.

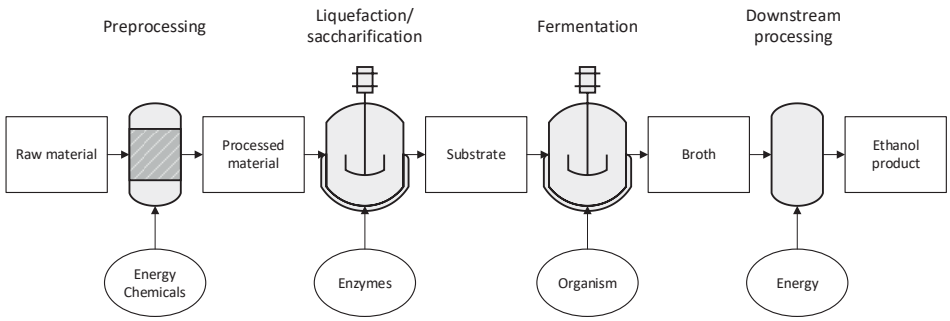

Figure 1. Flowchart representing a general bioethanol process.

2.2.1 Starch-based ethanol biorefineries

In the preprocessing stage of a starch-based ethanol biorefinery (SEB) the particle size of the cereal grains is reduced in order to increase exposure of the starch to subsequent treatment with enzymes. This can be achieved by various milling techniques. After milling, the grain is generally subjected to a cooking process, to sterilize the material and solubilize sugars [49].

The chemical breakdown of starch into glucose takes place in the liquefaction and saccharification stages of the process. During liquefaction, the starch is broken down into oligosaccharides by α-amylase enzymes and in the saccharification step glucoamylase enzymes break down the oligosaccharides into individual glucose subunits [49].

The saccharified slurry is transferred directly to a fermentation vessel. In the fermenter, glucose is transformed into ethanol by a fermenting organism. The most common practice is to use industrial strains of *Saccharomyces cerevisiae* which has high tolerance to ethanol and favorable product formation rates [49].

After fermentation, the broth is separated into different fractions by various downstream processing steps. First, the ethanol is recovered from the broth by distillation. However, due to the existence of an azeotrope for water-ethanol mixtures, all water cannot be separated from the ethanol through distillation alone. In order to produce pure ethanol, the distillation stage is followed by molecular sieving [49]. The bottoms after distillation contain residual carbohydrates, proteins, lipids and fiber from the wheat as well as yeast. To recover these solids the water is usually removed using a combination of centrifugation and evaporation [50]. The residual solids can be used to produce distillers grains with solubles (DGS) which is sold as animal feed [51].

2.2.2 Lignocellulose-based ethanol biorefineries

The main difference between an SEB and a lignocellulose-based ethanol biorefinery (LEB) stems from the recalcitrance of the lignocellulosic material. This is mainly observed in the preprocessing required in an LEB. A prerequisite condition for the economic production of products from lignocellulosic biomass is the efficient release of glucose from the cellulose fraction during the liquefaction/saccharification stage [52,53]. Structural factors of the biomass, such as the specific surface area and crystallinity of the cellulose and pore size in the biomass together with chemical factors such as the composition and content of lignin and hemicelluloses determine the efficiency of the enzymatic hydrolysis [52]. In order to make cellulose accessible to further degradation by enzymatic attack the structure of the lignocellulosic matrix has to be disrupted [54]. The methods commonly used to achieve this, is to utilize one or a combination of mechanical, thermal, chemical and biological pretreatment methods [18,55]. The purpose of these pretreatment methods is to break down the ultrastructure of the material, fractionate feedstock components and alter the chemical structure of the material to increase the efficiency of a subsequent enzymatic hydrolysis treatment [54].

After the feedstock has been pretreated, the next step is the transformation of the pretreated material into a fermentable substrate, i.e. monomeric sugars in the liquefaction/saccharification stage. This transformation can be achieved through either acid or enzymatic hydrolysis. Enzymatic hydrolysis has emerged as the preferred method due to the mild conditions at which the process can be performed, lower tendency for byproduct formation and high conversion efficiency [56]. The classic enzymatic hydrolysis process involves the action of three different types of enzymes: endo-1,4-β-glucanases, exo-1,4-β-glucanases and β-glucosidases [57]. Endo-1,4-β-glucanases cleave cellulose at random sites within the chain creating new cellulose chain ends. Exo-1,4-β-glucanases attack cellulose from either the reducing or nonreducing end of the polymer to release oligosaccharides. Lastly, β-glucosidases hydrolyze the oligosaccharide products from the exoglucanases into glucose. Recently a novel group of enzymes, lytic polysaccharide monooxygenases, have been identified as a candidate for increasing the efficiency of enzyme cocktails [57,58]. In addition to cellulolytic enzymes, adding enzymes with activity specific for breaking bonds in hemicelluloses, has also been suggested as a method for accelerating the rate at which cellulose becomes available for the cellulolytic enzymes [56].

Once a hydrolysate containing fermentable monosaccharides has been obtained, the substrate is transformed into ethanol in a fermentation process similarly to what is done in an SEB. However, while the carbohydrate content of the substrate used in SEBs is mainly composed of glucose, many LEB feedstocks have a high content of hemicelluloses and can therefore contain large amounts of pentoses.

Since natural strains of *S. cerevisiae* cannot metabolize pentose sugars, utilizing these carbohydrates requires the use of alternative organisms or genetically engineered hosts [59]. The main challenge with this approach has been to develop robust industrial strains that are tolerant to the inhibitory conditions presented by LEB substrate streams [59].

In addition to the choice of fermenting organism, there are two different approaches to configuring the fermentation generally considered in an LEB. These are simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) [60-62]. In the SSF mode, the hydrolytic enzymes and the fermenting organism are added concurrently, resulting in a steady release of fermentable substrate that can be directly converted into product by the fermenting organism. The advantages of this approach are twofold. Firstly, the continuous removal of sugars by the yeast decreases end-product inhibition of the enzymatic activity [63], which can improve the general rate of enzymatic hydrolysis as many cellulolytic enzymes have been reported to be inhibited by hydrolysis end-products such as glucose and cellobiose. Secondly, by running these two processes simultaneously, the overall residence time required to go from a pretreated feedstock to the finished product of ethanol can be reduced as the individual steps of hydrolysis and fermentation would otherwise have to be performed in succession [64]. In contrast to SSF, when operating a process in the SHF mode, hydrolysis and fermentation are performed in successive stages. Running the fermentation in SHF mode can however bring another set of advantages to the table. The optimal operating condition for hydrolysis and fermentation are usually not the same, which means that running the two processes simultaneously leads to compromises [63].

After fermentation, product recovery is generally handled in a similar manner to what was described for SEBs, with distillation and molecular sieving used in order to retrieve fuel ethanol [65]. The main difference lies in the potential coproducts that can be obtained. As opposed to SEB substrates, LEB substrates do not contain large amounts of protein or lipids, instead they do contain considerable amounts of lignin and hemicelluloses. The lignin can, for example, be dried and used as an energy source to power a combined heat and power plant. The produced heat and electricity can provide the energy required in the biorefinery as well as being sold to the grid [66]. Lignin is also a platform chemical in its own right and could potentially be further upgraded to other products. If the pentose sugars from the hemicelluloses are not converted to ethanol in the fermenter there are other ways of utilizing them. They can, for example, be used in other biochemical conversion processes such as anaerobic digestion to produce biomethane [67].

3 Parameters of importance for profitability

Even though an LEB has the potential to produce energy, chemicals and materials in a sustainable way from renewable raw materials, the development of the industry is still limited by technical and economic challenges [14]. Identifying and understanding which factors drive the profitability of ethanol producing biorefineries is paramount when directing the research focus for commercialization. The purpose of this chapter is to shed light on some of the main process parameters that affect the economy of an LEB. These parameters are the solids loading, the yield and the productivity. The parameters that are the focus of this chapter are general process engineering concepts that on their own cannot tell the whole story of the process. However, the purpose of using these concepts in the thesis is to bridge the gap between the fundamental biological and chemical processes taking place in individual stages of the biorefinery and the economic impacts they have on the overall process. Each subchapter, describing a process parameter, starts with a short elaboration on how the concept is defined in the context of this thesis, followed by an explanation of how it influences the process economy. This is followed by an attempt to map the main underlying processes that impact and limit these parameters and a consideration of the interdependencies between them. Each subchapter ends with a discussion about the potential impact that process integration of LEB and SEB substrates can have on the parameters. Results of previous studies as well as questions that remain unanswered are highlighted.

3.1 Solids loading

The solids loadings, i.e. the ratio of solids in proportion to water, in an LEB has a decisive impact on the cost of ethanol recovery. Besides the amount of water in the biomass, water is added in different stages of the process, for example during steam pretreatment. Increasing the concentration of the process streams in an LEB has the potential to significantly improve the economy of the process by decreasing both the operating and capital costs [68].

The overall energy costs of a biorefinery are directly influenced by product and substrate concentrations in the process streams. The main costs can be derived from the energy requirements of downstream processing [69,70], which is heavily dependent on the final ethanol concentration after fermentation. It has been reported that an ethanol concentration of at least 4% in the broth is required for a process to be economically viable [71]. Below this concentration, the energy requirement of distillation increases drastically, increasing operational costs beyond the point of profitability. Additionally, a more diluted process stream, means that a greater volume needs to be handled to produce the same amount of product, increasing the total investment cost required for hydrolysis reactors and fermenters [68]. A common approach to tackle these issues is to increase the concentration of the pretreated solids loaded into the enzymatic hydrolysis reactor, so called high solids enzymatic hydrolysis [72]. Enzymatic hydrolysis performed at a solids loading over 15% is generally considered as a high solids process [72]. However, when using a lignocellulosic feedstock, increasing the solids loading comes with challenges. These are mainly related to the operational constraints of enzymatic hydrolysis and fermentation [69].

It has been shown that there is a negative linear correlation between the conversion of cellulose and the dry matter content, i.e. solids loading, during hydrolysis of pretreated lignocellulose [73]. A number of factors have been connected to the poor performance during enzymatic hydrolysis observed at high solids loadings. The viscosity of pretreated lignocellulosic slurries at high solids loadings can lead to poor mass transfer characteristics and problems caused by insufficient mixing [74]. In other studies, the decrease in hydrolysis efficiency has been connected to the concentration of soluble carbohydrates in the slurry inhibiting enzyme activity [75]. It has also been suggested that the decrease in the efficiency of the hydrolysis is caused by high solids loadings having a constraining effect on water in the system [76]. Water is important as it facilitates the diffusion of enzymes, substrate and product. Additionally, water is a reactant in the actual hydrolysis reaction [76]. The concentration of certain degradation products from the pretreatment, specifically phenolics, have also been found to lower cellulose conversions [77]. Lastly, the non-productive binding of enzymes to lignin has been reported as a factor contributing to low conversion efficiency [78].

Another problem when using lignocellulosic hydrolysates at high concentration is the accumulation of pretreatment derived inhibitors (PDI) which can inhibit the fermentation. During pretreatment, different fractions of lignocellulose are degraded into a range of different weak acids, furan derivatives and phenolic compounds, which can negatively affect both the yield and productivity during fermentation [79]. The details of these effects will be elaborated on further in subsequent chapters.

