

# Conditioning of SO<sub>2</sub> - ethanol-water (SEW) spent liquor from lignocellulosics for ABE fermentation to biofuels and chemicals

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Evangelos Sklavounos



Conditioning of SO<sub>2</sub> -ethanol-water  
(SEW) spent liquor from  
lignocellulosics for ABE fermentation  
to biofuels and chemicals

**Evangelos Sklavounos**

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**Abstract**

This thesis introduces a biorefinery process to fractionate lignocellulosics followed by treatment of the produced hydrolysate for microbial fermentation to acetone, butanol and ethanol (ABE). The process utilizes SO<sub>2</sub>-Ethanol-Water (SEW) fractionation technology and a ‘conditioning’ protocol to treat SEW spent liquor for ABE fermentation by *Clostridia* bacteria.

It is found that SEW fractionation of spruce chips, mixed softwood biomass and Oil Palm Empty Fruit Bunch (OPEFB) at conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, liquor-to-feedstock (L/F) ratio of 3 L kg<sup>-1</sup>, 150°C, 30 min, is suitable for industrial scale application. SEW fractionation is followed by pulp washing and ‘conditioning’ to detoxify the spent liquor and to increase its monosugars content. The ‘conditioning’ scheme in its basic form comprises of the consecutive steps of vacuum evaporation, steam stripping, liming and catalytic oxidation.

‘Conditioning’ successfully removes most ABE fermentation inhibitors for *Clostridia*. It also allows almost total recovery of the cooking chemicals (ethanol and SO<sub>2</sub>) leading to an economical and environmentally benign process. Levels of residual inhibitory dissolved lignin in the final conditioned liquors correspond to only about 10% of the original lignin in the respective feedstocks. However, these levels are still too high for microbial ABE fermentation and therefore additional treatment with anion exchange resins followed by 4-fold dilution is employed before ABE fermentation to reach dissolved lignin levels of approximately 1 g L<sup>-1</sup> (tolerance limit for *Clostridia*). All the different feedstock-based hydrolysates that are produced after ‘conditioning’ are fermentable by *Clostridia* and ABE solvents (mostly butanol) are produced at satisfactory total concentration and yield.

Hydrolysis of OPEFB fibers in particular is impaired (compared to spruce) due to their high ash/alkali metals content. Acidic leaching of this feedstock did not remove sufficient amounts of metal cations leading to only marginally improved hydrolysis. However, it is possible to improve hydrolysis of this feedstock by adding inorganic acids (nitric, phosphoric) in the fresh fractionation liquor at a level to provide the required nutrients for *Clostridia*.

Finally, it is demonstrated that by introducing some small modifications to the basic SEW spent liquor ‘conditioning’ scheme and by performing nanofiltration instead of resins treatment it is possible to reach lower dissolved lignin levels (below 1 g L<sup>-1</sup> upon 4-fold dilution) in the feed liquor for fermentation. Furthermore, it is possible to significantly improve the production of solvents and ABE fermentation yield; total solvents concentration increases from 7 to 11 g L<sup>-1</sup>, yield increases from 0.26 to 0.30 g g<sup>-1</sup> sugars.

**Keywords** ABE fermentation, Biofuels, Biomass, Biorefinery, Butanol, Conditioning, SO<sub>2</sub>-ethanol-water fractionation

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# PREFACE

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Helsinki, April 8<sup>th</sup>, 2014

*Evangelos Sklavounos*

## LIST OF PUBLICATIONS

- Paper I** Sklavounos, E., Iakovlev, M., Yamamoto, M., Teräsvuori, L., Jurgens, G., Granström, T., van Heiningen A., (2011), Conditioning of SO<sub>2</sub>-ethanol-water spent liquor from spruce for the production of chemicals by ABE fermentation, *Holzforschung* 65(4):551-558.
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- Paper III** Sklavounos, E., Iakovlev, M., Survase, S., Granström, T., van Heiningen, A., (2013), Oil palm empty fruit bunch to biofuels and chemicals via SO<sub>2</sub>-ethanol-water fractionation and ABE fermentation, *Bioresource Technology* 117:102-109.
- Paper IV** Sklavounos, E., Iakovlev, M., Survase, S., Gouveia, S., Moldes, D., Sanromán, M. A., van Heiningen, A., (2014), Comparison of two conditioning schemes for detoxifying SO<sub>2</sub>-ethanol-water hydrolysate from lignocellulosics for ABE fermentation, *Nordic Pulp and Paper Research Journal*, accepted.

### **Author's contribution to the appended joint publications:**

- Papers I-IV** Evangelos Sklavounos was responsible for the experimental design, performed the experimental work, analyzed the results and wrote the manuscripts as principal author.



# LIST OF ABBREVIATIONS

ABE – acetone, butanol, ethanol  
AFEX – ammonia fiber explosion  
ARP – ammonia recycled percolation  
ASAM – alkaline sulfite anthraquinone methanol  
AVAP – American value added pulping  
CED – cupriethylenediamine  
CHN/S – carbon, hydrogen, nitrogen/sulfur  
CHP – combined heat and power  
DP – degree of polymerization  
GC – gas chromatography  
GGM – galactoglucomannan  
HMF – hydroxymethylfurfural  
HPAEC – high-performance anion exchange chromatography  
HPLC – high-performance liquid chromatography  
IC – ion chromatography  
ICP/AAS – inductively coupled plasma/atomic absorption spectroscopy  
L/F ratio – liquor-to-feedstock ratio  
LCC – lignin-carbohydrate complex  
 $M_w$  – weight average molecular mass  
MWL – milled wood lignin  
NF – nanofiltration  
OPEFB – oil pulp empty fruit bunch  
PCL – precipitated condensed lignin  
p-DADMAC – polydiallyldimethylammonium chloride  
PES – polyethersulfone  
PHL – prehydrolysis liquor  
PKC – palm kernel cake  
RO – reverse osmosis  
SEC HPLC – size exclusion high-performance anion exchange chromatography  
SEW – SO<sub>2</sub>-ethanol-water  
SPORL – sulfite pretreatment to overcome recalcitrance of lignocellulose  
SSL – spent sulfite liquor  
UF – ultrafiltration  
UV – ultraviolet

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# 1 INTRODUCTION

Rising crude oil prices, declining energy security due to diminishing conventional oil reserves and pressing environmental problems such as global warming attributed to the use of fossil fuels, have recently brought growing attention to the conversion of lignocellulosic biomass into transportation fuels and chemicals. The need for efficient conversion of biomass to biofuels has led to the development of many different biomass fractionation technologies. Their aim is to deconstruct biomass into its principal components and utilize pure sugar fractions, for example the hemicellulose fraction, as feedstock for bioconversion to biofuels. However, only a few of these technologies are economically viable for industrial applications. A promising fractionation technology for economic industrial production of biofuels and chemicals from biomass is the SO<sub>2</sub>-ethanol-water (SEW) fractionation originally introduced as pulping method in the 1950s (Schorning 1957).

Until recently, biofuels production has been mainly focused on the fermentative production of ethanol. However, butanol has better properties as a replacement fuel for gasoline as it has lower volatility, higher octane number and higher energy content. Furthermore, use of butanol as biofuel does not require any changes of the existing fuel transportation infrastructure. Butanol may be produced by fermentation via the acetone-butanol-ethanol (ABE) process. This technology, which was originally introduced in the 1920s to produce mainly acetone from starch, has been discontinued since the middle of the last century due to high feedstock costs and availability of cheap oil. The current rise in crude oil prices and the availability of cheap lignocellulosic biomass such as residues from the forest industry may renew interest in this process (Rakkolainen et al. 2009).

In the present work a new scheme to 'condition' SEW spent liquor from lignocellulosics for ABE fermentation is presented. The target is to produce a mixture of hemicellulose monosugars that is free of major fermentation inhibitors for use as substrate in microbial fermentation to organic solvents. The process is suitable for large scale application in a lignocellulosic biorefinery that will produce a wide range of bioproducts. The development of this process has followed an evolutionary approach which is presented in original Papers I-IV. Particular emphasis is given on:

- Carbohydrates mass balance during fractionation and 'conditioning'
- Lignin mass balance during fractionation and 'conditioning'
- Removal of ABE fermentation inhibitors (ethanol, SO<sub>2</sub>, furanic compounds, formic acid and dissolved lignin) during 'conditioning'
- Liquor purification step with resins or membrane filtration before ABE fermentation

## **2 BACKGROUND**

### **2.1 Lignocellulosic biomass constituents**

#### **2.1.1 Cellulose**

Cellulose is the main constituent of lignocellulosic biomass as it accounts for about 35-50% of its dry weight (Sjöström 1993). It is a homopolysaccharide that consists of D-glucopyranose units which are linked together by  $\beta$ -1,4-glucosidic bonds. The molecules form long linear chains (DP of 10,000-15,000) and have a strong tendency to form intra- and intermolecular hydrogen bonds. These bonds promote the formation of molecule aggregates known as microfibrils, in which crystalline regions alternate with amorphous regions. Microfibrils form the building blocks of cellulose fibers. As a result of its special structure cellulose has a high tensile strength and is insoluble in water and most solvents (Sjöström 1993).

#### **2.1.2 Hemicelluloses**

Hemicelluloses account for about 25-35% of the dry weight of lignocellulose (Stenius 2000). Like cellulose most hemicelluloses function as supporting material in the cell walls. Hemicelluloses are heteropolysaccharides that consist of D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, uronic acids and small amounts of L-rhamnose. The molecular chains are much shorter than in the case of cellulose (DP of 20-220), having side groups and being branched in some cases (Fengel and Wegener 1984). Softwoods have a lower hemicellulose content than hardwoods (25-30% vs 30-35%, respectively, Stenius 2000). Softwood hemicelluloses consist mostly of galactoglucomannans (about 20%) and arabinoglucuronoxylan (5-10%) whereas hardwood and annual plant hemicelluloses

consist mostly of xylan. Some hemicelluloses are soluble in water and most of them are relatively easily hydrolyzed by acids (Sjöström 1993).

### **2.1.3 Lignin**

Lignin accounts for about 20-30% of the dry weight of lignocellulose (Sjöström 1993). Its most commonly noted function is strengthening of the cell walls in plants. Lignin is a heteropolymer built from phenylpropane units (guaiacyl, syringyl and hydroxyphenyl) bound by ether and carbon-carbon bonds. It is bound to polysaccharides by covalent bonds. Benzyl ether, benzyl ester and phenylglucosidic bonds have been reported. It is generally known that softwoods have higher lignin content than hardwoods (25-30% vs. 20-25%, respectively, Stenius 2000).

Lignins are typically separated from polysaccharides in the form of ‘milled wood lignin’ (MWL), ‘dioxane lignin’, or ‘enzymatically liberated lignin’. However, there are several industrially based lignins that are by-products of the chemical pulping. Kraft lignin (or sulfate lignin), alkali lignin (or soda lignin) and lignosulfonates are derived from kraft, soda-anthraquinone and sulfite pulping of lignocellulosics, respectively (Stenius 2000). Lignosulfonates are also derived from the SEW process which is discussed in the current work.

## **2.2 Techno-economical potential of lignocellulosic biomass**

Lignocellulosic biomass exclusion from the human nutrition chain, its relatively low cost and its carbon neutrality make it a suitable candidate feedstock for the production of sustainable liquid transportation fuels (Huber et al. 2006). However, the lack of a proven economic technology and the high capital cost relative to the



production of fossil-based transportation fuels due to the much smaller scale of biomass processing (van Heiningen et al. 2011) have been key obstacles to its utilization for the production of biofuels. It is reported (van Heiningen 2006, Iakovlev 2011, Jurgens et al. 2012) that it is possible to improve the techno-economic potential of biomass for the production of liquid biofuels by integration within an existing industrial pulp and paper facility that will also produce other wood products (Integrated Forest Biorefinery, van Heiningen 2006). Integration within such a lignocellulosic biorefinery may allow for economies of scale as biomass harvesting and collection can be performed by utilizing the existing infrastructure to minimize feedstock cost and maximize supply. It may also lead to lower biomass processing costs by use of existing facilities such as steam/power and water effluent treatment plants. Use of an omnivorous fractionation process that simultaneously treats all lignocellulosic feedstocks (i.e. the SEW process, see below section) may also increase the scale of lignocellulosic biomass conversion and improve further its techno-economic potential (van Heiningen 2010, Iakovlev 2011).

### **2.3 Pre-treatment/fractionation of lignocellulosic biomass**

The production of biofuels via fermentation of lignocellulosic biomass sugars is facilitated when the biomass is cleanly separated into its principal constituents; cellulose, hemicellulose and lignin. This is a difficult task because the structure of biomass is very complex. The cellulose microfibrils are covered by a layer of hemicelluloses and are imbedded in a tight composite structure of lignin and hemicelluloses bound to each other by covalent bonds (Jurgens et al. 2012, Fengel

and Wegener 1984). Pre-treatment of lignocellulosics results in release of the cellulosic fibers and opening up of the cell wall structure by dissolution of lignin and hemicellulose between the cellulose microfibrils. Thus the cellulosic fibers are better accessible for hydrolysis by enzymes whereas the hemicellulose sugars can be used together with glucose from the fibers for subsequent fermentation or may serve as a feedstock for chemicals production. Pre-treatment/fractionation technologies have been extensively studied in the past since they constitute an expensive step for the production of lignocellulosic biofuels (Elander et al. 2009). Examples of such technologies include steam explosion, dilute acid hydrolysis, SPORL (acidic sulfite, Zhu et al. 2009), Lignol (ethanol-water with sulfuric acid), ammonia treatments i.e. AFEX (ammonia fiber explosion) and ARP (ammonia recycled percolation) and lime treatments. These processes increase the accessibility of cellulose to enzymes by removing the protective layers of either hemicelluloses (acidic processes) or lignin (alkaline processes) (Mosier et al. 2005). Reports (Mosier et al. 2005, Wyman et al. 2005) suggest that ammonia and lime treatments are considered less attractive due to the difficulty to recover ammonia/alkali. Also lime pre-treatment suffers from extensive scaling (Zhu and Pan 2010). On the other hand many of the acidic processes are energy intensive as they usually require temperatures higher than 150°C with associated high pressures (Zhu et al. 2010). Other drawbacks include a significant water requirement due to the high required liquid-to-solids ratios, the necessity to neutralize the acidic hydrolyzates before fermentation and associated gypsum disposal problem (Iakovlev and van Heiningen 2012a). Furthermore acidic processes cannot handle effectively softwoods, and they suffer from processing equipment failure and operational problems due to corrosion and formation of sticky lignin precipitates on reactor walls and piping (Leschinsky

2009).

An acidic process which is a hybrid between acid sulfite and organosolv (alcohol-water) processes is the SO<sub>2</sub>-ethanol-water (SEW) process introduced by Schorning as a pulping method in 1957. It was developed as an alternative process to traditional chemical pulping processes such as Kraft, soda and acid sulfite which suffer from serious drawbacks including high capital costs and complicated recovery of chemicals.

The SEW process uses a mixture of ethanol and water with dissolved SO<sub>2</sub> at moderate temperatures (130–150<sup>0</sup>C). The presence of SO<sub>2</sub> leads to hydrolysis of hemicelluloses producing monomeric sugars in relatively high yield. Furthermore, SO<sub>2</sub> sulfonates lignin to produce lignosulfonic acids which undergo solvolytic destruction resulting in dissolution of lignin. Ethanol increases the penetration rate of the cooking liquor into the lignocellulosic biomass, thereby minimizing lignin condensation and shortening the fractionation time. Ethanol reduces also the acidity somewhat (spent liquor pH of 1.0, at room temperature) to prevent excessive lignin condensation and carbohydrate hydrolysis. Finally, ethanol itself dissolves lignin. The fractionation mechanism and chemistry of delignification were studied in detail by Iakovlev and van Heiningen (2012a/b).

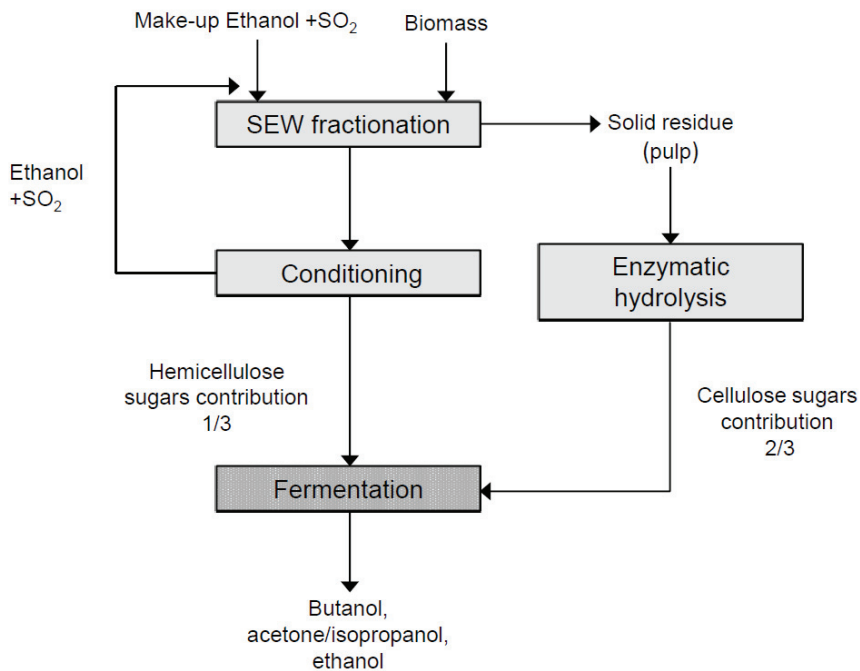
SEW fractionation has distinct advantages over other pre-treatment/fractionation/pulping processes (Table 1). For instance, hemicellulose sugars are fully usable as they are dissolved in high yields as monomeric sugars without suffering dehydration or oxidation (Iakovlev and van Heiningen 2012a). Production of monomers at a high yield eliminates the need for costly acid or enzymatic hydrolysis of hemicelluloses. On the other hand the cellulosic residue remains intact after fractionation and can be hydrolyzed by relatively low charge of

enzymes (Yamamoto et al. 2012). Other advantages include relatively low energy requirements because of the low temperature (130–150<sup>0</sup>C) and possibility to reduce the liquid-to-wood ratio to 2–3 L kg<sup>-1</sup>. Furthermore the absence of a base (Mg or Na) in the fractionation liquor eliminates the need for its energy intensive and therefore expensive recovery. The absence of formation of sticky lignin-based precipitates is another key benefit (Iakovlev 2011) as it allows for trouble-free operation of the digester. It is reported (Iakovlev et al. 2011, Yamamoto et al. 2011) that the method is omnivorous as it can digest softwoods, hardwoods and annual plants at similar delignification rates. This increases the potential feedstock supply and size of operation, and thus lowers capital cost per weight of product. Finally, the fractionation chemicals i.e. ethanol and SO<sub>2</sub> can be easily recovered by distillation. The above characteristics show that the SEW process is a method with good potential for application in an industrial process to produce biofuels from lignocellulosic biomass via microbial fermentation. Also the process fares better compared to acid sulfite process which is the only commercially operating lignocellulose fractionation process which produces biofuels (bioethanol) from the dissolved hemicelluloses via fermentation (Iakovlev and van Heiningen 2012a, Jurgens et al. 2012).

**Table 1.** Qualitative comparison of fractionation processes (Jurgens et al. 2012)

<b>Pre-treatment/ Fractionation</b>	<b>Full utilization of hemicellulo- ses</b>	<b>Low energy need</b>	<b>No sticky lignin issue</b>	<b>Omnivo- rous</b>	<b>Simple recovery</b>
Alkaline treatment	Intermediate	Yes	Yes	No	No
Steam explosion	Intermediate	No	Intermediate	No	Yes
Autohydrolysis	Intermediate	Yes	No	No	Yes
Acid hydrolysis	Intermediate	Intermediate	No	No	No
Lignol (EtOH-H <sub>2</sub> O)	Intermediate	No	Yes	No	Yes
SPORL	Intermediate	Intermediate	Yes	Intermediate	No
Sulfite pulping	Intermediate	Yes	Yes	Intermediate	Intermediate
SEW	Yes	Yes	Yes	Yes	Intermediate

SEW fractionation is currently used at a demonstration scale of several tonnes of biomass per day to hydrolyze cellulose to sugar monomers in a patented biorefinery process termed AVAP<sup>®</sup> by American Process Inc. (Retsina and Pylkkänen 2007, 2011). It has also been employed in a biorefinery process developed at Aalto University, Finland (Fig. 1) to fractionate lignocellulose for biofuels production via ABE fermentation. Part of this process utilizes the SEW fractionation and ‘conditioning’ scheme presented in the current work.



**Figure 1.** Flow diagram of the biorefinery process developed at Aalto University, Finland

## 2.4 Detoxification of hydrolysates before fermentation

Detoxification of biomass hydrolysates is the adequate removal of compounds that are inhibitory for subsequent fermentation. The identification of the main and relevant inhibitors present in the feedstock solution for fermentation is crucial in order to choose a specific, efficient and low-cost detoxification methodology. The maximum concentration allowed for each inhibitor, without losing fermentation efficiency, is very specific as it depends on several factors: the origin of the inhibitor, the inhibition mechanism, the microbial strain used and its physiological state, the fermentation technology adopted, the dissolved oxygen concentration in the medium and the pH (Mussato and Roberto 2004). Detoxification can be

performed by using different physical, chemical and biological methods. The most common detoxification technologies are presented below.