3.1.1 Solids loading and integration of LEB and SEB

Saccharification of starch is a mature process with a long history and plenty of industrial experience. Compared to lignocellulose, starch is highly susceptible to enzymatic breakdown even at high solids loadings and operating fermentation at carbohydrate concentrations well above 250 g/L is common practice in SEBs [80,81]. By integrating a process stream from an SEB into the hydrolysis or fermentation stage of an LEB, ethanol concentrations well above the minimum acceptable level of 4% w/w that is needed for economic downstream processing can be achieved without the need to use high loadings of lignocellulosic solids [82,83]. However, the effect that the integration of SEB and LEB streams could potentially have on the specific issues caused by high solids loadings depends on the manner of the integration, and the way in which such a process is configured. On one hand, the dilution effect of blending LEB substrate with SEB substrate could reduce problems caused by inhibitor concentration and viscosity. On the other hand, the high content of soluble carbohydrates from SEB could reduce hydrolysis efficiency due to the constraining of water [76] and product inhibition for enzymes [84]. Therefore, understanding the relative effect of different operating conditions when integrating LEB and SEB is important. The implications of integration on solids loading are discussed in Paper II and IV.

3.2 Ethanol yield

In this thesis, yield is defined as the amount of product formed in relation to the theoretical maximum that could be produced based on the raw material input within a specific system boundary. Increasing the yield of a process means that more product is generated with the same amount of raw material, thus increasing the margin between product sales revenue and feedstock related expenditures. Additionally, it can result in lower costs for waste handling when unutilized feedstock components cannot be recovered or reused. The importance of high yields is especially emphasized for the production of energy, fuels or bulk chemicals, which are low-value products produced in greater volume, as feedstock costs then make up a large portion of the operating costs [85], thus resulting in lower profit margins. Additionally, efficient material utilization is not only important from an economic perspective but is also a core tenet of the circular economy concept, which aims to minimize waste in all stages of production [86].

When analyzing the yield in a process, the system boundaries are important for the interpretation of any result. While individual conversion stages in a process have their own limitations and can be optimized separately in order to maximize the yield in each separate stage, it is important not to lose track of the yield of the

total process in the biorefinery. Choices that favor one subprocess can negatively impact another and if the entire process is not considered, important information about the actual efficiency of raw material utilization could be lost. In this section, the discussion about process yield will mainly focus on the production of ethanol. However, taking a holistic view on the process to maximize the utilization of all fractions is essential for the process economy of any biorefinery concept and will therefore be discussed further in this chapter.

Two different conversion processes have a large influence on the overall ethanol yield in an LEB, these are enzymatic hydrolysis, i.e. transformation of feedstock into fermentable substrate, and fermentation, i.e. transformation of substrate into ethanol. Challenges and obstacles for maximizing these yields can be traced to a couple of different factors. One set of issues stem from the trade-off between conditions that favor high hydrolysis yields and high fermentation yields. The other relates to the temporal aspects of these dynamic processes. Many factors affect the kinetics of these reactions and in case the reactions are too slow, aiming for high yields might not be economically justified.

3.2.1 Yield and hydrolysis

The manner in which lignocellulose is pretreated is one of the essential factors determining the efficiency of the conversion of lignocellulose to fermentable substrate during the enzymatic hydrolysis [54,53,18]. In order for a pretreatment method to increase the hydrolyzability of a lignocellulosic feedstock, the severity of the pretreatment has to be high enough to cause the structural and chemical changes necessary to increase enzyme accessibility. The pretreatment severity can be expressed as a function of time, temperature and catalyst concentration, in the case that a catalyst is used, during pretreatment [87]. However, high severity conditions during pretreatment result in degradation of the various fractions of the lignocellulosic material and the formation of unwanted byproducts [88]. The degradation of hexose and pentose sugars into hydroxymethylfurfural and furfural as well as the subsequent degradation of these compounds into formic acid and levulinic acid, is prevalent at elevated temperatures and acidic conditions [89,79,90]. The direct effect of this is that substrate for ethanol production is lost, which in turn limits the maximum achievable yield in the subsequent fermentation. Additionally, the degradation products that are formed are toxic to the yeast and can severely inhibit ethanol formation during fermentation affecting both the yield and the productivity of the process [91,79,92]. The inhibitory nature of lignocellulosic hydrolysates is further exacerbated by the release of acetic acid, which is a potent inhibitor in its undissociated form [93,94], due to the hydrolysis of acetyl groups of hemicelluloses [95]. Additionally, the degradation of the lignin and extractive fractions can result in the formation of various toxic aromatic and phenolic compounds [95].

Once the feedstock has been pretreated and enters the enzymatic hydrolysis stage, several factors can affect the product yield. An important factor affecting the efficiency of enzymatic hydrolysis is the choice of hydrolytic enzymes. Typically a mixture of enzymes with different types of complementary hydrolytic activity on cellulose is used together in order to maximize the efficiency of the hydrolysis [57]. Additionally, supplementing the enzyme preparation with enzymes that have activity specific to other carbohydrates than cellulose, such as xylan or pectin, can enhance the yield further by eliminating the inhibiting effect they have on cellulolytic enzymes [96]. The enzyme loading also has an impact on the achievable yield during hydrolysis [97,98]. This is especially emphasized in the case of that the residence time needs to be minimized [98].

There are some factors that are of general importance for enzyme performance during hydrolysis regardless of the enzymes used. Optimal performance of hydrolytic enzymes requires specific temperatures and pH [97,99]. Operating outside the optimal range can lead to decreased productivity and product yield can suffer as a result of enzyme inactivation [100]. Furthermore, feedstock properties can lead to unproductive binding of enzymes [101,102].

These issues can to some extent be mitigated by increasing the enzyme loading. However, increasing enzyme loading comes at the price of increased operational costs, and enzymes still represent a significant portion of LEB expenses [103]. Another factor to consider is end-product inhibition, a phenomenon where the activity of enzymes is diminished in the presence of high concentration of sugars [104].

3.2.2 Yield and fermentation

The yield that can be achieved during fermentation is largely a question about the fermenting organism. The fermenting organism and the specific metabolic pathway by which the desired metabolic product is generated decide the maximum theoretical yield. The use of various industrial strains of the yeast *S. cerevisiae* dominates the space of commercial ethanol production [105]. A disadvantage of *S. cerevisiae* is that it only ferments hexose sugars, mainly glucose and mannose. However, it should be noted that research efforts have been dedicated to engineering various yeast and bacterial hosts to give them the capability of fermenting pentose sugars [59]. This is an important field of research given that pentoses make up a significant part of many sources of lignocellulose.

In the context of lignocellulosic hydrolysates the main hexose sugars to consider are glucose, mannose and galactose. Out of these, glucose is the most important as cellulose, a glucose-based biopolymer, is the main source of hexoses in lignocellulose [106]. When *S. cerevisiae* metabolizes glucose at anaerobic conditions, glucose passes through the Embden-Meyerhof-Parnas pathway

resulting in two molecules of ethanol and two molecules of carbon dioxide [49]. This sets the hard metabolic limit on product yield when transforming hexoses into ethanol to 0.51 g ethanol per g hexose.

Even though yeast cells to some extent can be seen as catalysts facilitating the conversion of sugars to ethanol, as opposed to chemical catalysts, yeast cells are living organisms evolved to react to the environment in ways that will maximize their chance of survival and reproduction. The production of ethanol that occurs at anaerobic conditions is a way for yeast to produce energy that can be used to fuel its anabolism. However, yeast cells require more than just energy in order to grow and maintain their functions. During cell growth, the production of structural cell components require carbon, which is supplied by the substrate. Thus the first deviation from the theoretical maximum ethanol yield is achieved. Furthermore, cell growth is not a redox neutral process [107]. When cell-mass is produced a surplus of NADH is created. This creates an imbalance in the ratio of NADH/NAD+ in the cell, which needs to be rectified in order to maintain regular cell functions. The regeneration of NAD+ can be accomplished in yeast by the production of glycerol [107]. The production of glycerol requires carbon, thus adding an additional diversion of substrate from the main product.

The fact that yeast is a living organism has further implications on achievable ethanol yields during fermentation of lignocellulosic hydrolysates. Some of the degradation products generated during pretreatment have inhibitory effects on the metabolism of yeast [79]. The inhibitors affect the rate of ethanol production [108]. This has been connected to the inhibition of metabolic enzymes [109] and decreasing growth rates [91,94] or simply by causing cell death, thus completely terminating metabolic activity [91]. The effects on product formation rates might not directly affect the final product distribution, in the case that fermentation is allowed to continue until all substrate is depleted; however, the product formation rate adds a temporal aspect to the yield since it can increase the required residence time beyond what can be motivated economically, thus resulting in a decreased product yield.

Another factor that affects the final product yield are nutrients. Just like any other living organism, energy is not enough to sustain life and support reproduction. The production of vital molecules like proteins and cofactors require a source of elements such as nitrogen, phosphorous and potassium. Limited availability of a nitrogen source has been connected to slow or completely arrested alcoholic fermentation [110]. Furthermore, addition of nitrogen containing nutrient sources during fermentation of lignocellulosic hydrolysates has been shown to increase product formation rates [111], which would affect the product yield if excessive residence times are an issue.

3.2.3 Yield and integration of LEB and SEB

A question when blending LEB and SEB substrates during hydrolysis and fermentation is whether the blending can affect the product yield in some way. There are many factors affecting the answer to this question. Many of these questions come down to how the integration is implemented. What is the mode of operation, should the biorefinery be operated in a SSF or SHF fashion? What does this even mean in the context of integrating LEB and SEB substrate streams? Should all hydrolysis be performed separately and mixing only occur in the fermentation, or should the raw materials be mixed directly after pretreatment and then subsequently be subjected to their respective enzymatic hydrolysis simultaneously? Furthermore, at what ratio should the substrates be mixed?

There are several factors relevant for the process yield that could be affected by the integration of LEB and SEB substrate streams, which are covered in Paper II, III and IV :

- The hydrolysis of cellulose is known to be inhibited by high concentrations of carbohydrates [84]. Mixing a glucose-rich SEB substrate stream with an LEB substrate before the hydrolysis of cellulose could hamper the activity of cellulolytic enzymes.
- Cellulolytic hydrolysis has been shown to be negatively affected at high loadings of water-insoluble solids (WIS) [73]. Mixing LEB and SEB streams before hydrolysis could mitigate this effect by dilution.
- LEB process streams can contain PDIs which decrease productivity and inflicts a penalty on temporal yield or can even limit total yield if the yeast population crashes due to cell death. Mixing with an SEB substrate stream could lessen the impact of PDIs by dilution.
- SEB streams generally have a higher content of nutrients than LEB streams [80,112]. This can increase productivity, decrease the risk of arrested fermentation due to a nitrogen deficiency and help mitigate the effect of inhibitors[111].

3.3 Volumetric productivity

The volumetric productivity of a process is a measure of how much product can be produced per unit of time in a given reactor volume. The main impact of volumetric productivity in any process is its effect on the investment costs of the process. The lower the volumetric productivity of a process is, the higher the residence time required to reach the desired product yield. Looking at the mathematical description of an ideal reactor model, one can see that when a

specific residence time is required, the only way to increase the throughput in the reactor stage of a process is to increase the reactor volume; however, greater volumes mean larger equipment, which means higher investment costs.

In an ethanol-based biorefinery, the hydrolysis and fermentation processes are the main bottlenecks in the production line when it comes to time. A combined residence time of 5 days is commonly assumed as a benchmark for these processes [65]. The discrepancy in residence time between these sub processes and other parts of the biorefinery becomes apparent if compared to a process like acid-catalyzed steam pretreatment of the feedstock, where residence times in the range of 2-20 minutes are commonly reported [113,112].

The scaling advantages in hydrolysis and fermentation processes are limited due to the size of the production volumes in biorefinery processes [114]. Scaling up the production volume in a small process can have significant economic advantages, since doubling the volume of a reactor does not double the investment cost. However, due to the physical limitations of reactor size, above a certain production volume, one has to add more reactors rather than just increase reactor size in order to increase reactor volume. This also means that above a certain scale, investment costs for reactors will scale almost linearly with reactor volume [114].

Similarly to the case of product yield, when it comes to the productivity of the hydrolysis process, the main factor influencing it, is the enzyme itself. The inherent properties of the enzyme in the specific enzyme cocktail used for the hydrolysis together with the mechanism by which the substrate is converted to product make up the fundamental basis for what the rate of conversion will be [115]. The description and understanding of these kinds of processes is the domain of the field of enzyme kinetics. In the context of process design this extensive field of research boils down to a couple of central considerations. To start with, the question is if the right set of enzymes are used for the material in question, as different types of lignocellulosic materials have different types of composition and macrostructure. Tailoring the enzyme cocktail to have larger presence of enzymes with hydrolytic activity towards the bonds found in this structure will improve the performance of the process [116,117]. Furthermore, the amount of enzyme added during the hydrolysis will affect the productivity [118,97]. However, deciding on the scale of the enzyme loading is a trade-off between gains in productivity and the price associated with the costs of enzymes. The availability of the pretreated material for enzymatic attack is another consideration that has to be taken into account. The type of pretreatment used and the operating conditions have a large impact on this [53].

A factor that will affect the volumetric productivity of the fermentation is the product formation rate of the fermenting organism. The product formation rate of a microorganism can be divided into growth-associated and non-growth-associated production formation as described by equation 1 [119].

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (1)$$

Where P (g/L) denotes the product concentration, t (h) time, X (g/L) cell concentration, α (g/g) is a constant representing growth-associated product formation and β (g/g h) is a constant representing non-growth-associated product formation.

In the case of ethanol, which results in a net production of ATP, the main energy carrying molecule of living organisms [120], this illustrates that the product formation rate stems from intracellular energy production for cell maintenance or cell reproduction. If the total amount of cells in the fermentation broth is low, then the rate related to maintenance will be lower. If cell growth is inhibited, then the growth-related product formation will, of course, also be lower.

Regardless of the organism used, the amount at which the organism is added to the fermenter directly affects the base product formation rate, as increasing the amount of metabolic units inside of the reactor will increase the rate of production. Choosing the level of cell loading appropriate for any specific case is a trade-off between the cost of cell-mass production compared to the gain in productivity. The choice of fermenting organism can have a large influence on the volumetric productivity, with different strains exhibiting different traits such as inhibitor tolerance, giving them an advantage during the fermentation of lignocellulosic hydrolysates [121]. Furthermore, with metabolic engineering, the activity of specific metabolic enzymes in the organism can be altered in order to improve fermentation performance [122].

3.3.1 Productivity and integration of LEB and SEB

With regard to the productivity during the fermentation, mixing an SEB stream with an LEB stream presents an interesting question for how the process will respond. An SEB substrate stream provides many components necessary for the viability of the fermenting organism. It has a high concentration of glucose to act as an energy source and also contains nutrients and trace elements that are vital for cell growth. To some extent, an LEB substrate stream presents the opposite characteristics. While it does contain glucose, an LEB hydrolysate is a nutrient poor substrate that can often contain high concentrations of substances that are toxic to the fermenting organism and can severely inhibit its metabolism. The ways in which the characteristics of the individual substrates will interact and

affect the process in general is not obvious. The question of whether the nutritional qualities of the SEB substrate or the toxic qualities of the LEB substrate will have a larger effect on the fermentation is of utmost importance for the productivity of the process. The effect of integration on productivity was covered in Paper II, III and IV

4 Integration of starch- and lignocellulose-based biorefineries

In this thesis, different possible process configurations for the integration of SEBs with LEBs have been investigated. In Paper I, an overview of techno-economic considerations in LEBs is presented, and the potential of integration with SEBs is discussed. The experimental work was focused on two kinds of process integration: direct substrate co-fermentation and indirect material integration. Direct substrate co-fermentation refers to integrated processes where the main substrates from LEB and SEB are blended and fermented together. Two main configurations, SSF and SHF (shown in Figure 2), were studied. SSF and SHF are known concepts in the context of LEB. In this thesis, integrated SSF and SHF refers only to how the LEB substrate was handled. In all experimental studies, saccharification of the SEB substrate was performed separately. Differences between stand-alone and integrated SEBs and LEBs in the SSF configuration was studied in Paper II. The influence of operation mode (integrated or stand-alone SSF and SHF) on the performance of hydrolysis and fermentation was investigated in paper IV. Indirect material integration refers to a type of process integration where materials other than the main substrates are blended. In Paper III the protein-rich byproduct stream from an SEB was used as a nutrient source in an LEB process.

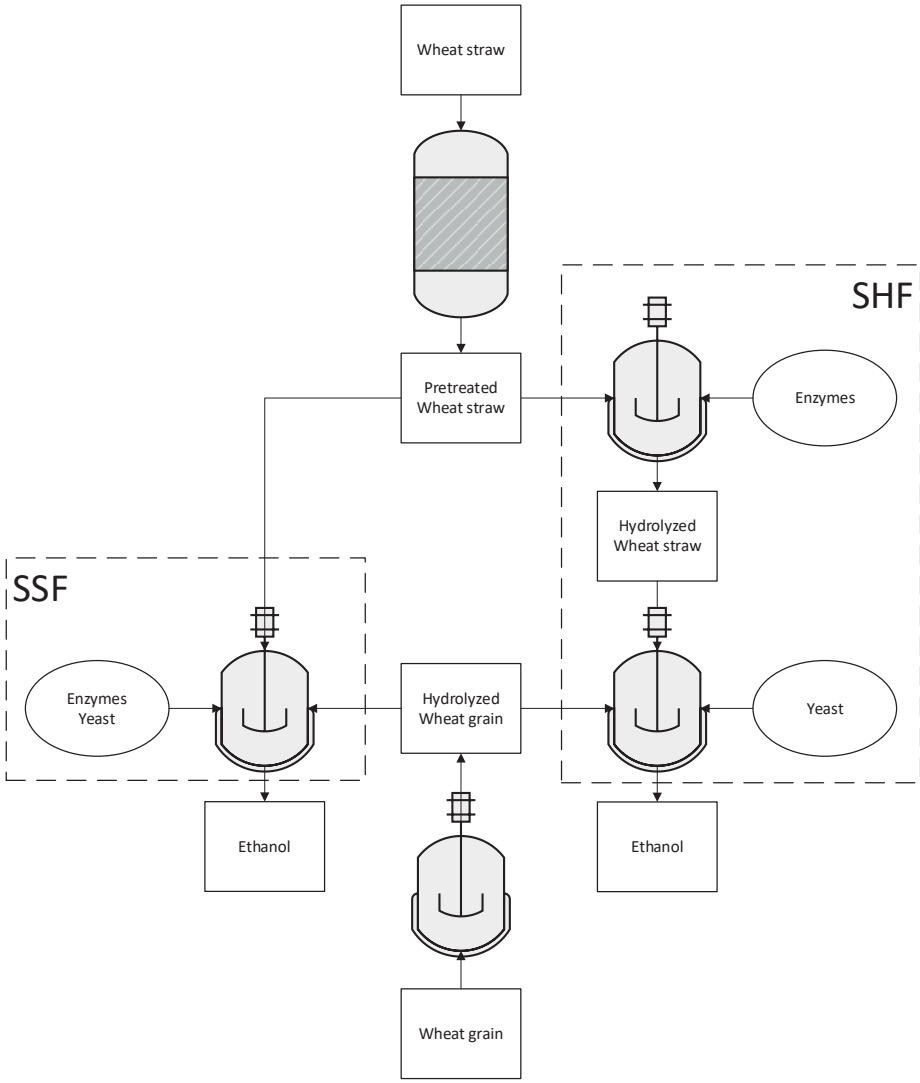

Figure 2. Schematic illustration of an integrated lignocellulose (wheat straw) and starch (wheat grain) biorefinery process.

4.1 Process configurations in ethanol production

In the design of an integrated LEB and SEB process there are many possible ways of connecting process streams. The way in which these streams are connected can have a significant impact on the performance of the process. Generally, the hydrolysis and fermentation stages of an ethanol producing biorefinery can be

designed according to the SSF or SHF process configuration. These process configurations present one way of integrating process streams in an integrated LEB and SEB process. Another possible way of integrating LEB and SEB processes is to utilize the protein-rich byproduct stream from SEB as a source of nutrients for the fermenting organism in an LEB. In this section, results are presented which show the impact of these design choices on the performance of ethanol production.

4.1.1 SSF and SHF

Direct comparison of SSF and SHF with regard to ethanol yield and total residence time is complicated by the fact that SSF is performed in one step and SHF is made up of two consecutive steps. It is common practice to present data on fermentation yield and residence time that represent the state when fermentation dynamics end, that is to say when no further increase in ethanol production is observed. When representing the fermentation performance in this way the potential for material utilization is highlighted, but it can also inflate the residence time beyond what is economically justifiable. Presenting SHF performance data in this way exacerbates the problem, as there are two consecutive conversion processes with individual endpoints. If both of these conversion processes have comparable kinetics, this can result in SHF processes being presented as requiring twice the residence time of SSF processes or more, as exemplified by results presented in previous work [60,64].

This representation is problematic for the following reasons: Most of the conversion dynamics in a batch process take place during a small portion of the runtime of the experiment, mainly in the beginning when the concentration of substrate is the highest, as the conversion rate is proportional to substrate concentration. This is characteristic of the Michaelis-Menten kinetic model that is commonly used as a basis for describing fermentation time courses as well as enzyme kinetics in general [123-125]. This means that each hour of increased residence time will give an incrementally smaller gain in material utilization. On the other hand, the investment costs due to increased residence time will scale almost linearly, assuming that reactor volume is proportional to residence time and the production volume is at a scale where an increase in volume has to be resolved by an additional piece of equipment rather than bigger reactors [114]. This means that in order to make a fair comparison between the configurations, a cost benefit analysis between material utilization and investment cost should be performed.

The cost benefit analysis is easily performed in the case of SSF, as each point during the time course represents the highest achievable yield at a given residence time. In the case of SHF, it is more complicated as there are many ways of combining the stop time for fermentation and hydrolysis, respectively. Given a

quick evaluation of a data set representing the time course for fermentation and hydrolysis in a SHF system, it becomes apparent that there are many combinations of stop times for the two conversion processes, which will lead to suboptimal performance with regard to process yield and combined residence time. To illustrate this point, the following example is considered: if hydrolysis is performed with the shortest possible residence time it would result in a shorter combined residence time. However, assuming that the hydrolysis process is terminated at the stop time, this would result in a low amount of available substrate and would therefore set a hard limit for achievable process yields regardless of the residence time in the fermentation step. Increasing the residence time during hydrolysis by just a small amount would result in a penalty on the combined residence time initially. However, as more fermentable substrate would have been made available, higher process yields could be achieved in the conversion of glucose to ethanol later in the time course. This means that at some point, a state with a combined residence time equal to the case with the shortest possible hydrolysis time but with higher process yield will occur in the case with an incrementally higher residence time in hydrolysis. Keeping this in mind, it becomes apparent that there exists a Pareto optimal set of combinations of hydrolysis and fermentation stop times that produce the highest possible yield at any given residence time. In Paper IV a method for producing this Pareto optimal representation of process yield and residence time for SHF is presented. An example of a Pareto optimal trajectory of combined stop times for ethanol production in an SHF configured process is shown in Figure 3. The main advantage of using this method is that it provides a simple structure for making direct comparisons of the techno economic trade-offs between SSF and SHF configurations.