### *Physical Methods*

Vacuum evaporation has been used to reduce the concentration of volatile compounds present in lignocellulosic hydrolysates, such as acetic acid, furfural and formaldehyde, and at the same time to increase sugars concentration (Fernandes et al 2012). However, this method also increases the concentration of non-volatile toxic compounds such as lignin derivatives. A balance between these two effects is needed to obtain good detoxification. Furthermore, the energy required for this process should be properly considered to achieve economical operation (Lawford et al. 1993, Mussato and Roberto 2004). The condensate obtained by this step is rich in furfural, acetic acid and other volatile compounds that can possibly be extracted and purified for selling as value-added products.

Steam stripping or steam distillation has been used to remove volatile organic compounds from solution by addition of steam. The effect of this treatment is that the boiling points of the compounds are depressed due to their lower partial pressure, allowing them to evaporate at lower temperatures and without any deterioration as with conventional distillation. It is a method that is commonly used in petroleum refineries and petrochemical plants but it has also been applied in pulping processes i.e. the ASAM process to recover cooking chemicals (methanol) from black liquor (Black 1991). Steam stripping has also been used to fully remove SO<sub>2</sub> from lignocellulosic hydrolysates in the AVAP<sup>®</sup> biorefinery process (Retsina and Pylkkänen 2007). Similar to all heat treatments steam stripping can be energy intensive and therefore uneconomical for industrial applications.

Solvent extraction has been used to detoxify hydrolysates i.e. aspen wood hydrolysates prior to fermentation (Wilson et al. 1989). Ethyl acetate extraction at a 1:1 (v/v) ratio of ethyl acetate to aspen wood hydrolysate removed low molecular weight phenolics, furfural, hydroxybenzoic acid and vanillin. One disadvantage of this method is the requirement for a large volume of solvent. Also the need to recover the solvent by distillation adds to production cost and time (Richardson et al. 2011).

Removal of fermentation inhibitors by ion exchange resins is another physical method to detoxify lignocellulosic hydrolysates (van Zyl et al. 1991, Larsson et al. 1999, Lee et al. 1999, Nilvebrant et al. 2001, Xavier et al. 2010). Ion exchange involves exchanging an ion from solution for a similarly charged ion attached to an immobile solid particle. Both cation and anion exchange resins have been used to remove inhibitors such as aliphatic acids, furan derivatives and phenolic compounds (Qureshi et al. 2007). It is reported (Larsson et al. 1999) that treatment of dilute-acid spruce hydrolysates by anion exchange resins made from styrene based matrices at pH 10 was effective at removing inhibitors and improving fermentation to ethanol, however, this treatment also resulted in 26% decrease in sugar content. This example stresses one key disadvantage of some resins i.e. non selective removal of inhibitors from hydrolysates. Another drawback is that the use of ion exchange resins is costly and may be uneconomical and impractical for large-scale biofuels production.

The use of activated charcoal or carbon to adsorb inhibitors from lignocellulosic hydrolysates has been studied extensively (Maddox and Murray 1983, Wang and Chen 2011, Sixta et al. 2012, Shen et al. 2013). This physical method has been applied to detoxify many different types of liquors i.e. Kraft prehydrolysis liquor



(PHL), dilute acid corn stover hydrolysate, sugarcane bagasse hydrolysate etc. to improve fermentation to ethanol. Chandel et al. (2007) reported that treatment of sugarcane bagasse hydrolysate with activated carbon removed significant amounts of furans, phenolic compounds and acetic acid (reduction of about 40, 60 and 50%, respectively) and resulted in doubling of fermentation yield. Other reports (Berson et al. 2005) stress the economic benefit of using activated carbons as they are less expensive than ion exchange resins and they can be regenerated by steam if the adsorbed components have a relatively low boiling point. It is possible though that the use of activated carbons might sustain significant sugar losses due to co-adsorption of sugars in their porous structure. Also the use of activated carbons might be uneconomical due to deactivation (Richardson et al. 2011).

Membrane filtration is a relatively new method to detoxify lignocellulosic hydrolysates. It is usually applied after a sequence of preceding steps to remove dissolved organics (i.e. lignin) that can impair membrane function (Koivula et al. 2012). It is reported (Restolho et al. 2009) that retention of lignosulfonate from eucalyptus spent sulfite liquor (SSL) that was filtered with ultrafiltration (UF) and nanofiltration (NF)/reverse osmosis (RO) membranes of different cut-off sizes was 41-80% and 92-99%, respectively. Retention of sugars ranged from 3-57% with UF membrane and 77-99% with NF/RO membranes. This broad range of retentions was due to the different membrane cut-off sizes used but also due to the different operating conditions i.e. pressure. A broad range of permeation fluxes was also observed indicating the presence of strong concentration polarization phenomena (fouling). It is concluded that none of the tested membranes can offer complete separation of lignosulfonates from sugars contained in SSL and that membrane filtration should be combined with ion exchange treatment to achieve better

separation efficiency. These findings serve as an example to illustrate that membrane filtration must be carefully optimized, preferably in conjunction with other treatments, to attain efficient and economical detoxification for application in an industrial process.

### *Chemical Methods*

A well-known chemical method to detoxify lignocellulosic hydrolysates is treatment with alkali. This treatment involves adjusting the pH to 9-10 by addition of an alkali or other bases i.e.  $\text{Ca}(\text{OH})_2$ , NaOH, KOH,  $\text{NH}_4\text{OH}$  followed by readjustment of the pH to neutral levels for fermentation (Leonard and Hajny 1945, Sjolander et al. 1938). The addition of  $\text{Ca}(\text{OH})_2$  to hydrolysates is specifically known as 'liming' or 'overliming'. Overliming produces a precipitate containing calcium salt (gypsum) that must be removed from the mixture. Heat is sometimes applied during the process as calcium salts, i.e. calcium sulfate, have decreased solubility at high temperatures and volatile inhibitors can be removed by evaporation from the hydrolysate solution (Perego et al. 1990). Overliming has been applied to remove acetic acid, furfural, HMF, soluble lignin and phenolic compounds from hydrolysates to improve yield of subsequent fermentation to ethanol (Lawford et al. 1993, Horváth et al. 2005, Ariksson 2006, Mohageghi et al. 2006, Helle et al. 2008, Sanchez et al. 2008, Qureshi et al. 2010). Martinez et al. (2001) reported for sugarcane bagasse hydrolysate at  $60^\circ\text{C}$  that the addition of  $\text{Ca}(\text{OH})_2$  to pH 9.0, promoted the precipitation of furanic compounds and phenolics. Their respective levels were about 50 and 40% lower after overliming. It was also reported that sugar losses of about 9% occurred. For removal of furans and soluble lignin the most commonly reported mechanism is adsorption on the gypsum.

Aliphatic acids i.e. acetic acid are reportedly not affected much by alkaline treatments. However, in specific cases (Person et al. 2002) it is reported that the removal of some inhibitors i.e. phenolics from hydrolysates may be due to chemical conversion at alkaline conditions rather than adsorption on gypsum and precipitation. To prevent excessive sugar losses and formation of amounts of potentially inhibitory aliphatic acids it has been suggested that overliming should be within pH range of 9-12 at temperatures under 30<sup>0</sup>C (Nilvebrant, 2003). This study reports that upon alkali treatment of spruce hydrolysate xylose gets somewhat more easily decomposed than other monosaccharides. More extensive sugar decomposition during alkaline treatment by overliming is attributed to the stabilization of reactive enolate intermediates by calcium ions (Jönsson et al. 2013).

Overliming is an effective detoxification method that can lead to improved fermentation yields when carefully optimized. Another key advantage of this detoxification method is that it is relatively inexpensive (Larsson et al. 1999, Ranatunga et al. 2000).

Other chemical methods to detoxify lignocellulosic hydrolysates include the use of cationic polymers such as polydiallyldimethylammonium chloride (p-DADMAC) or chitosan (Saeed et al. 2012, Fredheim and Christensen 2003, Saeed et al. 2011). p-DADMAC is used as a coagulant in water purification and as a pitch fixation agent in the pulp and paper industry. It is reported (Saeed et al. 2012) that treatment of Kraft PHL with small charge of p-DADMAC (about 0.5-1.0 mg g<sup>-1</sup> liquor) resulted in dissolved lignin and furfural removal of about 30 and 50%, respectively. A drawback of this method is that interaction of lignin with p-DADMAC may result in a soluble complex formation which is hard to precipitate from solution unless the concentration of p-DADMAC is sufficiently high to create insoluble lignin/p-

DADMAC complexes (Kekkonen et al. 2002, Strom et al. 1985, Li et al. 1992). Addition of p-DADMAC at a high charge can increase costs for detoxification. Chitosan is a very interesting biomaterial as it originates from deacetylation of chitin which is the second most abundant natural polymer in the world after cellulose (Rinaudo 2006). This natural cationic polymer can be used to flocculate and precipitate anionic lignosulfonate from solution by formation of polyelectrolyte complexes. It is reported (Saeed et al. 2011) that treatment of Kraft PHL with small dosage of different chitosans (about 2 mg g<sup>-1</sup> liquor) resulted in dissolved lignin and furfural removal of about 40 and 55%, respectively. A major drawback of treatment with chitosans is that they lose part of their positive charge at pH greater than 4.5 (Fredheim et al. 2002). Furthermore, their applicability is somewhat limited by their low solubility in aqueous solutions at pH greater than 6 or 7. However, there are some low molecular weight chitosans which are exceptions to this behaviour (Terbojevich and Muzzarelli 2000).

### *Biological methods*

An effective method to detoxify lignocellulosic hydrolysates is by the use of oxidative enzymes (Jönsson et al. 1995, Jurado et al. 2009). The most commonly used oxidase is laccase. Laccase oxidizes phenols and aromatic units in lignin to form water and unstable phenolic radicals that form insoluble high molecular mass polymerization products, resulting in a decrease in the concentration of low molecular mass phenolic compounds in the hydrolysate (Larsson et al. 2001). It is reported (Areskog et al. 2010, Gouveia et al. 2012, 2013) that laccases are very effective in inducing polymerization of sulfite or Kraft lignin as they increase molecular weight of lignosulfonates by a factor of 5 to 25. Furthermore, studies by

Jurado et al. (2009) showed that laccase treatment of acid hydrolysates from willow and wheat straw resulted in improved fermentability to ethanol due to removal of lignin from solution. A major drawback of treatment with laccase is that it only removes phenols (Chandel et al. 2007) and therefore the use of other detoxification methods that can remove more than one inhibitor simultaneously (i.e. ion exchange resins) could be more preferable. It is also generally known that enzymes can be expensive for industrial scale applications.

From the above discussion it is shown that all detoxification technologies suffer from limitations. It can also be concluded that choice of the optimal detoxification strategy requires balancing between inhibitor removal efficiency and costs. It is noted that the majority of the above detoxification methods are combined in the developed 'conditioning' process to detoxify SEW spent liquor from lignocellulosics for subsequent ABE fermentation which is described in the current work.

## **2.5 Inhibitory compounds for ABE fermentation by *Clostridia* bacteria**

For ABE fermentation of the hydrolysates produced under the developed SEW fractionation and conditioning scheme the following compounds have been identified by our group as inhibitors for fermentation by *C. acetobutylicum* in addition to SO<sub>2</sub> (10 ppm, Jurgens et al. 2012) and ethanol (50 g L<sup>-1</sup>, Jones and Woods, 1986):

- i. formic acid (0.5 g L<sup>-1</sup>)

- ii. furfural and hydroxymethylfurfural (1.0 and 1.5 g L<sup>-1</sup>, respectively)
- iii. dissolved lignin (1.0 g L<sup>-1</sup>)

Furfural and hydroxymethylfurfural (HMF) are common furan aldehydes derived from dehydration of pentoses and hexoses during acidic treatment of biomass (Liu et al. 2010). These furans may inhibit cell growth and respiration as is the case with specific ethanologens such as *Saccharomyces cerevisiae* (Palmqvist et al. 1999). Formic acid is a product of further degradation of HMF during acidic treatment as well as high temperature treatment of hydrolysates at both alkaline and acidic conditions. The inhibition of *C.acetobutylicum* by formic acid may be due to onset of 'acid crash' phenomena during ABE fermentation (Maddox et al. 2000, Wang et al. 2011). Dissolved lignin especially in its degraded form as phenolic compounds, can cause partition and loss of integrity of cell membranes of the fermenting microorganisms and lead to reduced cell growth and sugar assimilation. It is reported (Mussato and Roberto 2004) that the inhibitory effect of the above compounds is higher when they are present together due to a synergistic effect. Acetic acid and extractives are not identified as inhibitory for *C.acetobutylicum*.

## **2.6 ABE fermentation by *Clostridia* bacteria**

The research for sustainable biofuels has led to renewed investigations on the ABE process. This technology which is almost 100 years old (Weizmann 1915) is based on fermentation of different carbohydrate substrates by *Clostridia* bacteria to produce butanol, acetone and ethanol. Since the focus of the current work is on butanol production from lignocellulosics we will restrict this discussion on the most commonly used industrial solventogenic species used for this purpose i.e.

*Clostridium acetobutylicum*. These anaerobic species are particularly successful commercially because they are to some extent oxygen tolerant and because they are capable of degrading carbohydrates from various sources including not only plants but also animals and microbial materials (Jurgens et al. 2012). A key advantage for biofuels production from lignocellulosics is that *C. acetobutylicum* can use all pentose and hexose sugars derived from biomass with a preference for mannose and glucose, whereas the least preferred sugars are arabinose and galactose (Survase et al. 2011). Xylose uptake by *C. acetobutylicum* is suppressed by the presence of glucose (Fond et al. 1986, Marchal et al. 1992).

The metabolism of *C. acetobutylicum* is well understood and the genes and enzymes needed for butanol production have been studied extensively in the past (Dürre 2005, Jones and Woods 1986). In short, the metabolic mechanism during ABE fermentation by *C. acetobutylicum* is as follows; during exponential growth in the so-called acidogenic phase, *C. acetobutylicum* follows the standard butyric acid path producing acetate, butyrate, CO<sub>2</sub> and hydrogen. In addition, small amounts of ethanol and acetone are formed successively. Accumulation of the excreted acids leads to a rapid decrease in pH of the surrounding medium (pH below 5) which may cease cell growth. To avoid this deleterious effect a major metabolic shift takes place at the end of exponential growth where *C. acetobutylicum* takes up acetate and butyrate and converts these organic acids to acetone and butanol, respectively (solventogenic phase). It is reported (Qureshi and Blaschek 2001) that after biphasic ABE fermentation with corn steep liquor the mass ratio between solvents and gases produced is 3:6:1:17 for acetone, butanol, ethanol and gases, respectively (Jones and Woods, 1986; Zverlov et al. 2006). The fermentation off-gas composition usually is 60 % carbon dioxide and 40 % hydrogen by volume (Beesch 1953;

Zverlov et al. 2006).

Jones and Woods (1986), report that solvent toxicity is a major technological barrier for ABE fermentation by *C. acetobutylicum*. Butanol is the most toxic product of the fermentation. Butyric acid is even more toxic but due to the very low levels produced it does not impose a problem. The inhibitory level of butanol is around 12-16 g L<sup>-1</sup> (Jones and Woods 1986), above which cellular functions cease. The ability of butanol to inhibit cellular functions is due to the impact this alcohol has on the fluidity of the cell membrane and lipids dispersal. Acetone and ethanol are also inhibitory at levels of 40 and 50 g L<sup>-1</sup>, respectively (Jones and Woods 1986). The problem of solvent toxicity in ABE fermentation leads to low product concentrations and therefore high product recovery costs. In order to have an economically competitive/viable process a product concentration of about 25 g L<sup>-1</sup> should be achieved (Teräsvuori 2009). Currently, the main alternative to decrease the toxic effects of butanol on the bacterial cells is the removal of the fermentation products. This is possible by several methods as discussed in the below section.

## **2.7 Downstream processing after ABE fermentation**

The most common product recovery process is distillation carried out at the end of the bacterial cell growth. This process achieves separation on the basis of different boiling points (at standard pressure) of water (100<sup>0</sup>C), acetone (56<sup>0</sup>C), butanol (117<sup>0</sup>C) and ethanol (78<sup>0</sup>C). This method is still employed for batch fermentations despite high energy costs. Distillation is not suitable for continuous fermentations as they require integrated recovering techniques to avoid accumulation of butanol at toxic levels in the fermentation broth (Köpke and Dürre 2011).



Adsorption on resins is one possible alternative method which can be used *in situ* and has low energy requirements (Qureshi et al. 2005). However, this technique offers low selectivity and often cell growth nutrients are removed from the media as well. Furthermore, the price of resins and associated regeneration costs can be high.

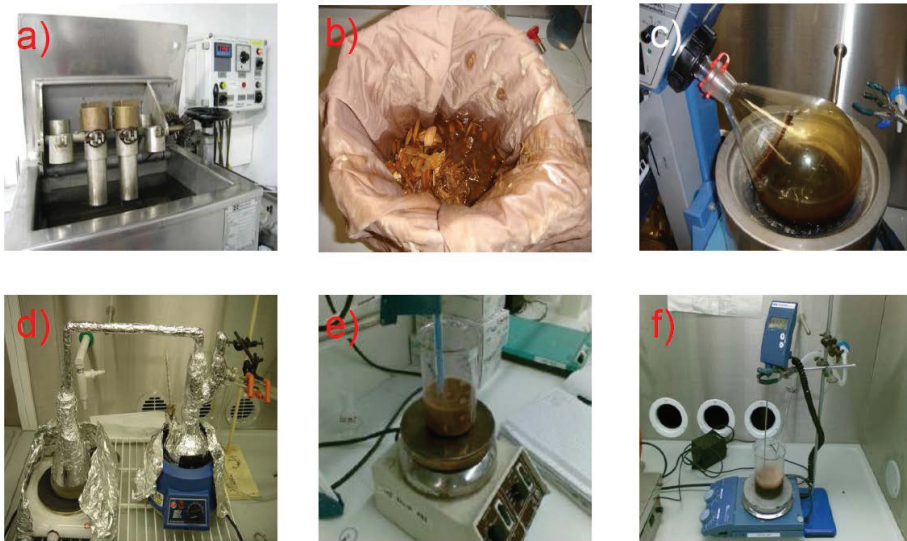
Another technique to recover ABE solvents is gas stripping. This method is relatively simple and inexpensive as it utilizes the CO<sub>2</sub> and H<sub>2</sub> gases that are generated during fermentation to capture the solvents from the broth (Ezeji et al. 2004, Groot et al. 1989). The gases are sparged through the fermentation broth, then cooled down in a condenser to strip-off the solvents and finally recycled back to the fermenter to recover more solvents. This technique suffers from low selectivity and does not allow complete removal of solvents from the fermentation broth.

Membrane based solvent recovery techniques such as perstraction, (Qureshi and Maddox 2005) pervaporation (Qureshi and Blaschek 1999, 2000) and reverse osmosis (Garcia et al. 1985) can also be used. However, these methods are generally expensive and separation of the solvents may be energy intensive.

The most promising solvent recovery method that offers high selectivity is liquid-liquid extraction, in which a water insoluble organic extractant is mixed with the fermentation broth (Ezeji et al. 2007). Since butanol is more soluble in organic than in aqueous solutions, it selectively accumulates in the organic phase of the extractant. A drawback of this method is that the number of non-toxic extractants i.e. oleyl alcohol (Qureshi and Maddox 1995), decanol (Evans and Wang 1988), etc. which are suitable for this application are limited.

### 3 MATERIALS AND METHODS

Air dried spruce chips, mixed softwood biomass and oil palm empty fruit bunch (OPEFB) fibers were used for the SEW fractionation experiments (dry matter content of approx. 92%). The spruce chips were screened to 2-4 mm thickness and a mixture of the accept fractions were used for the experiments. The air dried mixed softwood biomass was screened according to SCAN-CM 40:01 in order to remove humus and needles (7 mm and 13 mm hole screen accept fractions used). OPEFB fibers were not subjected to any processing before leaching or SEW fractionation experiments. The fresh fractionation liquor was prepared by injecting gaseous sulfur dioxide into a 55% (by volume) ethanol-water solution cooled in an ice bath. The concentration of SO<sub>2</sub> in the liquor was set to either 3% (Paper I) or 12% by weight (Papers II, III, IV), and the liquor-to-feedstock (L/F) ratio used was either 6 (Paper I) or 3 L kg<sup>-1</sup> (Papers II, III, IV). Fractionation was carried out in a HAATO 43427 silicon oil bath using six 220 mL bombs each filled with 25 g of oven dried feedstock at 150°C (±1°C) and 120 (Paper I) or 30 minutes (Papers II, III, IV), including the heating-up period of 8-9 minutes (Iakovlev et al. 2011) (Fig. 2a). After fractionation, the bombs were rapidly removed from the oil bath and cooled in cold water. SEW spent liquors were collected by squeezing the pulp suspensions contained in a washing sock. The pulps were then washed according to the procedures that are presented and discussed in sections 4.2 and 4.3. SEW spent liquors either alone (Paper I) or mixed with pulp washings (Fig.2b, Papers II, III, IV) were then subjected to ‘conditioning’. Conditioning in its basic form consists of the four consecutive steps of vacuum evaporation (Fig.2c), steam stripping (Fig.2d), liming with Ca(OH)<sub>2</sub> to pH 9.0 (Fig.2e) and catalytic oxidation (Fig.2f).