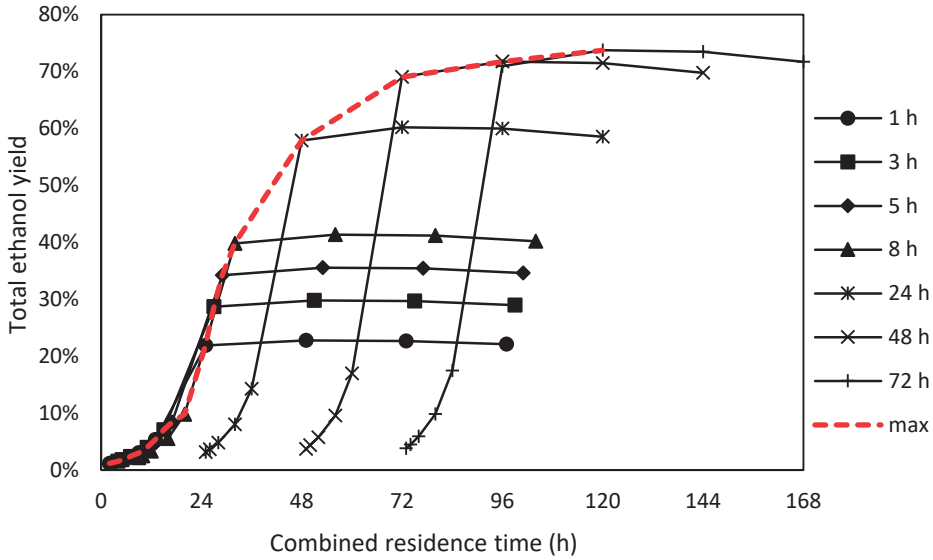

Figure 3. Example of how the Pareto optimal combined residence times for the SHF were obtained. The lines: 1 h (●), 3 h (■), 5 h (♦), 8 h (▲), 24 h (∗), 48 h (×) and 72 h (+) are theoretical fermentation trajectories with the number representing hydrolysis stop time. The red line (max) represents the Pareto optimal set of combined stop times optimizing for the highest ethanol yield at the shortest combined residence time. Adapted from paper IV.

In Paper IV, using the Pareto optimal representation of SHF trajectories, it was concluded that in the integrated case, SSF outperformed the SHF configuration with respect to ethanol production. The overall process yield and volumetric productivity were higher for SSF than SHF, as is shown in Figure 4. The increased process yield was attributed to an increase in the efficiency of hydrolytic enzymes due to improved conditions for hydrolysis. Operating the process in the integrated SSF configuration meant that enzymatic hydrolysis was performed at a lower WIS loading of 2-4% due to the dilution of the broth with starch-based substrate. In comparison, hydrolysis in the SHF configuration had to be performed at a WIS loading of 10% in order to achieve comparable levels of available glucose and glucan between the cases. As has been shown previously, WIS has a linear negative effect on hydrolysis yield [73].

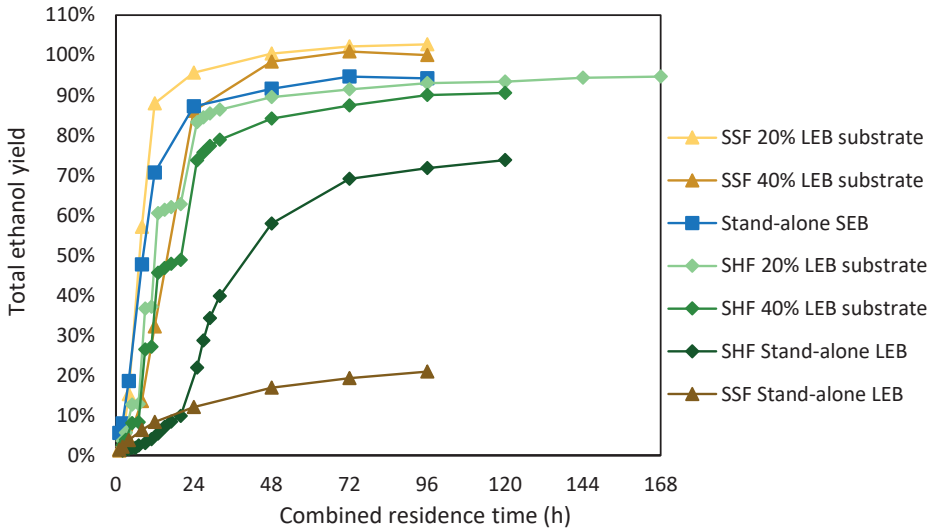

Figure 4. Pareto optimal fermentation trajectories with respect to ethanol yield and combined residence time for SSF and SHF. Stand-alone cases were performed with only one type of substrate. In the integrated cases 60% of the reaction mass consisted of a SEB substrate stream with either 20% or 40% of the total reaction mass consisting of an LEB substrate stream, the remainder was made up of deionized water. Adapted from Paper IV.

4.1.2 Nutrient stream integration

Another way of integrating material streams from SEBs and LEBs was investigated in Paper III. In conventional starch-based biorefineries, the protein fraction of the feedstock passes through the process relatively unaltered. The most common way of valorizing this fraction is to separate it from the broth after fermentation and sell it as a high protein animal feed, in the form of DGS [51]. However, in Paper III the prospect of hydrolyzing this protein fraction and utilizing the hydrolysate as a source of nutrients for the yeast was investigated. Common issues faced when fermenting lignocellulosic hydrolysates are related to the robustness of the ethanol fermentation process and low product formation rates. These issues are commonly related to the presence of PDIs in the hydrolysate [79], but can also be connected to deficiencies in the nutrient content, specifically assimilable nitrogen, in the lignocellulosic hydrolysate [111]. Utilizing the protein-rich side stream generated in an SEB could be an economically favorable alternative compared to the use of a nutrient source such as yeast extract (YE), the use of which could prove to be cost prohibitive [80]. The results presented in Paper III showed that it was possible to increase the product formation rate of ethanol during fermentation of a lignocellulosic hydrolysate using this wheat protein hydrolysate (WPH). The effects on fermentation performance using wheat protein hydrolysate as a nutrient source

was compared to the use of YE. In Figure 5 it is shown how these nutrient sources affected the fermentation performance at different inhibitory conditions.

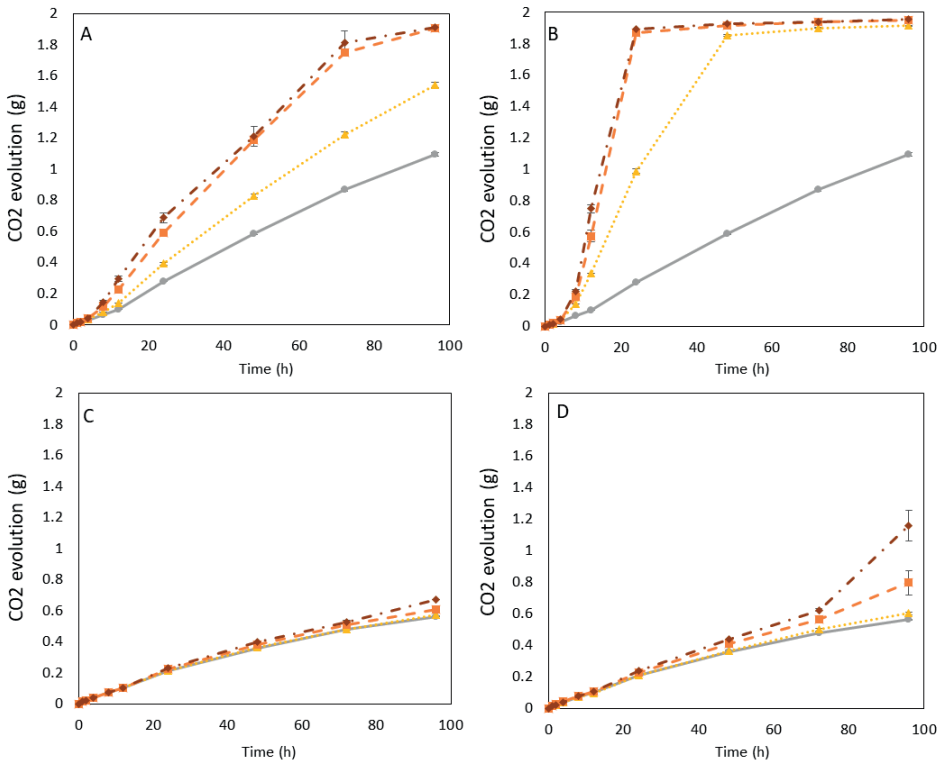

Figure 5. Fermentation time courses of mild and harsh lignocellulosic hydrolysates with addition of YE and WPH as nutrients. Nutrients were added at 0 g/L (●),1 g/L (▲), 4 g/L (■) and 7 g/L (♦). (A) WPH at mild conditions; (B) YE at mild conditions; (C) WPH at harsh conditions; (D) YE at harsh conditions. Adapted from paper III.

While the effects of WPH were not as great as those of YE, as shown in Figure 5, WPH still showed promise as a nutrient source. The differences in fermentation performance observed between WPH and YE were attributed to the relative difference in the abundance of assimilable nitrogen in the two nitrogen sources. The composition of the two nutrient sources was analyzed and presented in Paper III. While the YE was composed of 26% free amino acids with 17 different types of amino acids represented, WPH was composed of only 11% free amino acids with 9 different amino acids represented. However, analysis of the WPH showed that most of the amino acid content of the WPH was still bound in polypeptides, approximately 44%, with at least 14 different types of amino acids represented. As this fraction most likely was not assimilable by the yeast, this demonstrated the potential that optimization of the proteolytic hydrolysis could have on improving the efficacy of WPH as a nutrient source.

The assimilability of the wheat protein hydrolysate has interesting implications on the use of the byproduct stream from the SEB process. By implementing this type of configuration one can achieve a cascaded use of wheat protein nutrients. Since the protein is not lost in the process but rather incorporated into the yeast biomass, this resulting biomass could either be reused as yeast in the process or sold as animal feed in itself. This could be a way of increasing the overall process value of the wheat proteins as they first serve to increase volumetric productivity in fermentation thus increasing profitability without necessarily losing its value as animal feed. However, further investigation is needed in order to validate the viability of the byproduct stream as animal feed.

4.2 Effects of substrate blending ratio

A question of similar importance to how an integrated SEB and LEB process should be configured, is how the performance of such an integrated process is affected at different ratios of SEB to LEB substrate. There is a wealth of external economic and logistical factors that could produce scenarios where pursuing a range of different levels of substrate blending ratios would make sense. Availability of feedstock supply, current market pricing as well as taxation or subsidies related to specific types of raw materials are some examples of factors that could affect the optimal value of this ratio. Furthermore, the optimal process configuration could be different depending on the pursued blending ratio, as there is no guarantee that results from comparisons of process configurations at one blending ratio would be of general validity. Therefore, it is of interest to understand how the blending ratio of substrates in an integrated SEB and LEB process would affect the most important process parameters, in order to facilitate optimal process design choices for specific circumstances.

In this section, the effects of changing the blending ratio from pure LEB substrate all the way to pure SEB substrate are considered. The effects that the blending ratio had on material utilization was investigated in Paper II and Paper IV. The effects that the blending ratio had on the productivity of the process was considered in Paper II-IV.

4.2.1 Integration and substrate utilization

A distinct tendency towards improved substrate utilization was observed for integrated process configurations, as compared to stand-alone processes. In Paper II this was evidenced by a synergistic effect from substrate blending on ethanol yield, as shown in Figure 6.

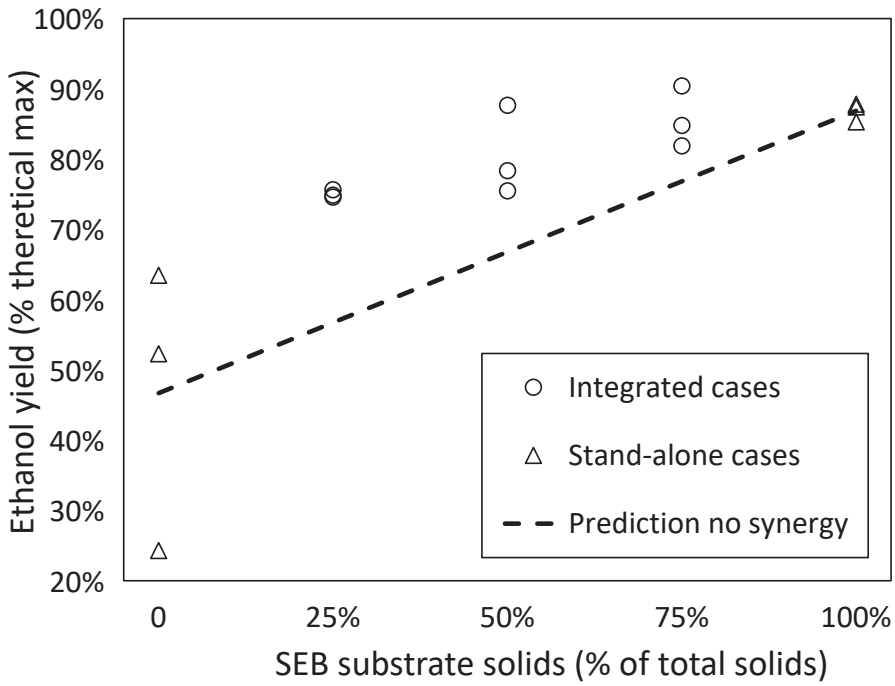

Figure 6. Ethanol yield after 96 hours of stand-alone SEB and LEB (empty triangle) and integrated SSF cases (empty circle). The dotted line is a prediction of the final ethanol yield in the case that it would be a linear combination of the yield in stand-alone-cases. A positive deviation from this prediction in the integrated cases indicates blending synergy. Adapted from paper II.

A similar effect was observed in Paper IV, where fermentation yields were higher in the integrated process cases than in the cases with pure SEB or LEB substrate, as shown in Figure 4. Similar synergistic behavior has been observed previously in studies investigating the integration of SEB and LEB substrates [82,126]. However, since SEB and LEB substrates have characteristics quite different from each other, these results open up two separate lines of inquiry. Firstly, why would substrate blending increase the yield compared to the pure LEB substrate case, and secondly, why would it increase the yield compared to the pure SEB case?

4.2.1.1 *Comparison between integration of LEB and SEB substrates and stand-alone LEB*

Comparing the fermentation dynamics observed in the pure LEB substrate cases with findings from the integrated cases, one thing becomes apparent: PDIs caused a major part of the observed discrepancies. Furthermore, the major part of the observed differences in material utilization was a product of temporal factors, that is to say growth and product formation kinetics. This could be seen in Paper II where the case of stand-alone LEB SSF was shown to be an unstable operating condition with great sample to sample variation with regard to ethanol yield (96 hour), as shown in Figure 6. This was further emphasized by the high concentrations of residual glucose observed at the end of fermentation in the case of SSF with pure LEB substrate, as shown in Figure 7.

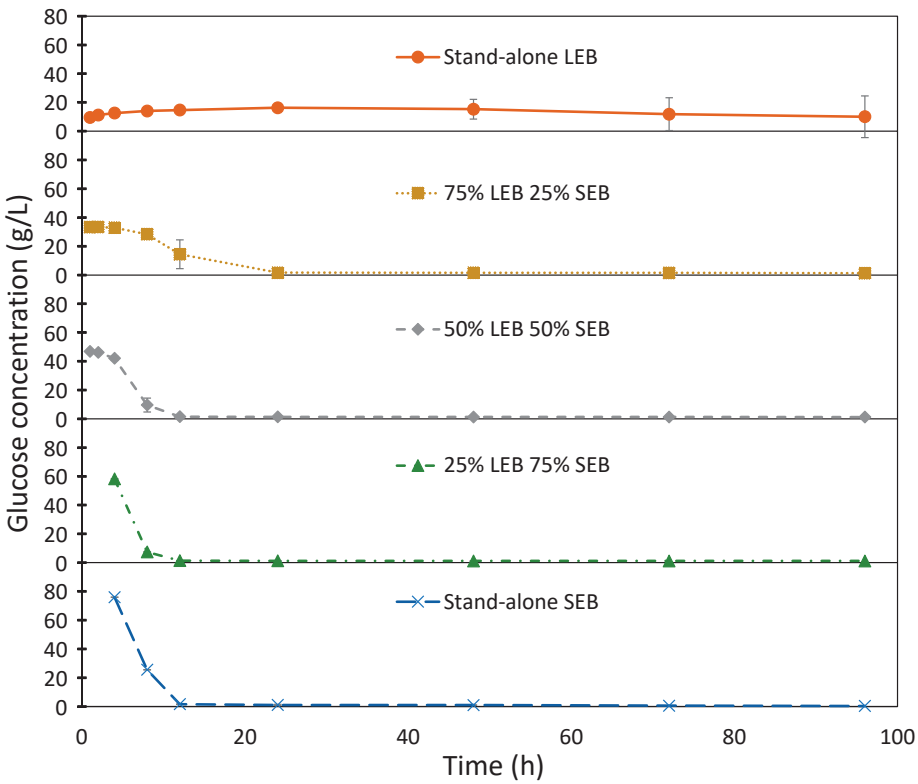

Figure 7. Average glucose concentration during SSF of stand-alone SEB and LEB and integrated cases. Adapted from Paper II.

The influence of PDIs in the stand-alone LEB case was further emphasized by the notable differences observed in inhibitor mitigation time. The time for complete removal of the PDI furfural from the system in the case of stand-alone

LEB was 3 to 5 times longer than in the case with a 25% lower initial concentration of PDIs, as shown in Figure 8. Similar behavior was observed in Paper III, where higher concentrations of PDIs showed clear negative effects on both the growth of yeast and the product formation rate.

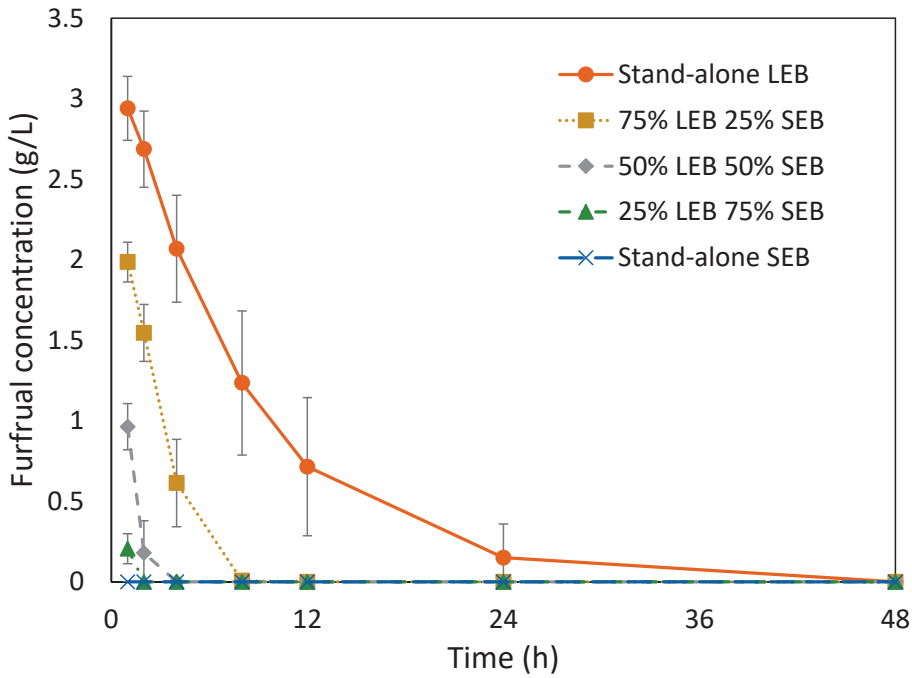

Figure 8. Average furfural concentration during SSF of stand-alone SEB and LEB and integrated cases. Adapted from Paper II.

The same behavior was observed in Paper IV where the pure lignocellulose cases were severely inhibited compared to all other cases, evidenced by low product formation rates and accumulation of glucose in the system. All these results together show that inhibition of the metabolic capacity of yeast contributed to the low degree of material utilization in the stand-alone LEB cases compared to the integrated cases.

Another observation made in Paper IV concerns the hydrolysis yield achieved in integrated cases using the SSF configuration. As mentioned in chapter 4.1.1, operating an integrated SEB and LEB process in the SSF configuration allowed for hydrolysis at comparatively low WIS loadings. This means that adjusting the blending ratio was in principle equivalent to controlling the WIS loading. However, shifting the blending ratio towards high SEB substrate loadings also meant an increase in the concentration of soluble carbohydrates, as the SEB substrate was an enriched source of glucose. This could lead to an increase in

end-product inhibition of hydrolytic enzymes [75]. This type of effect was observed in Paper II, where hydrolysis experiments were performed to determine whether blending SEB and LEB substrates would improve hydrolysis yields. The results from these experiments showed no significant effect of blending ratio on the hydrolysis yield. However, as soluble carbohydrates are continuously removed during SSF, the negative effect that they could have on hydrolysis would be mitigated in that case. This implies that in addition to the general effects of PDI dilution, the combination of lower WIS and continuous removal of soluble carbohydrates could explain the high degree of material utilization observed during SSF of integrated SEB and LEB substrates in Paper IV.

4.2.1.2 Comparison between integration of LEB and SEB substrates and stand-alone SEB

Results from Paper II and IV showed that integrating SEB and LEB substrate streams could improve material utilization in comparison to pure SEB substrate cases. This was shown in paper IV where fermentation yield was higher in the integrated cases compared to stand-alone SEB, as shown in Figure 4. Similar results were observed in Paper II, where the observed yield synergy indicates that integrated cases result in a higher degree of material utilization than in a stand-alone SEB case during SSF. In both cases this was associated with a decrease in the production of glycerol in the integrated cases compared to the stand-alone SEB cases.

In Paper II, increasing the ratio of SEB substrate to LEB substrate was correlated with an increase in the production of glycerol, as shown in Figure 9. Also, results from Paper IV show that the ratio of glycerol to ethanol increased as the amount of LEB substrate in the fermenter was decreased. In yeast cells, glycerol production requires the consumption of glucose [127,107]. Additionally, glycerol production is correlated with the production of cell-mass [127,128], which is also a glucose-consuming process. As glucose is the substrate for ethanol production, any increase in the production of other metabolites would lower the ethanol yield.