**Figure 2.** SEW fractionation (a) followed by pulp washing (b), vacuum evaporation (c), steam stripping (d), liming with  $\text{Ca}(\text{OH})_2$  to pH 9.0 (e), catalytic oxidation (f).

Details and the principal functions served by each conditioning step are presented below (Table 2). The liquors produced after the step of catalytic oxidation (so-called CATOX liquors) were subjected to a liquor purification step to further reduce dissolved lignin levels in the feed liquor for subsequent ABE fermentation. This step involved treatment with non-polar Amberlite XAD-4 (Paper I) or AS501G Cl<sup>-</sup> FINEX anion exchange resins (Papers II, III) at a resin-to-liquor ratio of 1:1.5 (Fig.3a).

An alternative CATOX liquor purification method where nano-/ultrafiltration (Fig.3b) was employed to produce clear permeate liquors for subsequent ABE fermentation, was used in Paper IV. Nanofiltration was performed at room temperature under  $\text{N}_2$  pressure of 5 bars by using Millipore Amicon 8200 stirred cell equipped with hydrophilic polyethersulfone (PES) membrane (Microdyn Nadir, molecular weight cut-off size of 1000 Da).

**Table 2.** Main steps for conditioning SEW spent liquor

conditioning step	conditions	function
Vacuum evaporation	300 mbar, 95°C, 1-2 h	Removal of SO <sub>2</sub> and ethanol, removal of soluble lignin as LCC
Steam stripping	2 h, steam temperature of 102°C, flow of 0.7 L h <sup>-1</sup>	Further removal of SO <sub>2</sub> to ca.100 ppm
Liming	addition of Ca(OH) <sub>2</sub> to pH 9.0, alkaline washing of precipitate and addition to mother liquor	Soluble lignin precipitation, pH adjustment
Catalytic oxidation	20 mg L <sup>-1</sup> FeSO <sub>4</sub> ·7H <sub>2</sub> O under air bubbling, 1 h, 60°C	SO <sub>2</sub> levels below 10 ppm, micronutrient supplementation for ABE fermentation

Ultrafiltration was performed at room temperature under 1 bar vacuum with a Millipore Amicon 8050 stirred cell equipped with hydrophilic regenerated cellulose membrane (Millipore, molecular weight cut-off size of 10 kDa). Ultrafiltration was preceded by enzymatic treatment of the CATOX liquor (see section 4.4, also Paper IV). The CATOX liquor was pH adjusted to 7.3 with sulfuric acid and left in an orbital agitator at 70°C and 150 rpm for 1 h. Then it was charged with 720 U of NS51003 laccase (*Myceliophthora thermophila*) to oxidatively polymerize lignin before membrane filtration.



**Figure 3.** Liquor purification stage to further remove dissolved lignin before ABE fermentation: treatment with resins (a) membrane filtration (b).

For comparison purposes all fermentation substrates were treated with the same method before fermentations; they were 4-fold diluted and pH controlled to pH 6.5 with sulfuric acid. Then they were supplemented with suitable growth medium according to the method described by Survase et al. (2011). Also glucose was added at a concentration of 35 g L<sup>-1</sup> to take into account the glucose feed stream, obtained by enzymatic hydrolysis of the pulp fibers, which will be mixed with the conditioned SEW spent liquor. Combination of the two feed streams may allow for an integrated approach where approximately 1/3 of monosugars originate from hemicellulose and approximately 2/3 of monosugars originate from cellulose. Glucose supplementation may raise the monosugars concentration to about 50-60 g L<sup>-1</sup> which is optimal for ABE fermentation by the microorganisms used in a batch process (*Clostridium acetobutylicum* ATCC 824).

All fermentations were performed in batch mode by using 125 mL screw cap bottles with 50 mL production medium according to Survase et al. (2011) (Fig.4a) except with spruce chips/mixed softwood biomass-based conditioned liquors (see section 4.3, also Paper II) where they were performed in continuous mode utilizing a patent pending fermentation column equipped with wood pulp as cell immobilization material (Fig. 4b, Survase et al. 2011).



**Figure 4.** ABE fermentation: batch mode (a) continuous mode (b).

The summary of the main analytical methods used in the current work is shown in Table 3. More detailed information on the methods can be found in the original papers (I-IV) indicated in the Table.

**Table 3.** Analytical methods used in the current work

analysis	method	standard	original Papers
Inorganics content of feedstocks and pulps	ICP/AAS		II, III
Protein content of feedstocks	CHN/S		II, III
Pulp and dissolved component yields for liquors	Gravimetric	SCAN-C3:78 and SCAN-N1:61	I, II, III
Ash content of feedstocks, pulps, liquors	Determination of Ash in Biomass	NREL/TP-510-42622	I, II, III
Extractives in pulps	Acetone extraction	SCAN 49:03	I, II, III
Kappa number	SCAN method for kappa number determination	SCAN-C 1:00	I, II, III
Intrinsic viscosity of pulps	Intrinsic viscosity in CED solution	T230 om-66	I, II, III

Total carbohydrates in pulps and liquors	Double-stage acid hydrolysis/HPAEC	NREL/TP-510-42618	I, II, III
Lignin in pulps	Double-stage acid hydrolysis/HPAEC	NREL/TP-510-42618	I, II, III
Acetyl groups in pulps	Double-stage acid hydrolysis/HPAEC	NREL/TP-510-42618	I, II, III
Uronic acids in pulps and liquors	Methanolysis/GC		I, II, III
Monosaccharides in liquors	HPAEC	NREL/TP-510-42623	I, II, III, IV
Acid soluble and acid insoluble lignin in liquors	UV/Double-stage acid hydrolysis	NREL/TP-510-42623	I, II, III, IV
Furfural, HMF, acetic acid, formic acid and ethanol in liquors	HPLC		I, II, III
Aldonic acids in liquors	HPAEC		I, II, III
Sulfur content in feedstocks, pulps, spent liquors and LCCs	Combustion in Schöniger flask/IC		II, III
SO <sub>3</sub> <sup>2-</sup> , SO <sub>4</sub> <sup>2-</sup> content in liquors	IC		I, II, III
ABE solvents quantification	GC		I, II, III, IV
Sugars consumption after fermentation	HPLC		I, II, III, IV
Mw of lignin during laccase treatment	SEC-HPLC		IV

## 4 RESULTS AND DISCUSSION

### 4.1 Chemical composition of the tested feedstocks (Papers I, II, III)

#### 4.1.1 Spruce chips

Norway spruce (*Picea abies*, Fig.5a) is a species of spruce native to Europe. It is one of the most economically important coniferous species with a large number of uses. The forest industry is one of the main users as Norway spruce (hereafter called 'spruce') is the main raw material for timber and paper production. Spruce chips are produced by mechanical reduction of wood to small pieces in a so called chipper.

Air dried and screened spruce chips were used for the SEW fractionation experiments. Their homogeneous composition (no presence of needles, bark or other impurities) facilitated relatively easy fractionation and trouble-free conditioning (see original SEW fractionation and conditioning scheme). Furthermore, spruce chips represent the benchmark feedstock used for comparison with the other feedstocks tested (see section 4.1.4). It is shown (Table 4) that the carbohydrate content is about 62% on dry feedstock (o.d.f.). Cellulose content is about 40% (o.d.f.) which is in agreement with reports in the literature (Fengel and Wegener 1984, Iakovlev and van Heiningen 2012a). The low extractives (1.5% (o.d.f.)) and ash content (0.3% (o.d.f.)) are also in agreement with previously reported values (Fengel and Wegener 1984, Stenius 2000, Iakovlev and van Heiningen 2012a). Analysis of the ash (Table 5) reveals that spruce chips contain low amounts alkali/alkali earth metals (calcium, potassium, sodium), magnesium, aluminium and silica. This is in agreement with reports (Fengel and Wegener 1984) suggesting that stem wood contains low amounts of inorganic components. The



predominant element is calcium; potassium, sodium and magnesium occur in secondary quantities. The dominance of calcium is due to the presence of calcium oxalate crystals, which are found in sieve cells and longitudinal parenchyma cells (Fengel and Wegener 1984). Protein content of 0.3% (o.d.f.) corresponds well to the commonly reported values of 0.2-0.8% (o.d.f.) for softwoods (Fengel and Wegener 1984). Lignin content is also within the typical range of values for softwoods (about 29% (o.d.f.), see section 2.1.3).

**Table 4.** Composition of different lignocellulosic feedstocks

<b>Feedstock composition (% o.d.f.)</b>	<b>spruce chips</b>	<b>mixed softwood biomass</b>	<b>OPEFB fibers</b>
Carbohydrates	62.2	51.6	58.4
Cellulose	39.6	31.2	36.2
<i>Glucan in hemicelluloses</i>	2.6	1.8	0.4
<i>Arabinan</i>	1.1	4.7	0.1
<i>Xylan</i>	5.6	6.0	21.0
<i>Mannan</i>	10.8	7.3	0.6
<i>Galactan</i>	2.5	3.1	0.0
Extractives	1.5	4.8	3.2
Lignin	28.9	29.8	21.6
<i>Acid insoluble</i>	28.3	28.7	17.3
<i>Acid soluble</i>	0.6	1.1	4.3
Ash	0.3	2.6	5.4
Acetyl groups	1.1	0.7	3.0
Uronic acids	3.0	3.0	5.4
Proteins	0.3	2.6	4.0
<b>Total</b>	<b>97.3</b>	<b>95.1</b>	<b>101.0</b>

#### **4.1.2 Mixed softwood biomass**

Mixed softwood biomass (Fig.5b) represents a cheap and available feedstock which can be used in a forest biorefinery. It is a heterogeneous feedstock as it contains forest residues such as needles, twigs, bark etc. that lead to variations in its chemical composition. It is shown (Table 4) that the carbohydrate content is about 52% (o.d.f.) which is in agreement with previous findings by Rakkolainen et al.

(2010). Lignin content is about 30% (o.d.f).

The predominant constituent of the present mixed softwood biomass is most likely pine as it is the most abundant tree species in Finland. Evidence of the latter is the high extractives content of 4.8% (o.d.f) which is higher than the upper end of commonly reported values for pine (2.5-4.5% (o.d.f), Stenius 2000) due to the presence of bark (about 20% (o.d.f)). The ash (2.6% (o.d.f)) and protein content (2.6% (o.d.f)) is rather high. This also is due to the high content of bark, twigs and needles in mixed softwood biomass. This finding is in agreement with reports (Werkelin et al. 2011, Fengel and Wegener 1984) suggesting that these biomass constituents are rich in such non-structural components. Table 5 shows that ash of the present mixed softwood biomass is rich in alkali and alkali earth metals; mostly calcium and potassium cations.



**Figure 5.** Different feedstocks used for SEW fractionation experiments: spruce chips (a), mixed softwood biomass (b), OPEFB fibers (c).

#### **4.1.3 Oil Palm Empty Fruit Bunch (OPEFB) fibers**

OPEFB fibers (Fig.5c) are a waste product of the oil palm industry in SE Asia. Together with other types of palm oil waste such as palm fibers, palm kernel cake (PKC), decanter cake, fronds, trunks, and shells they accounts for approximately 55.7 million tons/year of lignocellulosic agricultural waste that is produced in palm

oil mills (Abdullah et al. 2011). OPEFB fibers are obtained after removing the crude palm oil from fresh fruit bunches. They are commonly used in a wide range of applications; from combustion material to produce electricity and heat in CHP plants (Rahman et al. 2007) to use as fertilizer (Yusoff 2006). However, the potential of OPEFB fibers as a suitable feedstock for value-added products such as chemicals and biofuels has not been extensively investigated until recently.

OPEFB fibers represent a non-woody feedstock with similarities to annual plants. Analysis of the fibers composition indicates that the total carbohydrates content is about 58% (o.d.f.) which is in good agreement with data from the literature (Tan et al. 2012). The cellulose and lignin content of OPEFB fibers is about 36% and 22% (o.d.f.), respectively. The lignin content is in good agreement with the reported values (Sreekala et al. 1997) and it is similar to that of wheat straw (Iakovlev 2011). It is shown (Table 4) that the extractives (3.2% (o.d.f.)), protein (4% (o.d.f.)) and ash (5.4% (o.d.f.)) content are relatively high. The protein content of 4% (o.d.f.) is close to the lower end of commonly reported values for non-woody feedstocks (5-10% (o.d.f.), Stenius 2000). The high ash content in OPEFB fibers is mainly due to the presence of indigenous alkali and alkali earth metals (mostly potassium cations) and silica (Table 5). Content of the latter is particularly high (about 0.7% (o.d.f.)) which is in agreement with reports suggesting that many tropical plant species stand out by having a high percentage of silica (Fengel and Wegener 1984). High silica content is a problem for alkaline pulping since it leads to scale formation in the evaporators in the recovery cycle of pulping chemicals (Stenius 2000). Additionally to the presence of silica, the accumulation of dust and dirt before or after harvesting of the fibers contributes further to the high ash content of this feedstock.

#### **4.1.4 Comparison of the chemical composition of the feedstocks**

A comparison of the chemical composition of spruce versus mixed softwood biomass reveals significant differences (Table 4). The differences in chemical composition are due to the very heterogeneous nature of the latter (see section 4.1.2). Hence, mixed softwood biomass has a much lower carbohydrate content compared to spruce: 52 vs. 62% (o.d.f.). The much higher extractives, ash and protein content of mixed softwood biomass is explained by the presence of twigs and bark (see section 4.1.2). On the other hand, the lignin content of the two feedstocks is rather similar at about 30% (o.d.f.).

The carbohydrate content of OPEFB fibers (about 58% (o.d.f.)) is rather close to that of spruce chips, however, the dominant non-cellulosic polysaccharide is 4-O-methylglucuronoxylan at 21% (o.d.f.) whereas spruce and mixed softwood biomass hemicelluloses are represented both by galactoglucomannan (GGM) and some 4-O-methylglucuronoxylan. It is noted that lignin content of OPEFB fibers is the lowest among the three feedstocks tested at about 22% (o.d.f.). The protein content of OPEFB fibers at 4% (o.d.f.) is much higher than that of spruce and mixed softwood biomass. The extractives content of OPEFB fibers of 3.2% (o.d.f.) is in between the respective values for spruce chips and mixed softwood biomass. However, the most notable difference is in the ash content; OPEFB fibers have the highest ash content of 5.4% (o.d.f.). As mentioned earlier, the ash of OPEFB fibers is rich in silica and potassium whereas in the ash of spruce/mixed softwood biomass the dominant metal cations are calcium and potassium. This dissimilarity stems from differences in location, growth conditions (soil fertility, climate etc.) and plant physiology of each species (woody vs. non-woody structure).

The high ash content of mixed softwood biomass and OPEFB fibers in particular

has a detrimental effect on hydrolysis during SEW fractionation and spent liquor conditioning (see section 4.3).

**Table 5.** Elemental composition of the ashes of different feedstocks.

<b>Element</b>	% o.d.f.		
	<b>spruce chips</b>	<b>mixed softwood biomass</b>	<b>OPEFB</b>
K	0.041	0.141	1.047
Na	0.003	0.009	0.037
Al	0.001	0.038	0.020
Mg	0.012	0.050	0.110
Ca	0.113	0.577	0.192
Si	0.007	0.123	0.674
Total	0.177	0.938	2.080

## **4.2 Original scheme for SEW fractionation of spruce chips and spent liquor conditioning for ABE fermentation (Paper I)**

### **4.2.1 Process preview**

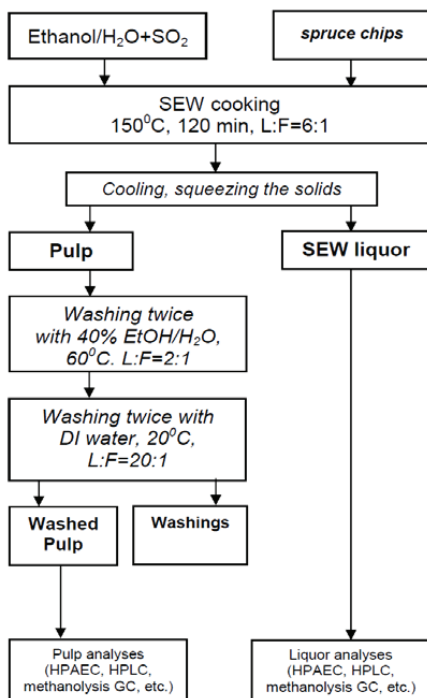
The originally developed scheme for SEW fractionation of spruce chips and spent liquor conditioning for ABE fermentation is presented (see also Paper I). SEW fractionation is performed at conditions of 3% SO<sub>2</sub> in 55 v/v% ethanol-water, L/F ratio of 6 L kg<sup>-1</sup>, 150<sup>0</sup>C, 120 min. These conditions are selected on the basis of previously reported kinetic studies at L/F ratio of 6 L kg<sup>-1</sup> (Iakovlev et al. 2009, Iakovlev and van Heiningen 2012b). These studies demonstrated that it is possible to achieve efficient fractionation of lignocellulosics in only 30 min at 150<sup>0</sup>C due to extremely fast impregnation and delignification of lignocellulosics by SO<sub>2</sub>-ethanol-water solutions.

SEW fractionation is followed by pulp washing to dissolve and recover hydrolyzed hemicellulose sugars for further processing. Figure 6 shows that the pulp is washed twice with 40 v/v% ethanol-water at 60<sup>0</sup>C and twice with deionized water at room temperature. The choice of ethanol-water washing is based on previous research by Iakovlev et al. (2009) suggesting that this mixture of non-polar and polar solvents is best for further removal of solubilized lignin and hemicellulose sugars respectively from the fibers after SEW fractionation.

The spent liquor resulting from SEW fractionation of lignocellulosics is rich in dissolved and hydrolyzed hemicellulose sugars. However, it cannot be directly subjected to ABE fermentation due to:

- i. high content of SO<sub>2</sub> which needs to be removed

- ii. high content of ethanol which needs to be removed
- iii. high content of oligomers



**Figure 6.** SEW fractionation and pulp washings in the original scheme (Paper I)

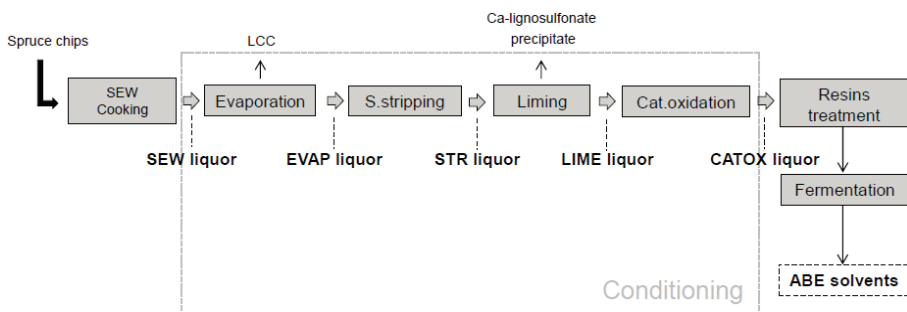
- iv. high acidity (pH of about 1.0)
- v. high lignin content

In order to remove ethanol and SO<sub>2</sub>, SEW spent liquor is subjected to vacuum evaporation. This step is performed to approximately 70% weight reduction and removes nearly all ethanol (98%) and about 90% of SO<sub>2</sub>. Levels of ethanol are below 10 g L<sup>-1</sup> after vacuum evaporation which is well below the ethanol inhibition level of 50 g L<sup>-1</sup> for *Clostridia* (Jones and Woods 1986). Quantification of the recovery of ethanol and SO<sub>2</sub> was not investigated in the present study, however, it is anticipated that nearly full recovery is possible by simple distillation. This step is

crucial to create an environmentally benign and also economical process as the recovered ethanol and SO<sub>2</sub> must be reused for fractionation. The step of vacuum evaporation leads to a 4-fold volume reduction due to removal of ethanol, SO<sub>2</sub> and some water. Also a major part of dissolved lignin is removed as char-like lignin-carbohydrate complex (LCC) during this step. Steam stripping removes further trace amounts of ethanol and SO<sub>2</sub>. These two consecutive high temperature treatments of the acidic liquor – vacuum evaporation and steam stripping – also decrease significantly the oligomeric sugar content of the liquor. Subsequent liming with Ca(OH)<sub>2</sub> to pH 9.0 results in some precipitation of soluble lignin as Ca-lignosulfonate. The final catalytic oxidation step to oxidize the remaining sulfite anions in the liquor to sulfate anions results in nearly total elimination of SO<sub>2</sub> as its concentration is reduced to levels below 10 ppm (tolerance limit by *Clostridia*). By the end of the conditioning process the monomeric sugars content in the conditioned liquor is increased 6-fold (compared to SEW spent liquor) which is beneficial for ABE fermentation stage as *Clostridia* bacteria consume mostly monomers.

Figure 7 presents a schematic diagram of the developed process. The liquors obtained after SEW fractionation and after each conditioning step are identified by different names. The final conditioned liquor (CATOX liquor) is treated with non-polar resins before ABE fermentation as non-resins treated conditioned liquor is not fermentable due to its high residual dissolved lignin content. Selection of the resins is based on previous reports (Schwartz and Lawoco 2010) suggesting that this treatment is effective for the removal of 90% of soluble lignin from hardwood hydrolysates.





**Figure 7.** Original SEW fractionation and spent liquor conditioning scheme

## 4.2.2 Lignin and sugars analysis

### *Lignin analysis*

SEW fractionation of spruce chips at conditions of 3% SO<sub>2</sub>, 150°C, 120 min results in lignin condensation presumably due to a slow rate of sulfonation at these conditions (Iakovlev and van Heiningen 2012b). Evidence of lignin condensation is provided by the dark color of the pulp (Kappa number of about 60) and the SEW spent liquor.

From the lignin amount in SEW spent liquor (about 23% (o.d.f.)) it is inferred that the majority of lignin from spruce chips (about 80%) is dissolved in the spent liquor during SEW cooking at the selected fractionation conditions (Table 6). The lignin amount in EVAP liquor is reduced to about 7% (o.d.f.). This major reduction in the total amount of soluble lignin is associated with the char-like lignin-carbohydrate complex (LCC) formed during the vacuum evaporation step. The acronym ‘PCL’ (Precipitated Condensed Lignin) instead of LCC is used in Paper I. The formation of char-like PCL (about 15% (o.d.f.) is probably due to lignin condensation induced by the low acidity of the spent liquor (pH 1.0) and the relatively initial low concentration of SO<sub>2</sub> (3%). Evaporation of ethanol to levels below 10 g L<sup>-1</sup> during the vacuum evaporation step further increases the effective acidity of the liquor and

this may explain the condensation of lignin. The amount of lignin in STR and LIME liquors is gradually reduced (Table 6) due to sticky precipitation on the wall and as lime precipitate, respectively. The amount of dissolved lignin remaining in CATOX liquor is about 4% (o.d.f.). The residual dissolved lignin in the conditioned liquor corresponds to about 13% of lignin in original spruce chips.

### *Sugars analysis*

Results of total sugars analysis (see Paper I) for the liquors obtained after each conditioning step reveal a major increase in total sugars concentration after vacuum evaporation. The 3.3-fold increase from about 40 g L<sup>-1</sup> (SEW liquor) to 130 g L<sup>-1</sup> (EVAP liquor) correlates well with volume reduction by factor of about 4 after removal of most of the ethanol, the SO<sub>2</sub>, and some water from the SEW liquor. Total sugar concentration changes during subsequent conditioning steps of steam stripping, liming and catalytic oxidation are not so high because the volume changes due to concentration and dilution phenomena are small. Total sugars concentration in CATOX liquor is at about 110 g L<sup>-1</sup>.

About 90% of the hemicellulose sugars are dissolved after SEW fractionation and their content in the SEW spent liquor is about 22% (o.d.f.) corresponding to about 1/3 of the total sugars present in spruce chips (Table 6). This result is in agreement with previously obtained results for SEW spruce spent liquor (12% SO<sub>2</sub>, 135°C, 80 min; Iakovlev and van Heiningen, 2012a). No significant sugar losses are observed under the quoted cooking conditions or under the present cooking conditions (3% SO<sub>2</sub>, 150°C, 120 min) as the numerical difference between carbohydrate content of spruce chips and carbohydrate content of the pulp is close to the amount of anhydrosugars found in SEW spent liquor. It is reported (see Paper I) that the sugar

losses of about 15% (relative to SEW spent liquor) that occur during vacuum evaporation are due to sugar degradation that is promoted by the low acidity of the liquor (pH 0.9). Later research work (see Paper III) revealed that carbohydrates are probably not affected much by the low acidity of the liquor and that the sugar losses are most likely due to entrapment of the sugars in the char-like LCC. Total amount of anhydrosugars after consecutive conditioning steps of steam stripping, liming and catalytic oxidation is gradually reduced to about 17% (o.d.f.) in CATOX liquor. The identified total sugar losses after conditioning, account for ca. 20% relative to SEW spent liquor. In summary, it is found that the largest total sugar mass balance loss occurs during vacuum evaporation as a result of the precipitation of the char-like LCC, while all the other liquor conditioning steps only lead to minor sugar losses.

From the oligomers analysis (see Paper I) it is shown that SEW liquor contains about 60% oligomers on average. Heat treatment of the acidic liquor during vacuum evaporation and steam stripping further reduce the oligomers share. Liming by  $\text{Ca}(\text{OH})_2$  induces entrapment of some oligomers in the Ca-lignosulfonate precipitate. Final oligomers content in CATOX liquor is about 13%. The monomers consist mainly of arabinose, xylose, and galactose. It seems that glucomannan is most resistant to hydrolysis. This is in agreement with the lower acid hydrolysis rate of methyl-mannopyranosides and glucopyranosides compared to methyl-xylopyranosides and galactopyranosides (Feather and Harris 1965). Monomeric sugars concentration in the final CATOX liquor is close to  $100 \text{ g L}^{-1}$ .

### 4.2.3 Removal of ABE fermentation inhibitors

#### *Removal of furfural, HMF and formic acid*

The amounts of inhibitory furfural and HMF in SEW spent liquor are below 0.5% (o.d.f.) (Table 6). Therefore, it is inferred that sugar dehydration during SEW fractionation is minimal. After vacuum evaporation, the furfural amount in the EVAP liquor is at zero levels due to evaporation as an azeotrope (Zeitsch 2000). On the other hand, the HMF amount in EVAP liquor is unchanged because HMF is not volatile by steam (Sjöström 1993). It is possible that small amounts of HMF may be formed during the vacuum evaporation stage from the C6 sugars and that part of it is further converted to levulinic acid, formic acid and humins (Girisuta 2006). Therefore a very small part of the earlier identified sugar loss occurring during vacuum evaporation may be due to formation and evaporation of furfural and formation and degradation of HMF. Evidence of the latter may be the formation of small amounts of formic acid during vacuum evaporation stage. Formic acid is totally removed after steam stripping. Furfural is not detected in the STR, LIME, and CATOX liquors confirming that furfural is totally removed after the vacuum evaporation step. The HMF concentration in STR, LIME, and CATOX liquors is gradually reduced to levels that are well below inhibition levels of  $1.5 \text{ g L}^{-1}$  for ABE fermentation.

#### *Removal of SO<sub>2</sub>*

Ion chromatography (IC) analysis results (see Paper I) reveal that SO<sub>2</sub> content is gradually reduced from about  $11 \text{ g L}^{-1}$  (7% (o.d.f.)) in the SEW spent liquor to 6 ppm in the CATOX liquor which is below the tolerance level of 10 ppm for *Clostridia*. It is also found that the majority of SO<sub>2</sub> is removed by vacuum

**Table 6.** Mass balance for original spruce chips, pulp, and conditioning liquors. In brackets: concentration (g L<sup>-1</sup>) of components in CATOX liquor.

<b>Solid (fiber) phase composition (% o.d.f.)</b>	<b>spruce chips</b>	<b>pulp</b>				
Carbohydrates	62.2	43.8				
<i>Arabinan</i>	1.1	0.0				
<i>Xylan</i>	5.6	1.0				
<i>Mannan</i>	10.8	1.1				
<i>Galactan</i>	2.5	0.0				
<i>Glucan</i>	42.2	41.7				
Extractives	1.5	0.3				
Lignin	28.9	2.6				
<i>Acid insoluble</i>	28.3	2.4				
<i>Acid soluble</i>	0.6	0.1				
Ash	0.3	0.0				
Acetyl groups	1.1	0.0				
Uronic acids	3.0	0.1				
Proteins	0.3	n.m				
<b>Total in solid phase</b>	<b>97.3</b>	<b>46.8</b>				
<b>Liquor composition (% o.d.f.)</b>	<b>SEW</b>	<b>EVAP</b>	<b>STR</b>	<b>LIME</b>	<b>CATOX</b>	
Carbohydrates	21.9	18.6	17.5	17.5	17.4 (111.0**)	
<i>Arabinose</i>	1.0	0.9	0.8	0.8	0.8 (5.4)	
<i>Xylose</i>	4.6	4.1	3.8	3.8	3.8 (24.8)	
<i>Mannose</i>	10.5	8.8	8.3	8.3	8.3 (52.3)	
<i>Galactose</i>	2.0	1.6	1.5	1.5	1.5 (9.7)	
<i>Glucose</i>	3.7	3.2	3.0	3.0	3.0 (18.8)	
Lignin	22.5	7.1	5.7	4.3	3.6 (20.5)	
<i>Acid insoluble</i>	19.9	2.9	1.7	0.5	0.4 (2.1)	
<i>Acid soluble</i>	2.6	4.2	4.1	3.7	3.2 (18.4)	
LCC*		15.1	15.1	15.1	15.1	
Furfural	0.4	0.0	0.0	0.0	0.0 (0.0)	
HMF	0.1	0.1	0.1	0.0	0.0 (0.3)	
Ash	0.6	0.3	0.5	2.4	2.4	
Acetic acid	1.0	0.4	0.0	0.0	0.0 (0.2)	
Formic acid	n.d	0.1	0.0	0.0	0.0 (0.2)	
Xylonic acid	0.0	0.0	n.d	n.d	n.d (n.d)	
Mannonic acid	0.0	0.0	0.0	0.0	0.0 (0.1)	
Glucuronic acid	0.5	0.5	0.4	0.4	0.4 (2.5)	
Galacturonic acid	1.0	0.8	0.7	0.7	0.8 (4.2)	
4-O-Me-gluc.A	0.4	0.3	0.3	0.3	0.3 (1.7)	
<b>Total in liquor</b>	<b>48.4</b>	<b>43.3</b>	<b>40.3</b>	<b>40.7</b>	<b>40.0</b>	
<b>Total in solid phase and liquors</b>	<b>95.2</b>	<b>90.1</b>	<b>87.1</b>	<b>87.5</b>	<b>86.8</b>	

n.d., not detected, n.m., not measured, \* LCC was removed from the liquor after vacuum evaporation and its amount is included in the mass balance for EVAP, STR, LIME and CATOX liquors \*\* Total sugars

evaporation (90%) and that nearly all remaining SO<sub>2</sub> is removed by steam stripping. The liming and catalytic oxidation steps result in total SO<sub>2</sub> elimination.

#### **4.2.4 Overall mass balance**

From the overall mass balance (Table 6) it is shown that the sum of the identified components in the pulp and SEW spent liquor is about 95% which is only slightly lower than the ca. 97% total for the original spruce chips. The sum of the mass of the components in the EVAP liquor, pulp, and LCC decreases to about 90% mostly due to sugar losses during the vacuum evaporation step. At the end of the conditioning process, the total solids identified account for ca. 87% of the original spruce chips weight.

#### **4.2.5 ABE fermentation results**

The conditioned CATOX liquor is treated with non-polar resins, 4-fold diluted and supplemented with glucose according to the procedure described earlier (see section 3). Batch fermentation tests produced ABE solvents at a ratio of 3:6:1 and at maximum total concentration of about 9 g L<sup>-1</sup> (total solvents yield of about 0.20 g g<sup>-1</sup> sugars).

#### **4.2.6 Summary of findings**

The originally developed scheme for SEW fractionation of spruce chips and spent liquor conditioning was successful at: i) removing fermentation inhibitors such as ethanol, SO<sub>2</sub>, furanic compounds and formic acid without creating new; ii) producing a suitable feed mixture of hemicellulose monomeric sugars for ABE fermentation. The results of fermentation tests were promising as ABE solvents

were produced at a reasonable total concentration and yield. The effectiveness of resins treatment was not evaluated at this stage. The original combined scheme provided a useful starting platform upon which the industry optimized scheme was developed. A summary of the numerical findings presented in section 4.2 is shown below (Table 7).

**Table 7.** Summary of numerical findings: section 4.2

<b>Conditioning process</b>
No significant sugar losses during SEW fractionation
Largest sugar losses during vacuum evaporation (15% relative to SEW liquor)
Total sugar losses of 20% (relative to SEW liquor)
Only 13% of original lignin in spruce chips remaining in conditioned liquor
Ethanol and SO <sub>2</sub> totally removed
<b>CATOX liquor composition</b>
Total lignin concentration at 21 g L <sup>-1</sup>
Total sugars concentration at 110 g L <sup>-1</sup>
Monomeric sugars concentration at 97 g L <sup>-1</sup>
SO <sub>2</sub> at levels below 10 ppm
Amounts of major fermentation inhibitors (formic acid, furanic compounds) below inhibition levels for ABE fermentation
<b>ABE fermentation (batch mode)</b>
Total solvents concentration at 9 g L <sup>-1</sup>
n-butanol, acetone and ethanol produced at a ratio of 6:3:1
Fermentation yield of 0.20 g g <sup>-1</sup> sugars

## **4.3 Industry optimized scheme for SEW fractionation of lignocellulosics and spent liquor conditioning for ABE fermentation (Papers II, III)**

### **4.3.1 Process modifications: overview**

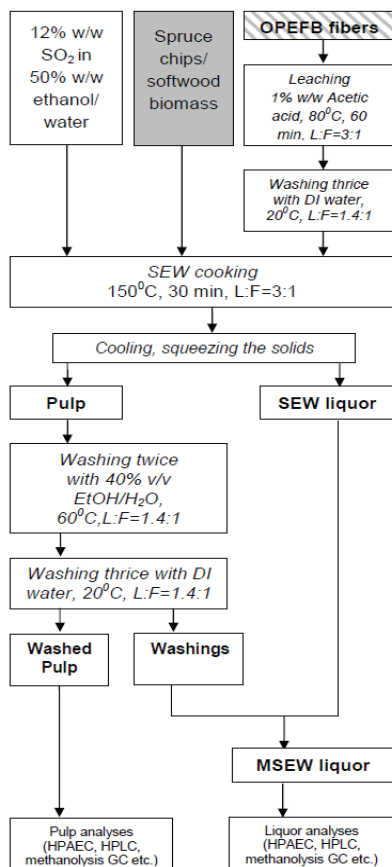
An industry optimized scheme for SEW fractionation of spruce chips/mixed softwood biomass and spent liquor conditioning for ABE fermentation is presented (see also Paper II). The fractionation conditions are modified to 12% SO<sub>2</sub> in 55 v/v% ethanol-water, L/F ratio of 3 L kg<sup>-1</sup>, 150<sup>0</sup>C, 30 min. The modifications are beneficial for the industry as they allow for:

- i. energy savings (less water and ethanol to be evaporated)
- ii. chemicals savings (less ethanol charged per weight of feedstock)
- iii. lower equipment capital costs (i.e. smaller digester size)
- iv. lower conditioning cost (higher dissolved wood concentrations)

The concentration of SO<sub>2</sub> is increased from 3% to 12% to compensate for the shorter fractionation time (120 vs. 30 minutes, respectively) and to allow for similar pulp delignification as in the original SEW fractionation scheme (Paper I).

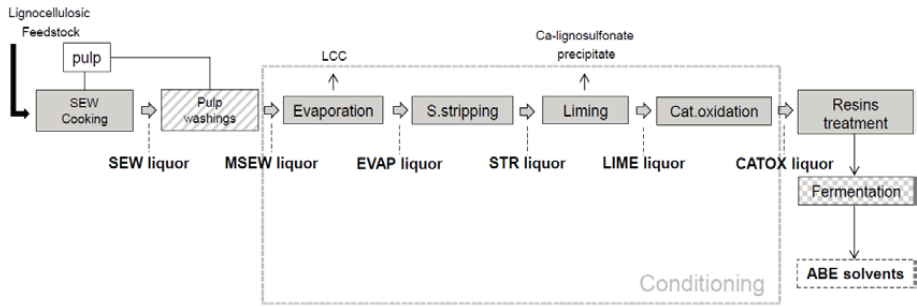
A modified pulp washing method is introduced to allow for better dissolution and recovery of the hemicellulose sugars after SEW fractionation. Better dissolution of sugars is satisfied by more intense washing as the pulp is washed twice with 40 v/v% ethanol-water at 60<sup>0</sup>C and three times with deionized water at room temperature (Fig. 8). Improved recovery of hemicellulose sugars after SEW fractionation is achieved by addition of the pulp washings to the SEW spent liquor to create dilute liquor (called MSEW liquor).





**Figure 8.** SEW fractionation and pulp washings in industry optimized scheme (Papers II, III, IV)

The general spent liquor conditioning process is kept the same as in the original scheme described previously (Fig. 9). The produced liquors after the step of catalytic oxidation are treated with anion exchange resins instead of non-polar resins to allow for better removal of dissolved anionic lignin. An investigation on sugar losses during this treatment is also performed. The process is tested with spruce chips which serve as our benchmark feedstock for comparison purposes and with mixed softwood biomass which is the actual feedstock considered for the present lignocellulosic biorefinery. SEW fractionation performance, chemical



**Figure 9.** Industry optimized SEW fractionation and spent liquor conditioning scheme (Papers II, III)

composition of the produced liquors and results of ABE fermentation tests are discussed below.

The following sections also present the results from a case study where a slightly modified (see section 4.3.2) version of the same industry optimized SEW fractionation and spent conditioning scheme is applied to non-woody lignocellulosics i.e. Oil Palm Empty Fruit Bunch (OPEFB) fibers to produce a suitable liquor for subsequent ABE fermentation (see also Paper III). The aim of this work is to demonstrate that the developed industry optimized scheme can handle also other, less conventional, lignocellulosic feedstocks for the production of biofuels and chemicals via ABE fermentation. This can increase the supply of suitable feedstocks for the SEW process.

In the following subsections the focus is on presenting and discussing different analysis results. However, some details are omitted and only those results which show important differences: i) compared to the original SEW fractionation and conditioning scheme (Paper I) ii) between different lignocellulosic feedstocks and respective conditioning liquors, are presented. The reader is referred to original Papers II and III for more detailed information.

### 4.3.2 SEW fractionation of different lignocellulosics

*SEW fractionation of spruce chips and mixed softwood biomass (Paper II)*

The results obtained after SEW fractionation at conditions of 12% SO<sub>2</sub>, L/F ratio of 3 L kg<sup>-1</sup>, 30 min (Table 8) suggest that hemicellulose dissolution at the new fractionation conditions is equally effective as in the original fractionation conditions (Paper I). Some apparent dissolution of cellulose seen only in the case of mixed softwood biomass could be due to calculation procedural inaccuracies or due to the presence of non-cellulosic glucans (Fengel and Wegener 1984).

**Table 8.** Sugars dissolution and pulp properties for spruce chips/mixed softwood biomass (industry optimized scheme)

	<b>spruce chips</b>		<b>mixed softwood biomass</b>	
	<b>‘Cellulose’</b>	<b>Hemicellulose</b>	<b>‘Cellulose’</b>	<b>Hemicellulose</b>
Original, % o.d.f.	39.6	22.6	30.9	19.7
MSEW liquor, % o.d.f.	1.5	19.4	5.4	17.0
Fraction dissolved	0.04	0.86	0.18	0.86
pulp intrinsic viscosity(mL g <sup>-1</sup> )	573		629	
pulp cellulose DP	2100		3260	
pulp kappa number	60		104	

The relative amount of oligomers is substantially higher in the mixed softwood biomass MSEW liquor compared to the spruce MSEW liquor (about 80% vs. 50%, see Paper II). The dehydration of sugars is generally not pronounced as inferred from the low amounts of furfural and HMF in spruce/mixed softwood biomass MSEW liquors (Tables 9, 10). Also only very small amounts of aldonic acids are formed indicating the near absence of hydrosulfite. More insight as to why very little SO<sub>2</sub> is converted to hydrosulfite ions can be found in Paper II.

Other phenomena observed during SEW fractionation of mixed softwood biomass (compared to fractionation of spruce) include less pulp delignification (Table 8). The lower delignification and less efficient fractionation of polysaccharides are explained by the lower acidity of cooking liquor in the case of mixed softwood biomass (pH of SEW spent liquor: 1.4 vs. 1.0, see Paper II). The lower acidity can be explained by the higher amount of alkali metals in mixed softwood biomass (Table 5) which dissolve in the fractionation liquor and neutralize the formed lignosulfonic acids.

*SEW fractionation of untreated OPEFB fibers: rational for leaching (Paper III)*

It is found that SEW fractionation of untreated OPEFB fibers at the industry optimized conditions suffers from poor hydrolytic performance as inferred by the high oligomers share of about 80% in MSEW liquor. The suppressed hydrolysis during SEW fractionation correlates with the low acidity of the SEW spent liquor (pH of 1.6). Low acidity indicates that a high fraction of the formed lignosulfonic acids are neutralized by metal cations present in OPEFB fibers. As discussed previously this phenomenon has also been observed during hydrolysis of mixed softwood biomass, however, it is much more pronounced with this feedstock. It is noted that the subsequent conditioning steps resulted in only limited reduction in oligomers content (see Paper III).

To improve polysaccharides depolymerization we performed leaching of OPEFB fibers before SEW fractionation with 1% w/w acetic acid solution (pH of 2.8) at a total L/F ratio of 3 L kg<sup>-1</sup> for 60 minutes at 80<sup>0</sup>C. More information on the rational for performing leaching with acetic acid can be found in Paper II.