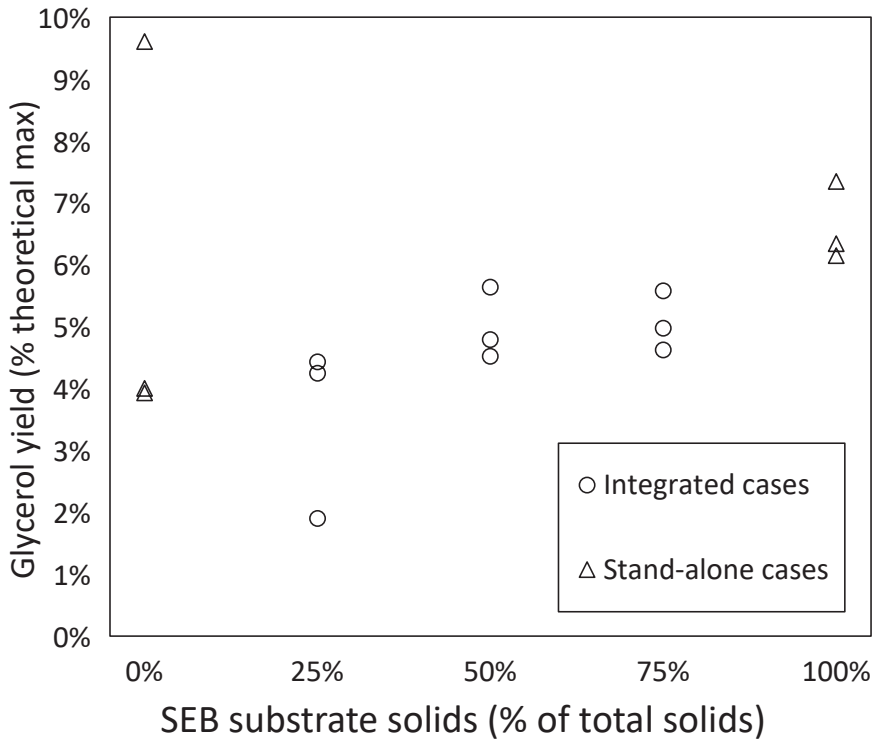

Figure 9. Glycerol concentration after 96 hours of stand-alone SEB and LEB (empty triangle) and integrated SSF cases (empty circle). Adapted from Paper II.

In order to explain the underlying causes leading to synergistic behavior in blended cases compared to the case of stand-alone SEB, a more thorough explanation of the mechanisms underlying glycerol production is required. The production of glycerol can fill several different functions in the metabolism of yeast. One of the main functions of glycerol production is to maintain the intracellular redox balance of the yeast. Many important cellular processes are driven by oxidative reactions, mainly biosynthesis of cell-mass [107]. These reactions depend on the reduction of the cofactor NAD^+ to NADH and $NADP^+$ to NADPH. The surplus NADH and NADPH that is produced has to be reoxidized in order for these processes to be sustained, which can be accomplished by the production of glycerol [107]. Based on these reactions, there are two different ways of explaining why blended substrate cases would result in lower glycerol production.

Firstly, one of the main differences between the blended cases and the pure SEB substrate case was the presence of PDIs in the blended cases, originating from the LEB substrate. It has been proposed that the mechanism by which PDIs, such as

furfural are biologically mitigated, is through a reduction reaction to the corresponding alcohol, which in the case of furfural is furfuryl alcohol [129]. This reduction reaction would be facilitated by oxidization of NADH to NAD^+. The surplus of NAD^+ produced in this process would decrease the need to produce glycerol which in turn would make more glucose available for ethanol production. A similar effect has been observed in previous studies investigating the influence of furfural on fermentation [130]. However, it has to be noted that results that contradict the logic of this argument were observed in the case of stand-alone LEB, as it had the highest concentrations of PDIs while resulting in a higher average glycerol concentration than some of the blended cases, as shown in Figure 9. However, considering the divergent behavior within samples with regard to ethanol production and glucose consumption it is possible that some other causal factor not accounted for affected the dynamics in this specific case.

The second way of explaining the change in glycerol production is mainly related to cell growth. Since biosynthesis reactions are largely responsible for driving the production of glycerol, a general decrease in the production of cell-mass would be expected to be accompanied by a decrease in glycerol production. The presence of PDIs during fermentation has been shown to reduce cell growth [91,79,130]. Since the initial concentration of PDIs would be directly proportional to the loading of LEB substrate, assuming that any negative effect of PDIs on growth would decrease as the ratio of SEB substrate was increased in the system, would be reasonable. This kind of effect on cell growth was observed in paper III, as shown in Figure 10. This indicates that the observed changes in glycerol concentration could have been an effect of inhibited growth.

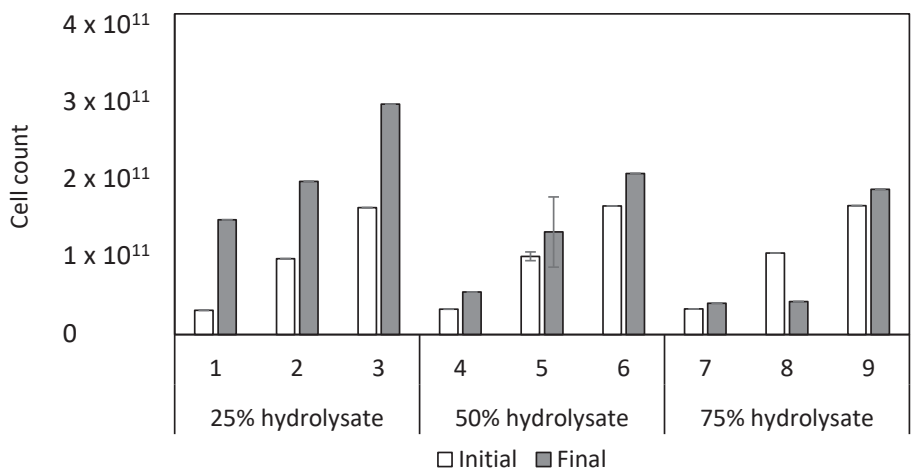

Figure 10. Initial and final cell count from factor experiments investigating the effect of lignocellulosic hydrolysate loading and initial yeast loading on fermentation performance. The number under the bars represent the experimental condition as described in Paper III. Adapted from paper III.

4.2.2 Integration and product formation rates

The product formation rate was found to be affected by the blending ratio of SEB and LEB substrate. In Paper II, it was shown that even a small shift away from stand-alone LEB towards increased SEB substrate ratio led to a shift in the rate-limiting conversion step from fermentation to hydrolysis. This observation was evidenced by the accumulation of glucose in the case of stand-alone LEB, as shown in Figure 7. In Paper III it was also observed that nutrients from an SEB material stream could be utilized to increase the rate of product formation during fermentation of lignocellulosic hydrolysates. The results from these papers clearly show the power of different integration strategies to significantly improve the volumetric productivity and decrease residence times in biorefineries.

Changing the blending ratio had an effect on several factors that would be expected to have an impact on the fermentation kinetics. These were mainly the concentration of PDIs and the concentration of nutrients. Out of these factors, PDI concentration was observed to have the most evident effect on product formation rates. In Paper III, the effects of PDI loading and yeast loading were investigated. As shown in Figure 11, fermentation at the highest level of PDIs resulted in a drastic reduction of the rate of ethanol production. This shows the clear advantage of inhibitor dilution that comes about naturally by shifting the blending ratio towards SEB substrate.

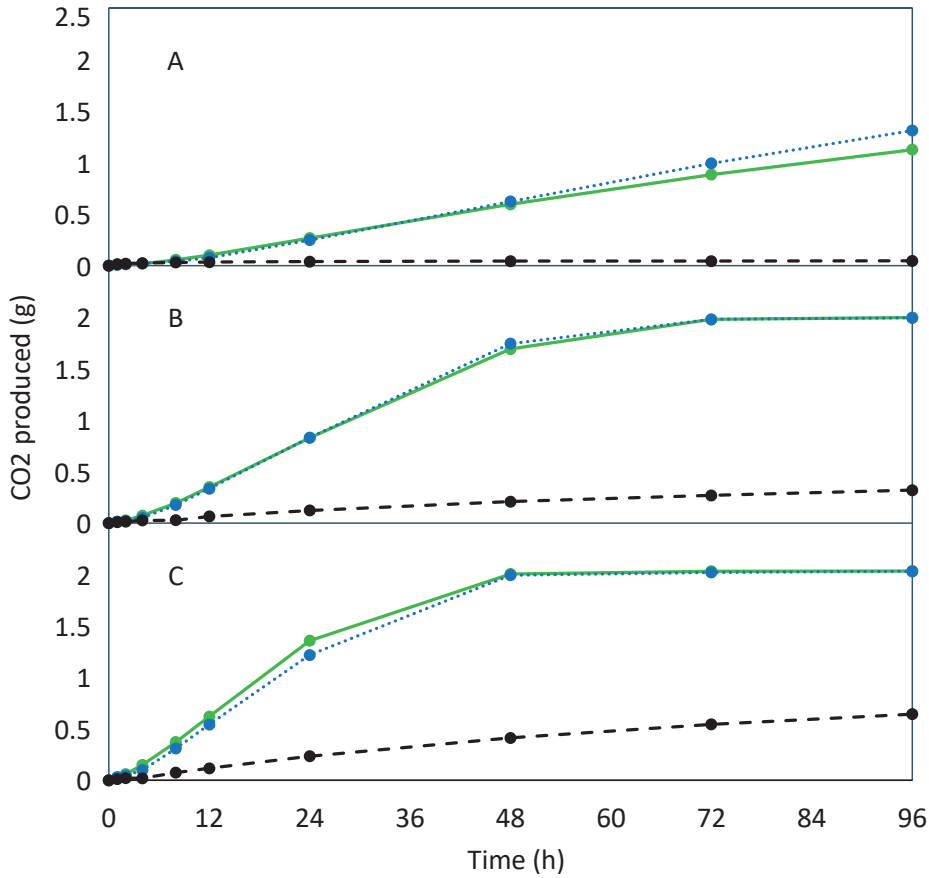

Figure 11. CO2 evolution during fermentation from experiments investigating the effects of lignocellullosic hydrolysate loading and initial yeast loading. Lignocellulosic hydrolysate made up 25% (solid line), 50% (dotted line) and 75% (dashed line) of total reaction mass. The initial yeast loadings were 0.5 g/L (A), 1.5 g/L (B) and 2.5 g/L (C). Adapted from Paper III.

What is interesting to note is that the responses in productivity was non-linear with respect to PDI concentration (Paper II-IV). The volumetric productivity was nearly unaffected by the PDI concentration below a certain threshold value at any yeast loading, as shown in Figure 11. Similar non-linearity was observed in Paper II, where a distinct increase in the volumetric productivity was observed when the SEB to LEB substrate ratio was increased above 25% SEB substrate, as shown in Figure 12. When comparing the performance of the integrated cases in the SSF configuration to the counterpart pure substrate cases in Paper IV, it was observed that the integrated cases displayed comparable or superior yield and volumetric productivity to the stand-alone SEB case, as shown in Figure 4. Conversely, in the stand-alone LEB SSF case, ethanol yield only reached approximately 20% within the timespan of the experiment, while significant amounts of glucose

(above 40 g/L) were still detected in the system. These findings all point to the existence of distinct concentration threshold values for inhibition of cellular functions caused by PDIs.