**Table 9.** Mass balance for original spruce chips, pulp and conditioning liquors (industry optimized scheme). In brackets: concentration (g L<sup>-1</sup>) of components in CATOX liquor.

Solid (fiber) phase composition (% o.d.f.)	spruce					
	chips	pulp				
Solid yield	100	48.7				
Carbohydrates	62.2	41.3				
Cellulose	39.6	38.1				
<i>Glucan in hemicelluloses</i>	2.6	0.4				
<i>Arabinan</i>	1.1	0.0				
<i>Xylan</i>	5.6	1.2				
<i>Mannan</i>	10.8	1.6				
<i>Galactan</i>	2.5	0.0				
Extractives	1.5	1.3				
Lignin	28.9	5.0				
<i>Acid insoluble</i>	28.3	4.7				
<i>Acid soluble</i>	0.6	0.3				
Ash	0.3	0.0				
Acetyl groups	1.1	0.0				
Uronic acids	3.0	0.2				
Proteins	0.3	n.m				
Total in solid (fiber) phase	<b>97.3</b>	<b>47.8</b>				
Liquor composition (% o.d.f.)	SEW	MSEW	EVAP	STR	LIME	CATOX
Dry solids content*	29.4	43.2	22.9	21.2	25.0	23.5
Carbohydrates	9.9	17.1	16.0	15.8	15.4	15.4 (94.8***)
<i>Arabinose</i>	0.5	0.8	0.8	0.8	0.8	0.8 (5.0)
<i>Xylose</i>	2.2	3.7	3.6	3.5	3.4	3.4 (21.4)
<i>Mannose</i>	4.7	8.2	7.6	7.5	7.3	7.3 (44.9)
<i>Galactose</i>	1.0	1.7	1.5	1.5	1.5	1.5 (8.9)
<i>Glucose</i>	1.5	2.7	2.5	2.5	2.4	2.4 (14.6)
Lignin	11.6	17.8	4.1	3.7	3.7	3.0 (16.5)
<i>Acid insoluble</i>	10.1	15.8	1.3	0.9	0.9	0.3 (1.4)
<i>Acid soluble</i>	1.5	2.0	2.8	2.8	2.9	2.7 (15.1)
LCC			12.7	12.7	12.7	12.7
Ca-Lignosulfonate precip.					1.8	1.8
Furfural	0.1	0.2	0.0	n.d	n.d	n.d (n.d)
HMF	0.0	0.1	0.1	0.1	0.1	0.1 (0.3)
Ash	0.2	0.5	0.4	0.4	3.4	2.4
Acetic acid	0.5	1.0	0.4	0.0	0.0	0.0 (0.3)
Formic acid	0.0	0.0	0.0	0.0	0.0	0.0 (0.0)
Xyloic acid	n.d	n.d	n.d	n.d	n.d	n.d (n.d)
Mannonic acid	0.0	0.0	0.0	0.0	0.0	0.0 (0.0)
Glucuronic acid	0.3	0.3	0.9	0.8	0.7	0.7 (3.9)
Galacturonic acid	0.4	0.6	1.9	1.5	1.3	1.3 (7.2)
4-O-Me-glucuronic acid	0.1	0.2	0.6	0.5	0.4	0.4 (2.4)
Total in liquor**	<b>23.1</b>	<b>37.8</b>	<b>37.1</b>	<b>35.5</b>	<b>39.6</b>	<b>37.8</b>
Total in all fractionation streams (% o.d.f.)	70.9	85.6	84.9	83.3	87.4	85.6

n.d., not detected; n.m., not measured. \* Dry solids content refers to those of liquors and excludes the precipitated materials (LCC and Ca-Lignosulfonate). \*\*Total in liquor includes the precipitated materials \*\*\*Total sugars

**Table 10.** Mass balance for mixed softwood biomass, pulp and conditioning liquors (industry optimized scheme). In brackets: concentration (g L<sup>-1</sup>) of components in CATOX liquor.

Solid (fiber) phase composition (% o.d.f.)	softwood					
	biomass	pulp				
Solid yield	100	55.2				
Carbohydrates	51.6	28.2				
Cellulose	31.2	25.5				
<i>Glucan in hemicelluloses</i>	1.8	0.3				
<i>Arabinan</i>	4.7	0.0				
<i>Xylan</i>	6.0	1.2				
<i>Mannan</i>	7.3	1.1				
<i>Galactan</i>	3.1	0.1				
Extractives	4.8	1.0				
Lignin	29.8	9.7				
<i>Acid insoluble</i>	28.7	9.5				
<i>Acid soluble</i>	1.1	0.3				
Ash	2.6	0.6				
Acetyl groups	0.7	0.4				
Uronic acids	3.0	0.7				
Proteins	2.6	n.m				
Total in solid (fiber) phase	<b>95.1</b>	<b>40.6</b>				
Liquor composition (% o.d.f.)						
	SEW	MSEW	EVAP	STR	LIME	CATOX
Dry solids content*	21.8	38.8	23.8	22.1	22.4	22.3
Carbohydrates	9.8	18.9	14.0	13.9	13.1	13.0 (61.6***)
<i>Arabinose</i>	1.1	1.9	1.5	1.5	1.4	1.4 (6.8)
<i>Xylose</i>	2.7	5.3	4.0	4.0	3.8	3.8 (18.1)
<i>Mannose</i>	2.9	5.6	4.1	4.1	3.8	3.8 (17.8)
<i>Galactose</i>	1.8	3.4	2.4	2.4	2.2	2.2 (10.4)
<i>Glucose</i>	1.4	2.7	2.0	1.9	1.8	1.8 (8.5)
Lignin	9.5	16.7	4.2	4.0	2.6	2.5 (10.6)
<i>Acid insoluble</i>	7.9	14.0	1.9	1.9	0.9	1.1 (4.6)
<i>Acid soluble</i>	1.7	2.7	2.3	2.1	1.8	1.4 (6.0)
LCC			11.0	11.0	11.0	11.0
Ca-Lignosulfonate precip.					3.2	3.2
Furfural	0.0	0.1	n.d	n.d	n.d	n.d (n.d)
HMF	0.0	0.0	0.0	0.0	0.0	0.0 (0.2)
Ash	0.4	1.2	0.6	0.6	2.2	2.2
Acetic acid	0.1	0.3	0.6	0.0	0.0	0.0 (0.0)
Formic acid	0.0	0.0	0.0	0.1	0.1	0.1 (0.4)
Xyloic acid	n.d	n.d	n.d	n.d	n.d	n.d (n.d)
Glucuronic acid	0.1	0.2	0.2	0.2	0.2	0.2 (0.6)
Glucuronic acid	0.0	0.0	0.0	0.0	0.0	0.0 (0.0)
Galacturonic acid	1.3	2.9	2.2	2.2	2.0	2.0 (7.5)
4-O-Me-glucuronic acid	0.5	1.1	0.9	0.9	0.9	0.9 (3.3)
Total in liquor**	<b>21.7</b>	<b>41.4</b>	<b>33.7</b>	<b>32.9</b>	<b>35.3</b>	<b>35.1</b>
Total in all fractionation streams (% o.d.f.)	<b>62.3</b>	<b>82.0</b>	<b>74.3</b>	<b>73.5</b>	<b>75.9</b>	<b>75.7</b>

n.d., not detected; n.m., not measured. \* Dry solids content refers to those of liquors and excludes the precipitated materials (LCC and Ca-Lignosulfonate). \*\*Total in liquor includes the precipitated materials \*\*\*Total sugars

*The effect of leaching on OPEFB fibers (Paper III)*

From Table 11 it is inferred that leaching reduces the overall alkali metals and silica content by approximately 25%. This acidic treatment removes ca. 40% of potassium and magnesium cations, while silica is almost completely retained in the fibers.

**Table 11.** Elemental composition of the ashes of original OPEFB fibers, OPEFB fibers after leaching, and OPEFB fibers after SEW fractionation.

Element	% o.d.f.		
	OPEFB	OPEFB after leaching	OPEFB after SEW fractionation
K	1.047	0.644	0.089
Na	0.037	0.037	0.004
Al	0.020	0.019	0.018
Mg	0.110	0.063	0.007
Ca	0.192	0.181	0.091
Si	0.674	0.648	0.467
Total	2.080	1.592	0.676

*SEW fractionation of leached OPEFB fibers (Paper III)*

SEW fractionation results in further dissolution of inorganics. The potassium, magnesium and calcium content of the SEW fractionated pulp is almost zero (Table 11). A comparison of the overall metals and silicon content of the leached vs. non-leached OPEFB fibers after SEW fractionation (see Paper III) reveals that the acidic pre-treatment followed by SEW fractionation is beneficial because it results in better removal of inorganics from OPEFB fibers. The positive effect of the higher reduction of metals content is reflected in the higher acidity of SEW, MSEW, EVAP and STR liquors (see Paper III). This suggests that hydrolysis conditions are more intense both during SEW fractionation and conditioning. This is confirmed by the

lower oligomers content in all conditioning liquors including final CATOX liquor (oligomers share lower by about 10%, see Paper III).

Table 12 shows that about 2/3 of hemicellulose sugars are dissolved in the MSEW liquor and that there is also some dissolution of cellulose. This may be the result of experimental and calculation procedural inaccuracies as the acidity during SEW fractionation is relatively mild.

**Table 12.** Sugars dissolution and pulp properties for SEW fractionation of leached OPEFB fibers

	‘Cellulose’	Hemicellulose
Original, % o.d.f.	36.2	22.2
MSEW liquor, % o.d.f.	3.9	14.2
Fraction dissolved	0.1	0.64
Pulp intrinsic viscosity (mL g <sup>-1</sup> )		553
Pulp cellulose DP		2920

The high acidity (pH of 1.0) of SEW spent liquor originating from leached OPEFB fibers suggests that only a small amount of the formed lignosulfonic acids is neutralized by the metal cations solubilized during SEW fractionation of leached OPEFB fibers. However, the relative amount of oligomers is still substantially higher for leached OPEFB-based SEW and MSEW liquors (oligomers content of about 70% for both, pH of 1.0 and 1.2, respectively) compared to spruce SEW and MSEW liquors (oligomers content of about 40 and 50%, pH of 1.0 and 1.1, respectively, see Paper III). This suggests that OPEFB fibers are more recalcitrant to SEW hydrolysis. It is noted that sugars dehydration is not pronounced as confirmed by the very low amounts of furans in the MSEW liquor (see section 4.3.5).



### **4.3.3 Lignin behavior during SEW fractionation and spent liquor conditioning**

*Tested feedstocks and respective conditioning liquors (Papers II, III)*

The following general remarks apply to all the tested lignocellulosic feedstocks and their conditioning liquors:

- i. delignification of spruce chips at the industry optimized SEW fractionation conditions is similar to delignification at the original SEW fractionation conditions (pulp kappa number of about 60 in both cases).
- ii. mixed softwood biomass delignification at the industry optimized SEW fractionation conditions is impaired compared to spruce chips (pulp kappa number of 104 vs. 60). An explanation for this difference is the presence of polyphenolic acids in the bark contained in the present mixed softwood biomass. It is reported (Goldstein 1975, Erman and Lyness 1965) that these flavonoids-derived polymers are present in amounts of 40-50% based on bark weight. It is therefore expected that their amount in the present mixed softwood biomass is significant (estimated at about 10% (o.d.f.)). According to Browning (1963) polyphenolic acids can constitute up to 50% of the material found in 'Klason' lignin. It is therefore presumed that these acids contribute also to the high observed kappa number. Removal of polyphenolic acids would therefore be necessary to further delignify mixed softwood biomass and facilitate easier enzymatic hydrolysis of the solid residue. It is known that polyphenolic acids dissolve in alkali but not in organic solvents such as acetone (Fengel and Wegener 1984). Research by Iakovlev (unpublished work) shows that a sequence of steps comprising acetone extraction, extraction with 1% NaOH and SEW fractionation at

conditions of 12% SO<sub>2</sub>, L/F ratio of 6 L kg<sup>-1</sup>, 60 min leads to the removal of about 90% of total lignin in original mixed softwood biomass. The latter is about 60% actual lignin and about 40% lignin-like material (polyphenolic acids).

- iii. OPEFB fibers are delignified less efficiently compared to both spruce chips and mixed softwood biomass. The reason for this is unknown.
- iv. addition of the pulp washings in the different feedstock-based SEW spent liquors results in approximate two-fold increase of the amount of dissolved lignin in the MSEW liquors (Tables 9, 10, 13).
- v. the low degree of sulfonation, quantified as S/C<sub>9</sub> molar ratio, for residual lignin in the pulp fibers and for dissolved lignin in MSEW liquor (Table 14) suggests that hydrosulfite anions which are reported to cause extensive lignin sulfonation (Iakovlev and van Heiningen 2012b) are not present in significant amounts during SEW fractionation.
- vi. the majority of dissolved lignin is removed during the conditioning step of vacuum evaporation (Tables 9, 10, 13) which is in agreement with previous findings from the original SEW spent liquor conditioning scheme. Dissolved lignin is removed either as colloidal precipitate or as char-like LCC upon evaporation of the respective spruce/mixed softwood biomass-based MSEW liquors and OPEFB fibers-based MSEW liquors. It is unclear however what the fundamental reason is for the state of the precipitated lignin, i.e. colloidal or char-like. The degree of sulfonation S/C<sub>9</sub> of lignin remaining in solution (about 0.5) is much higher than degree of sulfonation of the colloidal/char-like LCCs (0.03-0.07, see also Table 14) and corresponds to the lower end of the reported values for acid sulfite dissolved lignin (0.5-0.7,

Rydholm 1965, p. 467). The large difference in degree of sulfonation indicates that lignin is sulfonated non-uniformly, and the less sulfonated fraction precipitates when ethanol is removed to levels of  $10 \text{ g L}^{-1}$ .

- vii. the remaining amounts of dissolved lignin in STR, LIME, and CATOX liquors are progressively reduced in agreement with previous findings from the original conditioning scheme and they all reach final levels of about 3% (o.d.f). Thus the residual dissolved lignin in CATOX liquors corresponds to about 10-13% of lignin in original feedstocks. The majority of lignin present in CATOX liquors (80% on average) is found in acid soluble form (so-called UV lignin). The dissolved lignin levels in the spruce/mixed softwood biomass/leached OPEFB fibers-based CATOX liquors are at 17/11/13  $\text{g L}^{-1}$ , respectively.

**Table 13.** Mass balance for original OPEFB fibers, pulp after leaching & SEW fractionation and conditioning liquors (industry optimized scheme). In brackets: concentration ( $\text{g L}^{-1}$ ) of components in CATOX liquor.

Solid (fiber) phase composition (% o.d.f.)	OPEFB							
	fibers	pulp						
Solid yield	100	66.0						
Carbohydrates	58.4	40.4						
Cellulose	36.2	32.3						
<i>Glucan in hemicelluloses</i>	0.4	0.4						
<i>Arabinan</i>	0.1	0.0						
<i>Xylan</i>	21.0	7.6						
<i>Mannan</i>	0.6	0.3						
<i>Galactan</i>	0.0	0.0						
Extractives	3.2	2.4						
Lignin	21.6	10.5						
<i>Acid insoluble</i>	17.3	8.8						
<i>Acid soluble</i>	4.3	1.7						
Ash	5.4	1.5						
Acetyl groups	3.0	1.4						
Uronic acids	5.4	0.2						
Proteins	4.0	n.m						
Total in solid (fiber) phase	<b>101.0</b>	<b>56.4</b>						
Liquors composition								
(% o.d.f.)	SEW	MSEW	EVAP	STR	LIME	CATOX		
Dry solids content*	16.7	29.0	20.8	20.1	21.3	19.9		
	6.8	14.3	13.8	13.5	13.4	13.3		
Carbohydrates						(60.9***)		
<i>Arabinose</i>	0.4	0.8	0.8	0.8	0.8	0.8 (3.7)		
<i>Xylose</i>	5.9	12.2	11.9	11.7	11.5	11.5 (52.8)		
<i>Mannose</i>	0.1	0.2	0.2	0.2	0.2	0.2 (1.0)		
<i>Galactose</i>	0.2	0.5	0.4	0.4	0.4	0.4 (1.8)		
<i>Glucose</i>	0.2	0.6	0.4	0.4	0.4	0.4 (1.7)		
Lignin	5.8	11.4	5.1	4.4	3.8	3.3 (13.3)		
<i>Acid insoluble</i>	3.5	7.4	1.5	1.1	0.9	0.8 (3.3)		
<i>Acid soluble</i>	2.3	4.0	3.7	3.3	2.9	2.5 (10.0)		
LCC			9.2	9.2	9.2	9.2		
Ca-lignosulfonate precip.					2.0	2.0		
Furfural	0.0	0.1	n.d	n.d	n.d	n.d (n.d)		
HMF	n.d	0.0	0.0	0.0	n.d	n.d (n.d)		
Ash	0.7	1.3	1.4	1.4	2.3	1.7		
Acetic acid	0.3	0.8	0.7	n.d	n.d	n.d (n.d)		
Formic acid	n.d	n.d	n.d	n.d	n.d	n.d (n.d)		
Xylonic acid	0.3	0.8	0.6	0.8	0.8	0.6 (2.3)		
Gluconic acid	0.1	0.2	0.2	0.2	0.2	0.2 (0.6)		
Glucuronic acid	0.0	0.0	0.0	0.0	0.0	0.0 (0.0)		
Galacturonic acid	0.4	0.8	0.6	0.5	0.2	0.2 (0.9)		
4-O-Me-glucuronic acid	0.4	0.8	0.6	0.6	0.6	0.8 (3.1)		
Total in liquor**	<b>14.8</b>	<b>30.5</b>	<b>32.2</b>	<b>30.6</b>	<b>32.5</b>	<b>31.3</b>		
Total in all fractionation								
streams (% o.d.f.)	<b>71.2</b>	<b>86.9</b>	<b>88.6</b>	<b>87.0</b>	<b>88.9</b>	<b>87.7</b>		

n.d., not detected; n.m., not measured. \* Dry solids content refers to those of liquors and excludes the precipitated materials (LCC and Ca-Lignosulfonate). \*\*Total in liquor includes the precipitated materials \*\*\*Total sugars

**Table 14.** Lignin sulfur content

	<b>Feedstock</b>	<b>Pulp</b>	<b>MSEW liquor</b>	<b>LCC</b>	<b>EVAP liquor</b>
<b>spruce chips</b>					
Lignin, % o.d.f.	28.9	5.0	17.8	12.7	4.1
Sulfur, % o.d.f.	0.004	0.084	0.76	0.062	0.36
Sulfur, S/C9	–	0.10	0.25	0.029	0.52
<b>mixed softwood biomass</b>					
Lignin,% o.d.f.	29.8	9.7	16.7	11.0	4.2
Sulfur, % o.d.f.	0.024	0.13	0.75	0.097	0.36
Sulfur, S/C9	0.005	0.083	0.27	0.049	0.51
<b>OPEFB</b>					
Lignin, % o.d.f.	21.6	10.5	11.4	9.2	5.1
Sulfur, % o.d.f.	0.083	0.255	0.57-0.68	0.114	0.35-0.55
Sulfur, S/C9	0.023	0.144	0.30-0.35	0.074	0.41-0.63

#### 4.3.4 Sugar analyses

*Spruce/mixed softwood biomass (Paper II) and leached OPEFB fibers-based (Paper III) conditioning liquors*

The following general remarks apply for the different feedstock-based conditioning liquors:

- i. from Tables 9, 10, 13 it is inferred that the amount of anhydrosugars in the different feedstock-based MSEW liquors is nearly double the amount of anhydrodugars found in the respective SEW spent liquors indicating that a substantial amount of dissolved sugars remains in the pulps after drainage and squeezing when using a L/F ratio of 3 L kg<sup>-1</sup> (consistency of squeezed spruce, mixed softwood biomass, OPEFB pulps at 33, 38, 44%, respectively, spent liquor-to-feedstock ratio of 1.9, 1.6 and 1.2 for spruce, mixed softwood biomass and OPEFB fibers, respectively). The majority of these sugars are efficiently removed by pulp washing.

- ii. The majority of sugar losses during conditioning of all liquors happens during the step of vacuum evaporation presumably due to co-precipitation/entrapment of sugars in the colloidal precipitate/char-like LCC. These losses are small for spruce/OPEFB-based EVAP liquors, however, they are quite high for mixed softwood biomass-based EVAP liquors, standing at about 25% relative to MSEW liquor. An explanation is that the lower acidity of the mixed softwood biomass EVAP liquor (pH 1.6 vs. pH 0.8 for spruce EVAP liquor) results in suppressed hydrolysis of the LCC precipitate and thus lower amounts of sugars are released from the LCC colloidal precipitate.
- iii. the overall share of sugar dehydration products (furfural and HMF) and other degradation products to sugar losses is very small (see Tables 9, 10, 13).
- iv. the total amount of anhydrosugars after consecutive conditioning steps of steam stripping, liming and catalytic oxidation is gradually reduced but only marginally, in agreement with previous findings from the original conditioning scheme. Anhydrosugar amounts in CATOX liquors are in the range of 13-15% (o.d.f.). The identified total sugar losses after conditioning, account for about 10/30/7% relative to MSEW spent liquor (spruce/mixed softwood biomass/leached OPEFB fibers-based CATOX liquors, respectively).
- v. total sugars concentration in CATOX liquors is 95/62/61 g L<sup>-1</sup> (spruce/mixed softwood biomass/leached OPEFB fibers-based CATOX liquors, respectively). The lower total sugar concentrations for mixed softwood biomass/leached OPEFB fibers-based CATOX liquors (compared

to spruce-based CATOX liquor) originate from less effective hydrolysis of the respective feedstocks during SEW fractionation (see also below).

- vi. for all conditioning stages it is noted that monomeric sugars share in mixed softwood biomass/OPEFB fibers-based liquors is lower compared to monomers share in spruce-based liquors. The lower acidity of the former liquors is due to the high ash content of original mixed softwood biomass and OPEFB fibers, even when acidic leaching is performed for the latter (see section 4.3.2). Monomers share in CATOX liquors is about 80/70/50%, (spruce/mixed softwood biomass/leached OPEFB fibers-based CATOX liquors, respectively).
- vii. the relatively low monomers share in OPEFB fibers-based CATOX liquor suggests that the acidity and/or temperature during fractionation of high ash content feedstocks must be further increased to finally achieve an oligomers content of ca. 20% after conditioning as is found for spruce-based CATOX liquor.

#### **4.3.5 Removal of ABE fermentation inhibitors**

##### *Removal of furfural, HMF and formic acid*

From the furfural and hydroxymethylfurfural (HMF) analysis results (Tables 9, 10, 13) it is shown that the respective amounts of these compounds in different feedstock-based SEW spent liquors are close to their detection limit. This suggests that pentose and hexose sugar dehydration during SEW fractionation at the industry optimized conditions is also minimal. Both fermentation inhibitors are at zero/non-detect levels in the CATOX liquors produced after conditioning. Furfural is totally evaporated already at an early stage i.e. after the steps of vacuum evaporation and

steam stripping.

The amounts of formic acid are close to zero in all conditioning liquors suggesting that HMF degradation to formic acid is insignificant (Tables 9, 10, 13).

#### *Removal of SO<sub>2</sub>*

IC analysis results (Table 15) reveal that the SO<sub>2</sub> concentration in the fresh cooking liquor is about 120 g kg<sup>-1</sup> corresponding to SO<sub>2</sub> charge of about 33 % (o.d.f.) (density of fresh cooking liquor 0.957 kg L<sup>-1</sup>). This amount is approximately twice the amount of SO<sub>2</sub> charged in the fresh cooking liquor used for fractionation of spruce chips at conditions of 3% SO<sub>2</sub> in 55 v/v% ethanol-water, L/F ratio of 6 L kg<sup>-1</sup>, 150°C, 120 min (about 16% (o.d.f), density of fresh cooking liquor 0.924 kg L<sup>-1</sup>, see Paper I).

A small part of the original amount of SO<sub>2</sub> charged is consumed during SEW fractionation, mostly in lignin sulfonation reactions. The amount of SO<sub>2</sub> consumed corresponds to about 2.5% (o.d.f.) (van Heiningen et al. 2012). The measured concentration of SO<sub>2</sub> in SEW spent liquor is about 40 g L<sup>-1</sup> corresponding to only 6.5% (o.d.f). The amount of residual SO<sub>2</sub> in MSEW liquor (o.d.f) equals that in SEW liquor. The measured amounts of SO<sub>2</sub> in the SEW and MSEW liquors are low because significant SO<sub>2</sub> losses to the atmosphere occur during the handling of the liquors and the pulps.

It is shown (Table 15) that the SO<sub>2</sub> content is gradually reduced from about 40 g L<sup>-1</sup> in the SEW spent liquor to 10 ppm (tolerance limit by *Clostridia*)/non-detect levels in the CATOX liquor. It is therefore inferred that the current industry-optimized conditioning scheme is very effective at fully removing SO<sub>2</sub> from the SEW spent liquor despite the high initial SO<sub>2</sub> charge.



Similar IC analysis results are obtained for conditioning liquors derived from leached OPEFB fibers fractionated at the industry-optimized conditions (see Paper III).

**Table 15.** IC analysis results for conditioning liquors derived from SEW fractionated spruce/mixed softwood biomass (industry optimized scheme)

		fresh cooking liquor	spruce chips					CATOX
			SEW	MSEW	EVAP	STR	LIME	
concentration (mg L <sup>-1</sup> )								
	SO <sub>3</sub> <sup>2-</sup>	n.m	42780	10060	6080	126	25	12
	SO <sub>4</sub> <sup>2-</sup>	n.m	2670	880	2690	2780	3060	3780
	SO <sub>2</sub> *	115000	34220	8050	4860	101	20	10
	<b>SO<sub>2</sub> (% o.d.f.)</b>	<b>33.3</b>	<b>6.6</b>	<b>6.6</b>	<b>0.9</b>	<b>0.2</b>	<b>0.0</b>	<b>0.0</b>
		fresh cooking liquor	mixed softwood biomass					CATOX
			SEW	MSEW	EVAP	STR	LIME	
concentration (mg L <sup>-1</sup> )								
	SO <sub>3</sub> <sup>2-</sup>	n.m	44940	8740	6930	109	31	n.d
	SO <sub>4</sub> <sup>2-</sup>	n.m	2710	840	2410	2340	2650	3360
	SO <sub>2</sub> *	115000	35950	6990	5550	87	25	n.d
	<b>SO<sub>2</sub> (% o.d.f.)</b>	<b>33.3</b>	<b>5.7</b>	<b>5.7</b>	<b>1.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>

\* Calculated from sulfite anions, n.d., not detected, n.m., not measured

#### 4.3.6 Overall mass balance

From the overall mass balances (Tables 9, 10, 13) it is shown that the dry solids content of all liquors corresponds reasonably well to the total identified components. However, there are some discrepancies when comparing total mass balances after each conditioning step. For example, the sum of the identified components in the pulp and MSEW spent liquors is in the range of about 82 up to 87% which is less than the original 95-100% total for the original feedstocks. These

discrepancies might be due to components that are not possible to identify using the employed analytical techniques. It is also likely that part of the discrepancies is due to analytical errors. The sum of the mass of the components in the EVAP liquors, pulps, and LCCs is in the range of about 74 to 89%. At the end of the conditioning process, the total solids identified account for about 76 to 88% of the original feedstock weight.

#### **4.3.7 ABE fermentation results**

The conditioned CATOX liquors are treated with anion exchange resins, 4-fold diluted and supplemented with glucose according to the procedure described earlier (see section 3). Fermentation tests are performed either by using a patent pending fermentation column that uses wood pulp as cell immobilization material or on a batch mode as discussed previously. The column fermentation tests utilize spruce chips/mixed softwood biomass-based CATOX liquors and the batch fermentations tests utilize leached OPEFB fibers-based CATOX liquor. It is found that all conditioned liquors are fermentable by *Clostridia* bacteria. Column fermentation tests produce ABE solvents at a maximum total concentration of 13 g L<sup>-1</sup> (total solvents yield of around 0.25 g g<sup>-1</sup> sugars) and batch fermentation tests produce ABE solvents at a maximum total concentration of about 7 g L<sup>-1</sup>. Acetone, n-butanol and ethanol are produced at a ratio of 3:6:1, respectively, and total solvents yield is about 0.25-0.26 g g<sup>-1</sup> sugars (maximum theoretical yield is ca. 0.40 g g<sup>-1</sup> sugars).

An investigation on treatment with anion exchange resins reveals that this step removes about 50% of dissolved lignin leading to dissolved lignin levels of about 1.0 g L<sup>-1</sup> (tolerance limit by *Clostridia*) in the final liquors after 4-fold dilution.

However, high concurrent sugar losses of approximately 35% indicate that anion exchange resins are not sufficiently selective adsorbents for lignin removal. This finding is in agreement with previous reports from the literature (Larsson et al. 1999). It is therefore concluded that an alternative method is needed to remove selectively lignin and to improve ABE fermentation performance. This topic is thoroughly investigated in section 4.4 (see also Paper IV).

#### **4.3.8 Summary of findings on industry-optimized conditioning scheme**

The developed industry-optimized scheme for SEW fractionation of different lignocellulosics and spent liquor conditioning was successful at: i) removing fermentation inhibitors such as furans and formic acid without creating new; ii) removing ethanol; iii) removing SO<sub>2</sub> to levels at/below inhibition (10 ppm for *Clostridia*) despite the significantly higher initial SO<sub>2</sub> charge in the fresh SEW fractionation liquor (compared to original fractionation conditions). Furthermore, it was demonstrated that the developed scheme can process effectively spruce chips and mixed softwood biomass feedstocks to produce a suitable feed mixture of hemicellulose monosugars for ABE fermentation. However, there were some limitations with regards to fractionation of OPEFB fibers and to a lesser extent with fractionation of mixed softwood biomass. The former feedstock was particularly recalcitrant to hydrolysis due to the fibers high alkali and alkali-earth metals content which suppressed the hydrolysis of sugars to monomers during SEW fractionation and conditioning. An additional pre-treatment step (acidic leaching) was adopted before SEW fractionation to facilitate partial removal of alkali metals from the OPEFB fibers and better hydrolysis of sugars. This pre-treatment step resulted in

only a small increase of monomers content in OPEFB fibers-based CATOX liquor (increase of about 10%). This suggests that the acidity and/or temperature during fractionation must be further increased to achieve an oligomeric content in the final conditioned liquor that is comparable to spruce-based CATOX liquor.

The results of continuous and batch scale fermentation tests showed that all conditioned liquors can be fermented to ABE solvents at a reasonably good total concentration and yield despite their different sugar and lignin composition.

The current research work revealed also some limitations with regards to use of anion exchange resins for removal of dissolved lignin before ABE fermentation. It was found that resins are not selective lignin adsorbents due to high concurrent sugar losses. It is therefore concluded that an alternative method is needed to reduce selectively the levels of lignin dissolved in the feed liquor for ABE fermentation. A summary of the numerical findings presented in section 4.3 is shown below (Table 16).

**Table 16.** Summary of numerical findings: section 4.3 (values separated by ‘/’ for spruce, mixed softwood biomass, leached OPEFB fibers-based liquors, respectively)

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**Conditioning process**

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No significant sugar losses during SEW fractionation

Largest sugar losses during vacuum evaporation

Total sugar losses of 10/30/7% relative to MSEW spent liquor

Residual dissolved lignin in CATOX liquors corresponds to about 10-13% of lignin in original feedstocks

Ethanol and SO<sub>2</sub> totally removed

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**CATOX liquor composition**

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Total lignin concentration 17/11/13 g L<sup>-1</sup>

Total sugars concentration 95/62/61 g L<sup>-1</sup>

Monomeric sugars concentration 73/51/25 g L<sup>-1</sup>

SO<sub>2</sub> levels at zero/non detect levels

Amounts of major fermentation inhibitors (formic acid, furanic compounds) below inhibition levels for ABE fermentation

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**ABE fermentation**

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Total solvents concentration from 7 g L<sup>-1</sup> (batch mode, leached OPEFB-based CATOX liquor) to 13 g L<sup>-1</sup> (continuous mode, spruce/mixed softwood biomass based CATOX liquor)

n-butanol, acetone and ethanol produced at a ratio of 6:3:1

Fermentation yield of 0.25-0.26 g g<sup>-1</sup> sugars

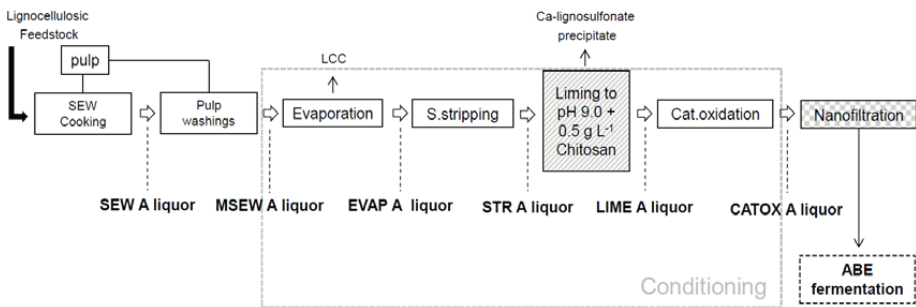
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## **4.4 Comparison of two modified conditioning schemes for detoxifying SEW hydrolysate from lignocellulosics for ABE fermentation (Paper IV)**

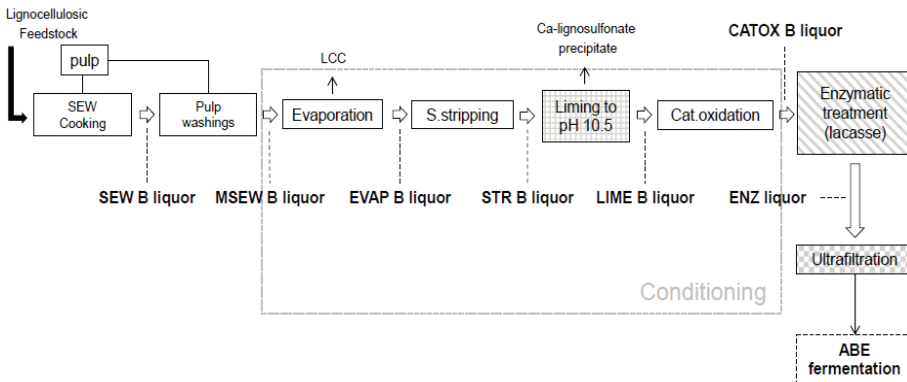
### **4.4.1 Aim of comparison and overview of modified conditioning schemes**

A comparison of two conditioning schemes (schemes A and B) for detoxifying SO<sub>2</sub>-ethanol-water hydrolysate from lignocellulosics for ABE fermentation is attempted. These conditioning schemes are modified versions of the industry optimized conditioning scheme presented in section 4.3 (see also Papers II, III). The target of the modifications is to selectively reduce dissolved lignin levels in the spruce-based liquor to levels below 4 g L<sup>-1</sup> so that upon 4-fold dilution the lignin concentration is below the tolerance limit of 1 g L<sup>-1</sup> for *Clostridia*. Also the modifications are designed to replace treatment with resins because this method suffers from serious drawbacks (see section 4.3). The ultimate goal is to improve performance of ABE fermentation i.e. total solvents concentration and fermentation yield. The major difference between the two tested schemes is in the final stages to reduce the dissolved lignin concentration. Scheme A incorporates addition of 0.5 g L<sup>-1</sup> low molecular weight (70-180 kDa) chitosan after the liming step (pH 9.0) and it is combined with nanofiltration after the step of catalytic oxidation. Scheme B incorporates liming to pH 10.5 and enzymatic treatment with laccase after catalytic oxidation to oxidatively polymerize dissolved lignin in order to improve its removal. It is combined with ultrafiltration. Figures 10 and 11 present the two modified liquor conditioning schemes including the names of the liquors after each conditioning step. A new name is introduced in scheme B i.e. 'ENZ' to denote

liquor after the enzymatic treatment. The permeate liquors after membrane filtrations are used for downstream ABE fermentation. They are totally free of all other ABE fermentation inhibitors ( $\text{SO}_2$ , ethanol, formic acid, furanic compounds) in accordance with previous reported results. Results of ABE fermentation tests are evaluated to determine which conditioning scheme and membrane filtration is more preferable.



**Figure 10.** SEW spent liquor conditioning scheme incorporating addition of  $0.5 \text{ g L}^{-1}$  chitosan after liming to pH 9.0 (scheme A). This scheme is combined with nanofiltration after catalytic oxidation step.



**Figure 11.** SEW spent liquor conditioning scheme incorporating: i) liming to pH 10.5 ii) laccase treatment after catalytic oxidation step (scheme B). This scheme is combined with ultrafiltration.

#### 4.4.2 Lignin removal during conditioning (conditioning schemes A and B)

The following general remarks apply for liquors produced under conditioning schemes A and B (Tables 17, 18):

- i. lignin amounts are quite similar for both liquors A and B until the step of steam stripping and in agreement with previous results (see section 4.3.3).
- ii. as expected the lignin amounts in the liquors are different after the different liming steps. It seems that liming to pH 10.5 results in higher dissolved lignin removal.
- iii. the final lignin amounts in CATOX A/CATOX B liquors are at about 2.6/2.1% (o.d.f.)
- iv. lignin in CATOX B liquor is found only in acid soluble form as the acid insoluble fraction is removed during the preceding overliming step.
- v. about 9/7% of original lignin in spruce chips remained in CATOX A/CATOX B liquors as dissolved lignin (lignosulfonate).

**Table 17.** Amounts of sugars and lignin in the original spruce chips, pulp and conditioning liquors (% o.d.f.). Liquor CATOX A subjected to subsequent nanofiltration.

Solid (fiber) phase (% o.d.f.)	spruce					
	chips	pulp				
<b>Carbohydrates</b>	<b>62.2</b>	<b>45.3</b>				
<b>Lignin</b>	<b>28.9</b>	<b>5.4</b>				
<i>Acid insoluble</i>	28.3	5.1				
<i>Acid soluble</i>	0.6	0.3				
<b>Conditioning liquors (% o.d.f.)</b>						
	SEWA	MSEWA	EVAP A	STRA	LIME A	CATOX A
<b>Carbohydrates</b>	<b>7.7</b>	<b>16.8</b>	<b>16.1</b>	<b>15.7</b>	<b>14.5</b>	<b>14.1</b>
<b>Lignin</b>	<b>8.5</b>	<b>17.4</b>	<b>3.7</b>	<b>3.3</b>	<b>2.7</b>	<b>2.6</b>
<i>Acid insoluble</i>	7.4	15.5	0.9	0.6	0.5	0.5
<i>Acid soluble</i>	1.1	1.9	2.8	2.6	2.2	2.1
LCC			10.6	10.6	10.6	10.6
Ca-Lignosulfonate precip.					1.8	1.8



#### **4.4.3 Sugar analyses (conditioning schemes A and B)**

The following general remarks apply for liquors produced under conditioning schemes A and B (Tables 17, 18):

- i. total amount of sugars (given as anhydrosugars) is quite similar in conditioning liquors A and B until the step of steam stripping. Monomeric sugars content is also at similar levels of about 70% on average, in both STR liquors.
- ii. liming to pH 9.0 followed by addition of chitosan results in total sugar losses of about 8% compared to STR A liquor. These sugar losses are significantly higher compared to sugar losses of about 2.5% after liming to pH 9.0 only. A likely explanation for these losses is the entrapment of sugars in the complex formed as a result of electrostatic interactions between cationic chitosan and anionic lignosulfonate (Saeed et al. 2011). Minor sugar losses of 5% relative to STR B liquor suggest that most of the total sugars are preserved after liming to pH 10.5.
- iii. liming to pH 9.0 followed by addition of chitosan also causes preferential precipitation of oligomers resulting in 10% higher monomers content in LIME A liquor. However, it seems that oligomers remain unaffected when liming is performed to pH 10.5.
- iv. catalytic oxidation of LIME B liquor results in reduced monomers content by about 9%. An explanation is that monosaccharides suffer oxidative degradation at alkaline conditions of pH 10.5 when air is supplied together with iron catalyst (Shen et al. 2011). Catalytic oxidation of LIME A liquor does not have any adverse effect on monomers.
- v. anhydrosugars in CATOX A/CATOX B liquors are at about 14/15.5%

(o.d.f).

vi. monomers share in CATOX A/CATOX B liquors is about 80/60%.

**Table 18.** Amounts of sugars and lignin in the original spruce chips, pulp and conditioning liquors (% o.d.f.). Liquor CATOX B subjected to subsequent laccase treatment.

Solid (fiber) phase (% o.d.f.)	spruce					
	chips	pulp				
<b>Carbohydrates</b>	<b>62.2</b>	<b>44.3</b>				
<b>Lignin</b>	<b>28.9</b>	<b>5.1</b>				
<i>Acid insoluble</i>	28.3	4.9				
<i>Acid soluble</i>	0.6	0.2				
Conditioning liquors (% o.d.f.)						
	SEW B	MSEW B	EVAP B	STR B	LIME B	CATOX B
<b>Carbohydrates</b>	<b>7.2</b>	<b>18.0</b>	<b>17.0</b>	<b>16.9</b>	<b>16.1</b>	<b>15.6</b>
<b>Lignin</b>	<b>8.1</b>	<b>17.6</b>	<b>4.6</b>	<b>4.3</b>	<b>2.9</b>	<b>2.1</b>
<i>Acid insoluble</i>	7.1	15.7	1.4	1.0	0.7	0.0
<i>Acid soluble</i>	1.0	1.9	3.1	3.3	2.2	2.1
LCC			12.0	12.0	12.0	12.0
Ca-Lignosulfonate precip.					3.6	3.6

#### 4.4.4 Enzymatic treatment of liquor produced under conditioning scheme B

CATOX B liquor is enzymatically treated with laccase according to the process described in section 3. The weight-average molecular mass ( $M_w$ ) of dissolved lignin in the liquor increases from 5 to about 50 kDa, after only 24 h. At the end of the enzymatic treatment (168 h) the  $M_w$  of dissolved lignin is about 70 kDa (ENZ liquor). This 14-fold increase in  $M_w$  of the remaining lignin is in agreement with previous reports (Areskog et al. 2010, Gouveia et al. 2012, 2013). Despite the substantial increase in the molecular weight after the laccase treatment, no lignin precipitation is observed. About 60% of dissolved lignin in ENZ liquor has a  $M_w$  over 10 kDa at the end of enzymatic treatment (168h) (see Paper IV).