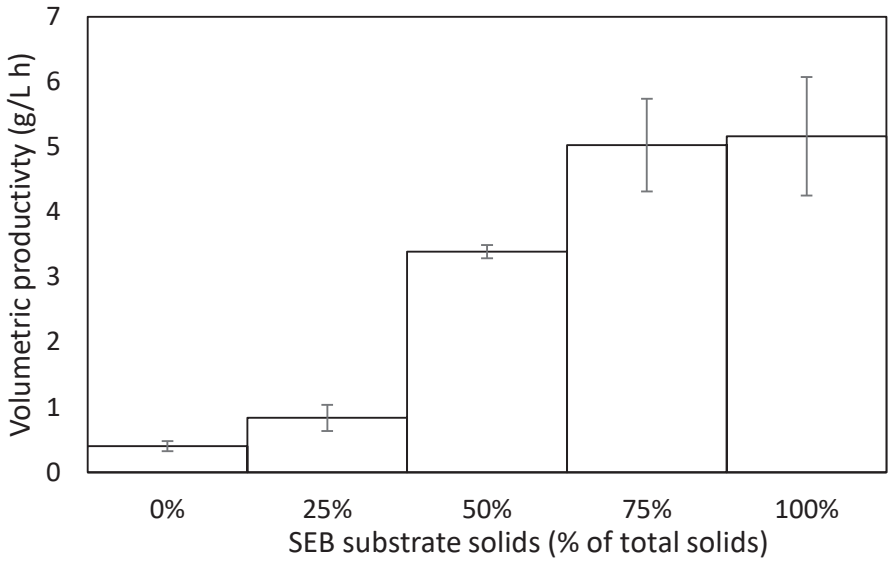

Figure 12. Average volumetric productivity after 24 hours at different substrate blending ratios from stand-alone LEB to stand-alone SEB.

In addition to the effects of PDIs, nutrients derived from SEB substrate streams were demonstrated to have the potential to significantly affect the volumetric productivity of an integrated SEB and LEB process. In Paper III it was shown that the protein from the SEB substrate stream could be hydrolyzed. The resulting hydrolysate could then be used to increase the productivity of fermentation inhibited by PDIs from an LEB process, as shown in Figure 5. However, as shown in Figure 5C and 5D, the results in Paper III also demonstrated the limitations of using nutrients to boost productivity at high concentrations of PDIs. These results highlight the potential that integrating SEB and LEB processes has to improve the productivity compared to a stand-alone LEB. Furthermore, it highlights the importance of rational decision making when choosing conditions such as blending ratio in order to maximize the benefits that could be gained from such an integration.

4.2.3 Process implications of substrate blending

When deciding the substrate blending ratio in an integrated SEB and LEB process, there are different considerations that must be made. Whether considering the design of a greenfield process or the implementation of a retrofit, this problem can be tackled from the perspective of either an LEB or SEB base case. From the perspective of an LEB, the integration of even a small stream of SEB substrate can have significant positive impacts on the robustness of the process, the achievable product concentrations, product formation rates, need for detoxification, and potential process yields. From the perspective of an SEB, adding a small portion of LEB substrate into the process can be done without expecting any significant losses in the robustness of the process with regard to product formation rates or ethanol yield. Integrating an LEB substrate stream could even be beneficial from the perspective of maximizing substrate utilization, as it appears to shift the distribution of metabolic products in favor of ethanol.

While the ratio at which the two process streams are mixed can have considerable implications on the performance of the hydrolysis and fermentation, the effects that it might have downstream from the fermentation also have to be considered. In the case of an SEB, an important revenue stream comes from the production of DGS which is sold as animal feed [51]. This product comes from the residual solids after fermentation, and is a mix of the unfermented fraction of the wheat grain, which is made up of proteins, lipids and fiber [131]. Additionally, it contains yeast from the fermentation [132] and other solubles such as residual sugars. Depending on the specific process configuration chosen when integrating an SEB and an LEB, the composition of the residual solids could be altered significantly, compared to what would be expected from a stand-alone SEB. The main concern stems from the increase in the lignin content of the residual solids that could come as a result of blending LEB substrate into the process. Such an increase could cause reduction of the nutritional value of the animal feed product, since lignin is generally considered to be indigestible [51]. The extent of this change would largely depend on the specific SEB to LEB substrate ratio in the process. This means that any gains in ethanol production performance should be weighed against potential losses in the quality of the animal feed product.

5 Maximizing feedstock utilization

Biorefineries based on the production of ethanol mainly focus on efficient utilization of cellulose and in some cases hemicelluloses in the case of LEBs, while starch is the main component of interest in SEBs. However, significant parts of the biomass are composed of other constituents that are not utilized in the production of ethanol. In addition to cellulose and hemicelluloses, a significant part of LEB feedstocks is made up of lignin, ash and extractives. In SEB feedstocks, a significant part consists of protein, lipids and lignocellulosic fibers. In traditional stand-alone plants, the issue of utilizing all of the biomass can be a challenge, and integration of an SEB and an LEB can make that challenge even more difficult. Integrating the processes in a way that results in blending of the substrates can increase the complexity of required separation processes for the isolation of individual components, or result in altered characteristics of existing byproduct streams from the original processes. Therefore, it is important to consider how an overall process can be configured in order to maximize the utilization of all fractions in order to valorize the material rather than create waste.

As mentioned in section 4.2.3, traditional SEBs commonly produce an animal feed coproduct in the form of DGS. This byproduct is composed of protein, lipids, residual soluble components, nondigested fiber and yeast. By combining the fermentation of SEB and LEB substrate, the characteristics of this coproduct stream will be altered. The way in which these characteristics are altered depends on the mode of integration. For example, operating the biorefinery in a SSF configuration as described in this thesis will result in lignin from the LEB feedstock being mixed with the residual solids from the SEB process. This has two negative consequences on the process. Firstly, the addition of lignin to the animal feed product will decrease the nutritional value on a mass basis, as lignin is not digestible by animals [51] and has been connected with the decrease of animal feed palatability [133]. Secondly, by mixing the lignin into the residual solids after fermentation, the potential for lignin valorization through other conversion routes is lost.

5.1 Sequential fractionation of lignocellulose components

In Papers V and VI the potential of mitigating the problem of material mixing by the introduction of a sequential fractionation of the LEB feedstock before hydrolysis and fermentation was investigated. The goal of the proposed process configuration was to remove hemicelluloses from the lignocellulosic feedstock after steam explosion (STEX) followed by lignin removal using hydrotropic extraction (HEX). Combining these fractionation processes opens up several new avenues for increasing the overall utilization of materials in the process. By recovering hemicellulose carbohydrates separately they can be be reintroduced at a later stage of the process or be used in an entirely separate process based on the pentose sugar platform. Likewise, by separating lignin from the LEB substrate it can be used for the production of lignin platform chemicals.

In Paper V, the impact of temperature, residence time and biomass solids loading on the extraction of lignin in wood chips was investigated. The study showed that up to approximately 70% of the lignin from an LEB substrate stream could be removed by HEX at optimal conditions when performed in the sequential fractionation configuration. However, it also showed that it was possible to extract a high amount of lignin at very mild conditions. In the same process configuration, approximately 50% of the lignin content of a pretreated LEB feedstock could be extracted by HEX at 25°C.

In Paper VI, the effect of sequential fractionation using wheat straw as feedstock was investigated as well as the effect that this kind of treatment would have on a subsequent SSF of the resulting cellulose fraction. The fractionation of wheat straw using the sequential fractionation method was successfully demonstrated resulting in three separate fractions enriched in hemicellulose, lignin and cellulose, respectively. A schematic illustration of an integrated wheat grain and wheat straw process with sequential fractionation prior to integration is shown in Figure 13.

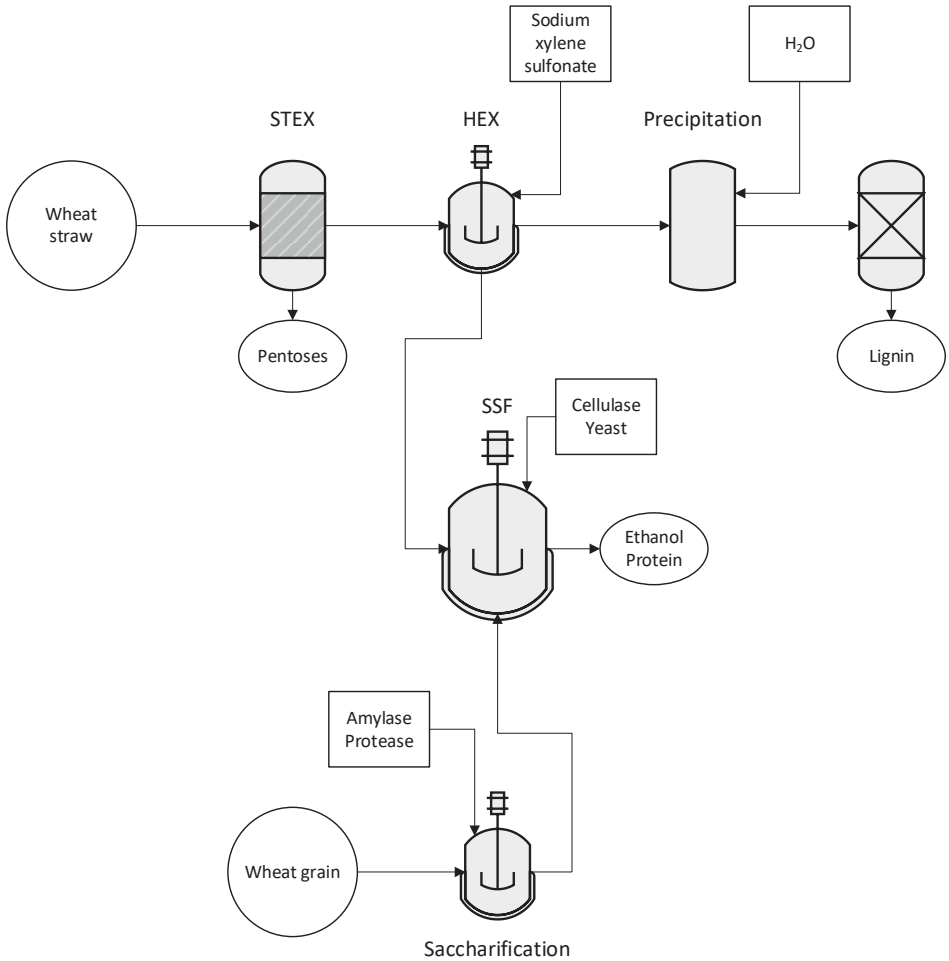

Figure 13. Schematic flowsheet of an integrated wheat grain and wheat straw biorefinery with sequential fractionation of the wheat straw prior to integration.

In Paper VI, it was also shown that the resulting cellulose fraction had improved hydrolyzability characteristics compared to substrate only subjected to STEX. The resulting cellulose fraction also displayed favorable fermentation characteristics resulting in higher ethanol yield and productivity compared to the STEX reference case. A comparison of the hydrolysis and fermentation time courses during SSF for STEX treated, and sequentially fractionated wheat straw is shown in Figure 14. The removal of lignin from the biomass by HEX was most likely the cause of the improved hydrolyzability of the material. The presence of lignin subjected to STEX has previously been shown to increase the non-productive binding of enzymes for bioconversion of lignocellulose [134]. While

STEX treated lignin will be present in both cases, the amount was lower in the material also treated with HEX.