#### 4.4.5 Membrane filtrations

From Tables 19 and 20 it is shown that the target of reaching total lignin concentration at levels below  $4 \text{ g L}^{-1}$  is achieved as total lignin concentration in the permeate liquor after nano-/ultrafiltration is  $3.5$  and  $3 \text{ g L}^{-1}$ , respectively. From the lignin mass balance calculations around each membrane (see paper IV) it is inferred that nanofiltration retains about 15% more dissolved lignin compared to ultrafiltration due to the smaller molecular weight cut-off size of the former (1000 Da), as expected. From Table 19 it is shown that total and monomeric sugars concentrations before nanofiltration are at about  $110$  and  $80 \text{ g L}^{-1}$ , respectively, in CATOX A liquor. Total and monomeric sugar concentrations in the permeate liquor are at about  $100$  and  $80 \text{ g L}^{-1}$ , respectively. Sugar mass balances around the nanofiltration membrane reveal that about 60% of sugars are found in the permeate liquor whereas the remaining 40% of sugars remain in the retentate liquor. The limited permeation of sugars through the membrane is probably due to mass transfer limitations at the maximum applied pressure of only 5 bars.

**Table 19.** Composition of the liquors before and after nanofiltration: sugars and lignin.

Liquor	BEFORE	AFTER NANOFILTRATION	
	CATOX A	RETENTATE	PERMEATE
Volume (mL)	37	13	24
pH	7.0	n.m	7.0
Monomeric sugars ( $\text{g L}^{-1}$ )	83.0	86.2	80.3
Total sugars ( $\text{g L}^{-1}$ )	106.7	119.8	99.3
Lignin ( $\text{g L}^{-1}$ )	17.3	44.3	3.5
<i>Acid insoluble</i>	3.3	11.1	0.0
<i>Acid soluble</i>	14.0	33.2	3.5

n.m, not measured

It is shown (Table 20) that total and monomeric sugars concentrations before ultrafiltration (ENZ liquor) are at about 80 and 45 g L<sup>-1</sup>, respectively. Total and monomeric sugar concentrations in the permeate liquor after ultrafiltration are at about 90 and 50 g L<sup>-1</sup>, respectively. Sugar mass balances around the ultrafiltration membrane reveal that almost all sugars pass through the 10 kDa membrane to the permeate liquor (see paper IV).

#### 4.4.6 ABE fermentation results

Results of batch ABE fermentation tests show that the two permeate liquors after nano-/ultrafiltration are fermentable by *Clostridia*. This is expected since they both contain dissolved lignin at levels below 1 g L<sup>-1</sup> (tolerance limit by *Clostridia*) after 4-fold dilution. The permeate liquors are supplemented with 35 g L<sup>-1</sup> glucose as in previous fermentations. However, the fermentation microorganisms are adapted i.e. the permeate liquors were added in the production medium to allow for better tolerance of *Clostridia* to low molecular weight lignin (see Paper IV).

**Table 20.** Composition of the liquors before and after ultrafiltration: sugars and lignin

Liquor	BEFORE	AFTER ULTRAFILTRATION	
	ENZ	RETENTATE	PERMEATE
Volume (mL)	56	10	46
pH	4.4	n.m	4.4
Monomeric sugars (g L <sup>-1</sup> )	44.0	17.9	49.9
Total sugars (g L <sup>-1</sup> )	78.0	26.8	89.6
Lignin (g L <sup>-1</sup> )	8.3	32.7	3.0
<i>Acid insoluble</i>	5.0	27.0	0.0
<i>Acid soluble</i>	3.3	5.7	3.0

n.m, not measured

Maximum produced solvent concentrations after ABE fermentation with nanofiltration and ultrafiltration permeate liquors are at 11 and 7 g L<sup>-1</sup>, respectively (acetone:n-butanol:ethanol ratio of 3:6:1 for both, total solvents yield of about 0.30 and 0.24 g g<sup>-1</sup> sugars, respectively). The inferior performance of ABE fermentation with permeate liquor from ultrafiltration is most likely due to microbial inhibition by higher amounts of residual low molecular lignin. The reader is referred to original Paper IV for a more detailed explanation.

From the above results it is suggested that total solvents production and fermentation yield can be improved if a suitable membrane with cut-off size below 10 kDa is selected to allow for smaller amounts of low molecular weight lignin in the permeate liquor after ultrafiltration. It is also possible that total solvents production and fermentation yield with permeate liquor from nanofiltration can be further improved if filtration conditions are optimized to allow for better permeation of sugars through the membrane as the current retention of sugars is at similar levels to sugar losses with resins treatment.

It is observed that microbial inhibition is much more pronounced when permeate liquors are subjected to non-adapted ABE fermentation. This suggests that the adaptation approach is beneficial for ABE fermentation with the specific liquors. It is noted that previous ABE fermentation tests with spruce-originating conditioned liquors that were treated with anion exchange resins to remove dissolved lignin (see Papers I, II) did not require adaptation of the microbial seed culture to give satisfactory fermentation yield and good total production of ABE solvents. This fact suggests that perhaps anion exchange resins are better at removing the low molecular weight lignin fraction compared to membrane filtration.

#### 4.4.7 Summary of findings

It is shown that it is possible to improve ABE fermentation performance by introducing modifications to the industry optimized SEW fractionation and spent liquor conditioning protocol described in section 4.3.

It is demonstrated that the use of a modified scheme (scheme A) that incorporated supplementation of a small dosage of chitosan after the liming step (pH 9.0) and combination of this scheme with nanofiltration after the step of catalytic oxidation, produced a conditioned liquor of suitable chemical composition for ABE fermentation. The final step of nanofiltration removed the majority of soluble lignin and as a result the final levels of dissolved lignin in the liquor were below  $1 \text{ g L}^{-1}$  upon 4-fold dilution (tolerance limit for ABE fermentation by *Clostridia*).

It is noted that fermentation tests with the above conditioned liquor required adaptation of the microbial seed culture to allow for better tolerance of the fermentation microorganisms to low molecular weight dissolved lignosulfonate possibly because the latter is still present in significant amounts in the permeate after nanofiltration. Adaptation-assisted batch ABE fermentation with the produced spruce-based liquor gave  $11 \text{ g L}^{-1}$  of total solvents at a yield of  $0.30 \text{ g g}^{-1}$  sugars. This result is a significant improvement over previous results of batch ABE fermentation tests with similar spruce-based liquor that was conditioned and treated with anion exchange resins.

Optimization of the nanofiltration conditions i.e. higher operating pressure may allow for better permeation of the sugars through the membrane and possibly further improve performance of subsequent ABE fermentation. This will clearly make this liquor purification step a superior alternative to resins treatment.

A summary of the numerical findings concerning the most promising approach

(scheme A followed by nanofiltration) is presented below (Table 21).

**Table 21.** Summary of numerical findings: section 4.4 (scheme A followed by nanofiltration)

<b>Conditioning process</b>
Largest sugar losses after liming to pH 9.0 followed by supplementation of low molecular weight chitosan (13% relative to MSEW A liquor) due to entrapment of sugars in lignin-chitosan complexes
Total sugar losses of 16% (relative to MSEW A liquor)
Ethanol and other fermentation inhibitors (SO <sub>2</sub> , furans, formic acid) totally removed
<b>Composition of liquor before nanofiltration</b>
Total lignin concentration of 17 g L <sup>-1</sup>
Total sugars concentration 107 g L <sup>-1</sup>
Monomeric sugars concentration 83 g L <sup>-1</sup>
<b>Composition of liquor after nanofiltration</b>
Total lignin concentration below 4 g L <sup>-1</sup>
Total sugars concentration 99 g L <sup>-1</sup>
Monomeric sugars concentration 80 g L <sup>-1</sup>
<b>ABE fermentation (permeate after nanofiltration)</b>
Total solvents concentration 11 g L <sup>-1</sup> (batch mode)
n-butanol, acetone and ethanol produced at a ratio of 6:3:1
Fermentation yield of 0.30 g/g sugars

## 4.5 Further process optimization

### 4.5.1 Improved hydrolysis of feedstocks with high ash content

*Supplementation of inorganic acids in fresh SEW spent liquor to improve hydrolysis of OPEFB fibers (unpublished)*

From the above discussion (see section 4.3) it is shown that introducing a feedstock pre-treatment step to reduce its high ash content (acidic leaching of OPEFB fibers) leads to only marginal improvement of hemicellulose depolymerization during SEW fractionation and conditioning. It is also generally known that introducing an additional pre-treatment step increases capital investment. It is therefore suggested (see section 4.3) that the acidity and/or temperature during SEW fractionation must be further increased to reduce oligomers share in the SEW spent liquor derived from high ash content feedstocks. An elevation in temperature at the currently employed fractionation conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, 30 min, L/F ratio of 3 L kg<sup>-1</sup> is not desirable as:

- i. it will cause extensive lignin condensation which is already evident by the dark color of the pulp (kappa number of 88 for leached SEW fractionated OPEFB pulp). Extensive lignin condensation can lead to formation of sticky precipitates that can cause blockage of process equipment (reactors, pipework).
- ii. it will cause significant sugars degradation leading to formation of furfural and HMF (temperatures over 160<sup>0</sup>C).
- iii. it will lead to increased capital investment and operational costs.

Therefore, it was considered whether supplementation of acids into the fresh SEW liquor could increase the degree of hydrolysis of the dissolved hemicelluloses.



Experiments with supplementation of inorganic acids (nitric and phosphoric acid) in the fresh SEW liquor for fractionation of OPEFB fibers at conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, 30 min, L/F ratio of 3 L kg<sup>-1</sup> (Iakovlev et al. 2013) showed that this method can solve the problem of suppressed hydrolysis of feedstocks with high ash content by neutralization of the dissolving metal cations that are present in the feedstock. The resulting cation-free lignosulfonic acids and the presence of SO<sub>2</sub> in hydrated form, lead to increased acidity and successful fractionation. This is clearly evident after vacuum evaporation of the SEW spent liquor mixed with pulp washings (MSEW liquor) as the monomers share in EVAP liquor at about 50% is comparable to that of spruce-based EVAP liquor. Another advantage of the proposed method is that the amounts of inorganic acids added correspond to the amounts of N and P needed for downstream ABE fermentation.

Supplementation of inorganic and or even organic acids i.e. acetic acid in the fresh SEW liquor can be applied to any lignocellulosic feedstock with high ash content (mixed forest biomass, annual plants, various straws) to solve the problem of suppressed hydrolysis during SEW fractionation. This step can be easily incorporated in the developed industry optimized SEW fractionation and conditioning scheme without any added capital investment. Also it can increase significantly supply of suitable feedstocks for our process.

#### **4.5.2 Optimization of membrane filtration**

Membrane filtrations performed as described above (see section 4.4.5) demonstrated that it is possible to further reduce soluble lignin levels in the detoxified liquor before ABE fermentation by introducing a final liquor purification stage after the step of catalytic oxidation. It is noted though that the chosen batch

membrane filtration module, membrane characteristics (cut-off size, material of construction) and operating conditions (temperature, pressure) were not optimized for industrial scale use. This is particularly evident in the case of membrane nanofiltration as the operating pressure was limited to only 5 bar while the industrial set-ups generally apply pressure exceeding 10 bar. This had a profound effect on the membrane filtration selectivity since separation of carbohydrates from dissolved lignin was incomplete. Furthermore, important membrane filtration parameters such as critical flux and membrane fouling performance were not investigated as they were beyond the scope of the present thesis. However, scaling-up of the filtration process requires that also these process parameters are thoroughly investigated to allow for reduced operational and capital costs.

Despite the above shortcomings, it is expected that careful optimization of the membrane filtration for industrial scale application will further assist selective removal of dissolved lignin from the conditioned liquor to allow for maximum ABE fermentation performance.

## **4.6 Creation of value-added products**

### *Butanol and other solvents*

The developed SEW fractionation and conditioning scheme, combined with enzymatic hydrolysis of the cellulose, introduces a new process to valorize C5 and C6 sugars from cheap lignocellulosic feedstocks for the production of biofuels and chemicals in a lignocellulosic biorefinery. Most of the value can be generated by the production of n-butanol which can be used as biofuel when mixed with gasoline or diesel for use in internal combustion engines. Table 22 shows that this alcoholic

fuel is a perfect replacement for gasoline and a superior alternative to currently produced lignocellulosic bioethanol (Köpke and Dürre 2011).

ABE fermentation produces also a small amount of ethanol and as well as acetone. Ethanol can be used as biofuel or make-up chemical for SEW fractionation while acetone can be sold directly to the market as a chemical solvent to create revenue. However, it may be preferable that fermentation produces isopropanol instead of acetone because the former can also be used as biofuel. This is possible by metabolic engineering of the fermentation microorganisms. It is reported (Jurgens et al. 2012) that genetically modified *C.acetobutylicum* DM792-pADH1 strain can produce up to 14.3 g L<sup>-1</sup> of isopropanol, butanol, ethanol (IBE) solvents from standard glucose media and about 5 g L<sup>-1</sup> of IBE solvents from spruce-based SEW spent liquor that was conditioned according to the original scheme described in section 4.2.

**Table 22.** Properties of gasoline, butanol and ethanol (Köpke and Dürre 2011)

	gasoline	biobutanol	bioethanol
Energy density (MJ L <sup>-1</sup> )	32-35	21.2	29.2
Air-fuel ratio	14.6	9.0	11.2
Mileage (%)	100	61-66	83-91
Research octane number (RON)	91-99	129	96
Motor octane number (MON)	81-89	102	78
Vapour pressure (20 <sup>0</sup> C, hPa)	35-90	58	6.7
Enthalpy of vaporization (MJ kg <sup>-1</sup> )	0.36	0.92	0.43
Flashpoint ( <sup>0</sup> C)	< -20	12	35-37
Kinematic viscosity (20 <sup>0</sup> C, mm <sup>2</sup> s <sup>-1</sup> )	0.4-0.8	1.5	3.6

Solvents recovery after microbial ABE fermentation of the liquors produced by use of the developed process scheme was not investigated as it is beyond the scope of this thesis.

#### *Acetic acid*

Acetic acid is an aliphatic acid that originates from deacetylation of hemicellulose. It is released upon SEW fractionation and sufficient amounts can be recovered as a side product within an integrated process by steam distillation of the SEW spent liquor mixed with pulp washings (MSEW liquor).

#### *Acidic solid catalyst*

Research work by Lê Huy et al. (unpublished work) suggests that the char-like LCC produced during the vacuum evaporation stage of the SEW spent liquor conditioning process can be used as a novel acidic solid catalyst for different biomass chemical conversion processes, including saccharification of hemicellulose solutions and /or dehydration of hemicellulose solutions to produce furanic compounds. The solid catalyst can be produced after SEW cooking of spruce chips at the original fractionation conditions of 3% SO<sub>2</sub> in 55 v/v% ethanol-water, L/F ratio of 6 L kg<sup>-1</sup>, 150°C, 120 min (see section 4.2) or at fractionation conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, L/F ratio of 6 L kg<sup>-1</sup>, 150°C, 60 min, followed by vacuum evaporation of the produced SEW spent liquor to remove ethanol and SO<sub>2</sub>. It is therefore a by-product of the developed SEW fractionation and conditioning scheme which can be made with little production costs. A key advantage of the acid solid catalyst is that it is renewable i.e. it can be reused upon re-activation.

The acidic solid catalyst can be used to substitute conventional homogenous acid catalysts, particularly sulfuric acid, for which the recovery is expensive (at high concentrations) or the acid is not recycled (as low concentrations).

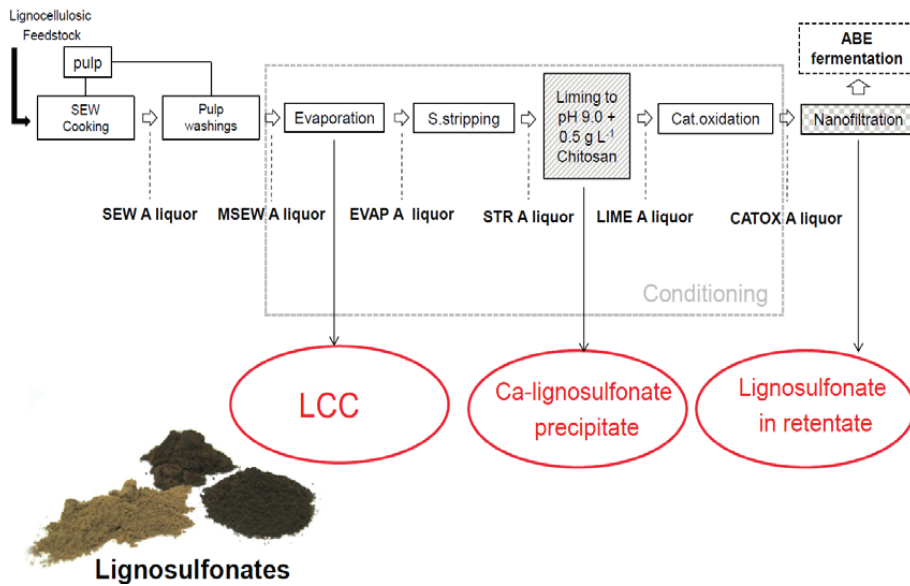
### *Lignosulfonates*

The developed process removes significant amounts of sulfonated lignin which is present as dissolved lignosulfonate in the SEW spent liquor. Lignosulfonate is removed at different stages of the conditioning process (Fig. 12):

- i. vacuum evaporation: removes the highest molecular weight lignosulfonate. This fraction has relatively low degree of sulfonation (S/C9 of 0.03-0.07). Lignosulfonate is removed either as colloidal LCC or as char like-LCC (11-15% (o.d.f.), see Tables 9, 10, 13).
- ii. liming with  $\text{Ca}(\text{OH})_2$ : removes residual dissolved lignosulfonate (2-3% (o.d.f.)) by precipitation as calcium salt. The formed precipitate contains also complexes of calcium with sulfite and sulfate ions ( $\text{CaSO}_3/\text{CaSO}_4$  complexes). Since the inorganics content is about 10% it can be concluded that most of the Ca-lignosulfonate is sulfonated lignin.
- iii. nanofiltration after catalytic oxidation step: removes about 2% (o.d.f.) of highly sulfonated lignin. This lignosulfonate fraction remains in the retentate liquor after nanofiltration.

Some of the above lignosulfonate fractions could be marketed as a substitute to traditional sulfite lignosulfonates for applications as concrete admixtures, road base, oil drilling muds, etc. Alternatively the lignosulfonates can be burned to produce steam and electricity or to recover the  $\text{SO}_2$  (Iakovlev et al. 2007). The former option could make the present lignocellulosic biorefinery process energy mostly self-

sufficient.



**Figure 12.** Removal of lignosulfonates during SEW spend liquor conditioning and after nanofiltration

### *Other products*

In the integrated process shown in Fig. 1 the solid cellulose residues that are produced after SEW fractionation of the tested lignocellulosics at conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, 30 min, L/F ratio of 3 L kg<sup>-1</sup> are intended for use as feedstock for the production of glucose. Glucose can be mixed with the conditioned hemicellulose sugars stream to create a feed for subsequent ABE fermentation. Alternatively the produced cellulosic pulps could be used directly for the creation of pulp and paper products. However, a detailed study of the pulp properties (mechanical strength, optical properties etc.) is needed to determine their suitability for this purpose. This was not explored in the current research work. However, previous research (Iakovlev et al. 2010, Iakovlev et al. 2014) suggests that SEW

solid residues have generally good pulp and papermaking properties, comparable to sulfite, and that their applications can be extended further i.e. for the production of dissolving pulps and nanocellulose.

The above value-added products can be sold together with fermentation products such as acetic and butyric acid, hydrogen and CO<sub>2</sub> to increase revenue of the process.

## 5 CONCLUSIONS

A new biorefinery process to fractionate lignocellulosics and to treat the produced hydrolysate for microbial fermentation to butanol, acetone/isopropanol and ethanol is presented. The process comprises of two distinct stages: i) SO<sub>2</sub>-ethanol-water (SEW) fractionation ii) a conditioning protocol to treat SEW spent liquor for ABE fermentation by *Clostridium acetobutylicum*.

SEW fractionation is proven fractionation technology which has been extensively studied in the past by our research group. The current research work reaffirms its potential as it shows that the method may be used at conditions applicable to large scale efficient fractionation of different lignocellulosic feedstocks and produce spent liquor of suitable chemical composition for ABE fermentation.

The present SEW fractionation study at two different sets of conditions reveals that the most preferable option for industrial SEW fractionation of spruce chips, mixed softwood biomass and Oil Palm Empty Fruit Bunch (OPEFB) is cooking at conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, 30 min, 150<sup>0</sup>C, L/F ratio of 3 L kg<sup>-1</sup>. Dissolution of the hemicellulose sugars from the different feedstocks is almost complete after SEW fractionation at the above conditions followed by washing of the produced pulps twice with 40% ethanol-water at 60<sup>0</sup>C and thrice with deionized water at room temperature. Addition of the pulp washings to the SEW spent liquor recovers most of the dissolved hemicellulose sugars.