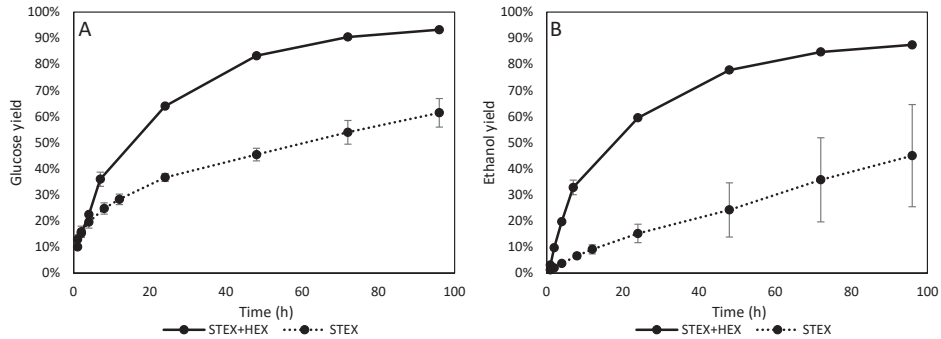

Figure 14. Glucose yield (A) and ethanol yield (B) during SSF of STEX pretreated wheat straw with and without HEX treatment. The WIS was 10% for both the STEX and STEX+HEX cases. Adapted from Paper VI.

5.2 Isolation of lignin

By removing the lignin before SSF, an enriched lignin side stream can be created. This fraction can be used for the production of chemicals via thermochemical pathways. Furthermore, by separating this lignin from the rest of the material, issues of lignin inhibiting hydrolysis and fermentation as well as contaminating the residual solids after fermentation are minimized. The composition of the cellulose and lignin fractions from sequentially fractionated birch-wood chips (Paper V) and wheat straw (Paper VI), is shown in Table 3.

Table 3. Composition of fractions retreived from sequential fractionation.

Feedstock	Cellulose	Hemicelluloses	Lignin
Birch			
Raw material	43.5	24.3	27.4
Lignin fraction	7.0	8.1	85.0
Cellulose fraction	72.8	6.8	15.4
Wheat straw			
Raw material	38.9	35.7	16.2
Lignin fraction	51.9	10.8	37.3
Cellulose fraction	69.0	1.6	20.0

In the case of wheat straw, a relatively large part of the cellulose was extracted with the lignin fraction. The HEX conditions used for the extraction of lignin from wheat straw were not optimized and were most likely too severe for the material in question. However, the findings that a large amount of the lignin in STEX pretreated birch chips could be extracted at mild conditions (Paper V)

indicates that further investigation of conditions for HEX of wheat straw could result in more favourable lignin recovery and selectivity.

The enriched lignin recovered after HEX could be used for synthesis of lignin platform biorefinery products. In Paper V, it was shown that the properties of the extracted lignin could be altered depending on the temperature during HEX. Performing HEX at low temperatures resulted in a lignin with higher molecular weight and higher oxygen content, while higher temperatures resulted in a lignin with lower molecular weight and lower oxygen content. These results present a trade-off that has to be considered when choosing the operating conditions for the HEX.

5.3 Factor screening experiments

An experiment was designed to investigate the effect of three different factors on the lignin recovery during HEX of STEX treated birch (Paper V). The experimental series was designed according to the 311B screening design developed by Roquemore [135]. The investigated factors were residence time, temperature and biomass loading. Multiple linear regression was used to fit the data from the experiments to a quadratic response surface model. The results from these experiments are presented in Figure 15 as response surfaces.

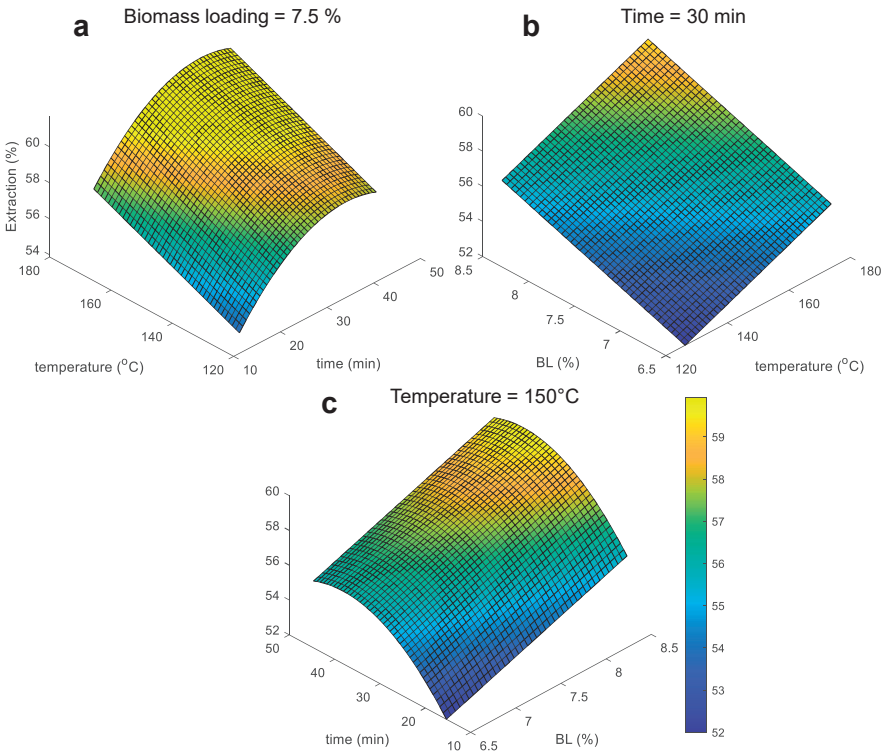

Figure 15. Response surfaces representing the effects of residence time, temperature and biomass loading on lignin recovery at (A) constant biomass loading of 7.5%, (B) constant residence time of 30 minutes and (C) constant temperature of 150°C. BL is biomass loading. Adapted from Paper V.

The results presented in Paper V showed that the residence time, temperature and biomass loading all had an impact on the lignin recovery during HEX of STEX treated wood chips. However, the effect of each of these factors was limited. Higher lignin recoveries could be achieved by increasing the residence time but a saturation of the effect was observed when increasing the residence time above 30 minutes, represented by the curvature of the response surface shown in Figure 15. Increasing the biomass loading was also shown to increase the lignin recovery. This was attributed to an increased solubility of lignin at higher lignin concentrations, an effect previously observed by McKee et.al [136]. Increasing the temperature during HEX was shown to increase lignin recovery. This was attributed to further hydrothermal degradation of the lignocellulosic material at elevated temperatures. Further investigation of the effect of temperature on lignin recovery in Paper V revealed that in the temperature span between 25-100°C the temperature had no discernible effect on the lignin recovery. This indicated that a significant portion of the lignin was made available for extraction by STEX. Furthermore, it was shown that HEX at temperatures above 200°C led to

excessive degradation of the lignocellulose and subsequently to a loss of selectivity with regard to lignin recovery.

6 Conclusions

Integration of a process stream from an SEB into an LEB can improve the performance of the LEB process compared to a stand-alone LEB. Blending the streams of these two processes can improve the productivity during fermentation of the LEB process stream by diluting PDIs while maintaining a high substrate concentration. Furthermore, hydrolysis of proteins in the SEB process stream can improve the productivity by providing nutrients which help the yeast to cope with PDIs. By improving the productivity, the residence time can be reduced, which can lower investment costs and improve material utilization.

The results from the integration of LEB and SEB showed that most of the negative effects on the productivity and yield of ethanol production associated with processing the LEB process stream were absent above a certain blending ratio. Furthermore, the results showed evidence of improved material utilization in the integrated cases also when compared to stand-alone SEBs. This shows that the integration of LEB and SEB processes could be beneficial from the perspective of an existing SEB.

Comparing the SSF and SHF configurations, in an integrated LEB and SEB process, showed that SSF performed better with regard to both product yield and combined residence time. This observation was partly attributed to the SSF configuration resulting in more favorable operating conditions for hydrolysis with reduced insoluble solids loading and product inhibition by soluble carbohydrates.

While SSF presented favorable performance with regard to ethanol production, operating the process in this configuration would result in blending of the residual solid components from the LEB and SEB process streams. This would remove the possibility of valorizing the lignin in the LEB substrate stream as well as diminishing the value of the residual solids as an animal feed. The prospects of introducing a sequential fractionation of the LEB substrate stream by STEX and HEX in order to alleviate this problem was investigated. It was shown that this method of sequential fractionation could be used to produce enriched streams of cellulose and lignin which addresses both the concern of lignin valorization and animal feed value in an integrated LEB and SEB process. Furthermore, it was shown that sequential treatment of the LEB substrate resulted in a cellulose fraction with improved hydrolyzability during SSF.

6.1 Future prospects

While the use of hydrolyzed proteins from the SEB substrate stream was shown to be effective in increasing the productivity during fermentation of LEB substrate, analysis of the protein hydrolysate revealed that the full potential of this nutrient source had not been unlocked. Further optimization of the hydrolysis procedure and conditions, enzyme loading as well as the composition of the enzyme cocktail used for hydrolysis could potentially improve the properties of the protein hydrolysate to the point where it could compete with YE as a nutrient source.

Sequential fractionation of the LEB substrate was shown to work as a method of producing enriched fractions of the different lignocellulosic components. However, the selectivity towards lignin extraction during HEX of pretreated wheat straw was low compared to what was achieved with birch wood chips. The conditions used for the STEX treatment of the wheat straw were based on conditions previously optimized for a subsequent enzymatic hydrolysis rather than a fractionation process. A thorough investigation of the combined effect of STEX and HEX conditions on the fractionation of wheat straw would be valuable for further optimization of the process.

Investigating the effect that integration of LEB and SEB substrate streams would have on the resulting residual solids after fermentation and the value these solids would have as an animal feed product would be of interest. Doing this would help determine how to integrate these processes without affecting existing revenue streams. Factors such as the process configuration, blending ratio and the implementation of a sequential fractionation stage could all have an impact on the quality of the residual solids. Furthermore, utilizing the technique of protein hydrolysis for generating fermentation nutrients implies the transformation of SEB substrate stream proteins into yeast protein. Investigating the impact that this would have on the nutritional value of the residual solids could also be of interest.

The results presented in this thesis provide an empirical basis for how different design choices could affect the performance of specific sub-processes in an integrated LEB and SEB process. However, a full evaluation of the economic viability of the proposed process designs would require rigorous techno-economic evaluations of the entire process in order to take all potential implications on operational and capital expenditures into account.

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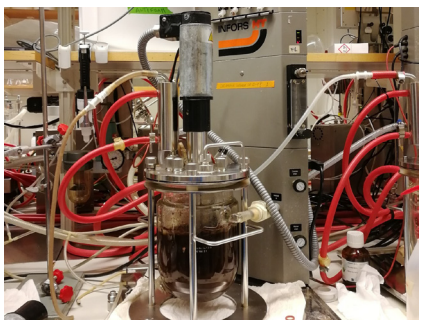

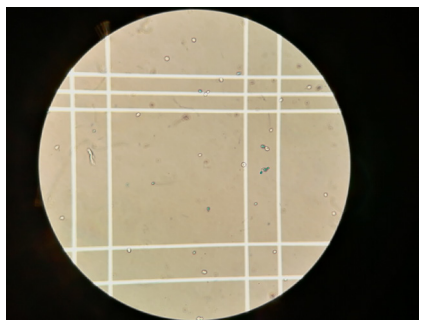

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