SEW fractionation of the above lignocellulosics at conditions of 12% SO<sub>2</sub> in 55 v/v% ethanol-water, 30 min, L/F ratio of 3 L kg<sup>-1</sup> is combined with a novel conditioning scheme to detoxify SEW spent liquor and to increase its monosugars content as *Clostridia* consume mostly monomers. This conditioning scheme in its



basic form comprises the consecutive steps of vacuum evaporation, steam stripping, lining and catalytic oxidation. It is found that the conditioning process is successful at fully removing the following ABE fermentation inhibitors for *Clostridia*: i) furans and formic acid ii) ethanol iii) SO<sub>2</sub>. Almost all ethanol (98%) and SO<sub>2</sub> (90%) are removed after the step of vacuum evaporation and can be used as make-up chemicals for fractionation. The subsequent conditioning steps result in total ethanol and SO<sub>2</sub> elimination from the final conditioned liquors (so-called CATOX liquors). It is also found that inhibitory dissolved lignin is removed mostly during the step of vacuum evaporation (as colloidal or char-like LCC) and to a lesser extent after the lining step (as Ca-lignosulfonate). Levels of residual lignin (lignosulfonate) in the final conditioned liquors correspond to only about 10% of the original lignin in the respective feedstocks but they are still too high for ABE fermentation by *Clostridia*. To reach dissolved lignin levels of approximately 1 g L<sup>-1</sup> (tolerance limit for *Clostridia*) a liquor purification step comprising of treatment with anion exchange resins followed by 4-fold dilution is applied before ABE fermentation. The 4-fold dilution simulates supplementation of glucose to account for the sugar stream that is obtained from enzymatic hydrolysis of the pulps. All the different feedstock-based hydrolysates that are produced after conditioning as described above are fermentable by *Clostridia* as ABE solvents are produced at a maximum total concentration of 13 and 7 g L<sup>-1</sup> in continuous and batch mode, respectively (yield of 0.25-0.26 g g<sup>-1</sup> sugars). ABE fermentation produces mostly butanol as this is the target biofuel in the present process.

It is found that particularly OPEFB fibers suffer from poor hydrolytic performance (compared to spruce) due to their high alkali metals content as inferred by their low monosugars content in the produced SEW spent liquor. Acidic leaching pre-

treatment of the feedstock resulted in only marginally higher monosugars content in the SEW spent liquor. This problem can be solved by supplementing inorganic acids (nitric, phosphoric) in the fresh fractionation liquor to produce OPEFB SEW spent liquor of monosugars content that is similar to monosugars content of its spruce-based counterpart (50%).

Furthermore, it is found that by maintaining the basic conditioning scheme except for addition of a small amount of chitosan after the step of liming (pH 9.0) and by performing nanofiltration after the step of catalytic oxidation it is possible to obtain better removal of dissolved lignin and reach levels below tolerance limit for bacteria after 4-fold dilution. Sugar losses are at similar levels with losses after resins treatment (40% vs 35%, respectively), however, they will be much lower when nanofiltration is optimized for industrial scale use. Therefore a more selective method than resins treatment has been found to purify the produced conditioned liquors before ABE fermentation. Our results show that this alternative method increases production of solvents and fermentation yield (total solvents concentration of  $11 \text{ g L}^{-1}$ , yield of  $0.30 \text{ g g}^{-1}$  sugars) when combined with adaptation of *Clostridia* to tolerate better inhibitory low molecular weight lignin.

## 6 REFERENCES

- Abdullah, M. A., Nazir, M. S., Wahjoedi, B. A., (2011), Development of value-added biomaterials from oil palm agro-wastes, *IPCBEE* 7, 32–35.
- Ariksson, B., (2006), Ethanol from lignocellulose. Licentiate Thesis, Karlstad University.
- Areskog, D., Li, J., Gellerstedt, G., Henriksson, G., (2010), Investigation of the molecular weight increase of commercial lignosulfonates by laccase catalysis, *Biomacromolecules*, 11:904-910.
- Beesch, S. C. (1953), A Microbiological Process Report: Acetone-Butanol Fermentation of Starches, *Applied Microbiology*, 1(2):85-95.
- Berson, R. E., Young, J. S., Kamer, S. N., Hanley, T. R., (2005), Detoxification of actual corn stover hydrolysate using an activated carbon powder, *Applied Biochemistry and Biotechnology*, 121(124):923-934.
- Black, N., (1991), ASAM alkaline sulfite pulping process shows potential for large-scale application, *Tappi*, 87-93.
- Browning, B. L., The chemistry of bark, In: Chapter 12: The chemistry of wood, pp. 587–666, Interscience Publishers, New York, NY, USA, 1963.
- Chandel, A. K., Kapoor, R. K., Singh, A., Kuhad, R. C., (2007), Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM 3501, *Bioresource Technology*, 98:1947-1950.
- Dürre, P., (2005), Formation of solvents in clostridia, In: *Handbook on Clostridia*, CRC. Press, Boca Raton, 671–693.
- Elander, R. T, Dale, B. E, Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Mitchinson, C., Saddler, J. N, Wyman, C. E., (2009), Summary of findings from the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI): corn stover pretreatment, *Cellulose*, 16(4):649-659.
- Erman, W. F., Lyness, W. I., (1965), The isolation, purification and structure determination of a phenolic acid fraction from slash pine (*Pinus Elliottii*) bark, *Tappi*, 48(4):249-256.
- Evans, P. J., Wang, H. Y., (1988), Enhancement of butanol formation by *Clostridium acetobutyricum* in the presence of decanol-oleyl alcohol mixed extractants, *Applied and Environmental Microbiology*, 54(7):1662-1667.
- Ezeji T. C., Qureshi, N., Blaschek, H. P., (2004), Butanol Fermentation Research: Upstream and Downstream Manipulations, *The Chemical Record*, 4:305-314.
- Ezeji, T., Qureshi, N., Blaschek, H. P., (2007), Production of acetone–butanol–

ethanol (ABE) in a continuous flow bioreactor using degermed corn and *Clostridium beijerinckii*, *Process Biochemistry*, 42:34-39.

Feather, M. S., Harris, J. F., (1965), The acid-catalyzed hydrolysis of glycopyranosides, *Journal of Organic Chemistry*, 30(1):153-157.

Fengel, D., Wegener, G., *Wood—Chemistry, Ultrastructure, Reactions*, Walter de Gruyter & Co., Berlin, 1984.

Fernandes, D. L. A., Pereira, S. R., Serafim, L. S., Evtuguin, D. V., Xavier, A. M. R. B., Second generation bioethanol from lignocellulosics: processing of hardwood sulphite spent liquor, Chapter 6, *Bioethanol*, InTech, 2012.

Fond, O., Engasser, J. M., Matta-El-Amouri, G., Petitdemange, H., (1986), The acetone butanol fermentation on glucose and xylose. II: Regulation and kinetics in batch cultures, *Biotechnology and Bioengineering*, 28:160-166.

Fredheim, G. E., (2002), Polyelectrolyte complexes: Interactions between lignosulfonate and chitosan, *Biomacromolecules*, 4:232-239.

Fredheim, G. E., Christensen, B. E., (2003), Polyelectrolyte Complexes: Interactions between Lignosulfonate and Chitosan, *Biomacromolecules* 4(2):232-239.

Garcia, A., Lanotti, E. L., Fischer, J. L., (1985), Butanol fermentation liquor production and separation by reverse osmosis, *Biotechnology and Bioengineering*, 28:785-791.

Girisuta, B., Janssen, L. P. B. M., Heeres, H. J., (2006), A kinetic study on the decomposition of 5-hydroxymethylfurfural into levulinic acid, *Green Chemistry*, 8(8):701-709.

Goldstein, I. S., (1975), Perspectives on production of phenols and phenolic acids from lignin and bark, *Applied Polymer Symposium*, 28:259-267.

Gouveia, S., Fernández-Costas, C., Sanromán, M. A., Moldes, D. (2012). Enzymatic polymerisation and effect of fractionation of dissolved lignin from *Eucalyptus globulus* Kraft liquor, *Bioresource Technology* 121:131-138.

Gouveia, S., Fernández-Costas, C., Sanromán, M. A., Moldes, D., (2013), Polymerisation of Kraft lignin from black liquors by laccase from *Myceliophthora thermophila*: Effect of operational conditions and black liquor origin, *Bioresource Technology*, 131:288-294.

Groot, W. J., van der Lans, R. G. J. M., Luyben, K. Ch. A. M. (1989), Batch and continuous butanol fermentation with free cells: integration with product recovery by gas stripping, *Appl Microbiol Biotechnol*, 32:305-308.

Helle, S., S., Lin, T., Duff, S. J. B., (2008), Optimization of spent sulfite liquor fermentation, *Enzyme and Microbial Technology*, 42(3):259-264.

Horváth, I. S., Sjöde, A., Alriksson B., Jönsson L. J., Nilvebrant, N. O., (2005), Critical conditions for improved fermentability during overliming of acid hydrolysates from spruce. *Applied Biochemistry and Biotechnology*, 121-124:1031-1044.

Iakovlev, M., van Heiningen, A., Pykkänen, V., (2007), SO<sub>2</sub>-ethanol-water pulping of softwoods, 6<sup>th</sup> Biennial Johan Gullichsen Colloquium, Espoo, Finland, November 15<sup>th</sup> 2007, 39-45.

Iakovlev, M., Pääkkönen, T., van Heiningen, A., (2009), Kinetics of SO<sub>2</sub>-ethanol-water pulping of spruce. *Holzforschung*, 63(6):779-784.

Iakovlev, M., Sixta, H., van Heiningen, A., (2011), SO<sub>2</sub>-Ethanol-Water (SEW) pulping: II. Kinetics for spruce, beech, and wheat straw, *Journal of Wood Chemistry and Technology*, 31(3):250-266.

Iakovlev, M., van Heiningen, A., (2011), SO<sub>2</sub>-Ethanol-Water (SEW) pulping: I. Lignin determination in pulps and liquors. *Journal of Wood Chemistry and Technology*, 31(3):233-249.

Iakovlev, M., SO<sub>2</sub>-ethanol-water fractionation of lignocellulosics, Doctoral Thesis, 2011, Aalto University, Finland.

Iakovlev, M., van Heiningen, A., (2012a), Efficient fractionation of spruce by SO<sub>2</sub>-Ethanol-Water treatment: closed mass balances for carbohydrates and sulfur, *ChemSUSChem* 5:1625-1637.

Iakovlev, M., van Heiningen, A., (2012b), Kinetics of fractionation by SO<sub>2</sub>-Ethanol-Water (SEW) treatment: understanding the deconstruction of spruce wood chips. *RSC Advances*, 2: 3057-3068.

Iakovlev, M., Sklavounos, E., van Heiningen, A., (2013), Efficient fractionation of lignocellulosics with variable ash content using SO<sub>2</sub>-organic solvent-water (SOW) solutions supplemented by inorganic/organic acids, U.S. provisional patent No.61/823,923.

Iakovlev, M., You, X., van Heiningen, A., Sixta, H., (2014), SO<sub>2</sub>-ethanol-water (SEW) fractionation of spruce: kinetics and conditions for paper and viscose-grade dissolving pulps, *RSC Advances*, 4:1938-1950.

Janson, J. Analytik der Polysaccharide in Holz und Zellstoff. *Faserforsch. Textiltech.* 1974, 25:375–382.

Jones, D. T., Woods, D. R., (1986), Acetone-butanol fermentation revisited, *Microbiological Reviews*, 50:484-524.

Jönsson, L., Sjöström, K., Häggström, I., Nyman P. O., (1995), Characterization of a laccase gene from the white-rot fungus *Trametes versicolor* and structural features of basidiomycete laccases *journal, Biochimica et Biophysica Acta*, 1251(2):210-

Jönsson, L., Ariksson, B., Nilvebrant, N. O., (2013), Bioconversion of lignocellulose: inhibitors and detoxification, *Biotechnology for Biofuels*, 6:16.

Jurado, M., Prieto, A., Martínez-Alcalá, Á., Martínez, Á. T., Martínez, M. J., (2009), Laccase detoxification of steam-exploded wheat straw for second generation bioethanol, *Bioresource Technology* 100:6378–6384.

Jurgens, G., Survase, S., Berezina, O., Sklavounos, E., Linnekoski, J., Kurkijärvi, A., Väkevä, M., van Heiningen, A., Granström, T., (2012), Butanol production from lignocellulosics, *Biotechnology Letters*, 34:1415-1434.

Kekkonen, J., Lattu, H., Stenius, R., (2002), Formation, adsorption and interactions of poly-DADMAC/Pectic acid complexes, *Journal of Pulp and Paper Science*, 28(1):6-12.

Koivula, E., Kalioinen, M., Mänttari, M., (2012), Methods to remove foulants from wood extracts prior to ultrafiltration, 4<sup>th</sup> Nordic Wood Biorefinery Conference, October 23-25, Helsinki, Finland.

Köpke, M., Dürre, P., Biochemical production of biobutanol, Chapter 10, *Handbook of biofuels production-processes and technologies*, Woodhead Publishing, 2011.

Larsson, S., Reimann, A., Nilvebrant, N., Jonsson, L. J., (1999), Comparison of different methods for the detoxification of lignocellulose hydrolysates of spruce, *Applied Biochemistry and Biotechnology*, 77–79, 91–103.

Larsson, S., Cassland, P., Jönsson, L. J., (2001), Development of a *Saccharomyces cerevisiae* Strain with Enhanced Resistance to Phenolic Fermentation Inhibitors in Lignocellulose Hydrolysates by Heterologous Expression of Laccase, *Applied and Environmental Microbiology*, 67(3):1163–1170.

Lawford, H. G., Rousseau, J. D., (1993), Production of ethanol from pulp mill hardwood and softwood spent sulfite liquors by genetically engineered *Escherichia coli*. *Applied Biochemistry and Biotechnology*, 39:667-685.

Lee, W. G., Lee, J. S., Shin, C. S., Park, S. C., Chang, H. N., Chang, Y. K., (1999), Ethanol production using concentrated oak wood hydrolysates and methods to detoxify, *Applied Biochemistry and Biotechnology*, 78:547-559.

Leonard, R.; Hajny, G., (1945), Fermentation of wood sugars to ethyl alcohol, *Industrial and Engineering Chemistry*, 37:390-395.

Leschinsky, M., (2009), Water prehydrolysis of *Eucalyptus globulus*: Formation of lignin-derived precipitates that impair the extraction of hemicelluloses. PhD thesis, University of Hamburg, Hamburg, Germany.

Li, P., Pelton, R., (1992), Wood pulp washing. 1. Complex formation between kraft lignin and cationic polymers, *Colloids and Surfaces*, 64:217-222.

Liu, Z., Ying, Y., Li, F., Ma, C., Xu, P., (2010), Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran, *Journal of Industrial Microbiology and Biotechnology*, 37:495–501.

Maddox, I. S., Murray, A. E., 1983, Production of butanol by fermentation of wood hydrolysate, *Biotechnology Letters*, 5(3):175–177.

Maddox, I. S., Steiner, E., Hirsch, S., Wessner, S., Gutierrez, N. A., Gapes, J. R., Schuster, K. C., (2000), The cause of “Acid Crash” and “Acidogenic Fermentations” during the batch Acetone-Butanol-Ethanol (ABE) fermentation process, *Journal of Molecular Microbiology and Biotechnology*, 2(1):95-100.

Marchal, R., Ropars M., Pourquié J., Fayolle F., Vandecasteele J. P., (1992), Large-Scale Enzymatic Hydrolysis of Agricultural Lignocellulosic Biomass. Part 2: Conversion into Acetone-Butanol, *Bioresource Technology* 42:205-217.

Martinez, A., Rodriguez, M. E., Wells, M. L., York, S. W., Preston, J. F., Ingram, L. O., (2001), Detoxification of dilute acid hydrolysates of lignocellulose with lime, *Biotechnology Progress*, 17(2):287-293.

Mohagheghi, A., Ruth, M., Schell, D. J., (2006), Conditioning hemicellulose hydrolysates for fermentation: effects of overliming pH on sugar and ethanol yields. *Process Biochemistry*, 41:1806-1811.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., Ladisch, M., (2005), Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technology*, 96(6):673-686

Mussatto, S. I., Roberto, I. C., (2004), Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review, *Bioresource Technology*, 93:1-10.

Nilvebrant, N. O., Reimann, A., Larsson, S., Jönsson, L. J., (2001), Detoxification of lignocellulose hydrolysates with ion exchange resins. *Applied Biochemistry and Biotechnology*, 91-93(1):35-49.

Nilvebrant, N. O., Persson, P., Reimann, A., de Sousa, F., Gorton, L., Jönsson, L. J., (2003), Limits for alkaline detoxification of dilute-acid lignocellulose hydrolysates, *Applied Biochemistry and Biotechnology*, 105–108:615–628.

Palmqvist, E., Almeida, J. S., Hähen-Hägerdal, B., (1999), Influence of furfural on anaerobic glycolytic kinetics of *Saccharomyces cerevisiae* in batch culture, *Biotechnology and Bioengineering*, 62:447-454.

Perego, P., Converti, A., Palazzi, E., Del Borghi, M., Ferraiolo, G., (1990), Fermentation of hardwood hemicellulose hydrolysate by *Pachysolen tannophilus*, *Candida shehatae* and *Pichia stipites*, *Journal of Industrial Microbiology*, 6:157–164.

Persson, P., Andersson, J., Gorton, L., Larsson, S., Nilvebrant, N. O., Jönsson, L. J., (2002), Effect of different forms of alkali treatment on specific fermentation

inhibitors and on the fermentability of lignocellulose hydrolysates for production of fuel ethanol, *Journal of Agricultural and Food Chemistry*, 50:5318-5325.

Qureshi, N., Maddox, L. S., (1995), Continuous production of acetone-butanol-ethanol using immobilized cells of *Clostridium acetobutylicum* and integration with product removal by liquid-liquid extraction, *Journal of Fermentation and Bioengineering*, 80(2):185-189.

Qureshi, N., Blaschek, H. P., (1999), Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation, *Biotechnology Progress*, 15:594-602.

Qureshi, N., Blaschek, H. P., (2000), Butanol production using hyper-butanol producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation, *Applied Biochemistry and Biotechnology*, 84-86:225-235.

Qureshi, N., Blaschek, H. P., (2001), ABE production from corn: a recent economic evaluation, *Journal of Industrial Microbiology and Biotechnology*, 27:292-297.

Qureshi, N., Maddox, I. S. (2005), Reduction in butanol inhibition by perstraction: Utilization of concentrated lactose/whey permeate by *Clostridium acetobutylicum* to enhance butanol fermentation economics, *Food and Bioproducts Processing*, 83:43-52.

Qureshi, N., Hughes, S., Maddox, I. S., Cotta, M. A., (2005), Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption, *Bioprocess and Biosystems Engineering*, 27:215-222.

Qureshi, N., Saha, B. C., Cotta, M. A., (2007), Butanol production from wheat straw hydrolysate using *Clostridium beijerinckii*, *Bioprocess and Biosystems Engineering*, 30:419-427.

Qureshi, N., Saha, B. C., Hector, R. E., Dien, B., Hughes, S., Liu, S., Iten, L., Bowman, M., J., Sarath, G., Cotta, M. A., (2010), Production of butanol (a biofuel) from agricultural residues: part II—use of corn stover and switchgrass hydrolysates, *Biomass and Bioenergy*, 34:566-571.

Rahman, S. H., Choudhury, J. P., Ahmad, A. L., Kamaruddin, A. H., (2007), Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose, *Bioresource Technology*, 98:554-559.

Rakkolainen, M., Pylkkänen, V., van Heiningen, A., (2009), Commodity Chemicals from Forest Biomass, *Proceedings 7<sup>th</sup> Biennial Johan Gullichsen Colloquium*, November 19<sup>th</sup> 2009, Espoo, Finland, 57-63.

Rakkolainen, M., Iakovlev, M., Teräsvuori, A.-L., Sklavounos, E., Jurgens, G., Granström, T.B., van Heiningen, A., (2010), SO<sub>2</sub>-ethanol-water fractionation of forest biomass and implications for biofuel production by ABE fermentation, *Cellulose Chemistry and Technology*, 44(4-6):139-145.



Ranatunga, T. D., Jarvis, J., Helm, R. F., McMillan, J. D., Wooley, R. J., (2000), The effect of overliming on the toxicity of dilute acid pretreated lignocellulosics: The role of inorganics, uronic acids and ether-soluble organics, *Enzyme and Microbial Technology*, 27:240-247.

Restolho, J. A., Prates, A., Norberta de Pinho, M., Dina Alfonso, M., (2009), Sugars and lignosulfonates recovery from eucalyptus spent sulphite liquor by membrane processes, *Biomass and Bioenergy* 33(11):1558-1566.

Retsina, T., Pylkkänen, V., (2007), Back to the biorefinery: a novel approach to boost pulp mill profits. Paper 360°, February issue, 18-19 <http://www.avapco.com/strategy.html>

Retsina, T., Pylkkänen, V., (2011), Method for the production of fermentable sugars and cellulose from lignocellulosic material, U. S. patent. US 8030039 B1 20111004.

Richardson, T. L., Harner, N. K., Bajwa, P. K., Trevors, J. T., Lee, H., Approaches to deal with toxic inhibitors during fermentation of lignocellulosic substrates, Chapter 7, Sustainable production of fuels, chemicals, and fibers from forest biomass, ACS, 2011.

Rinaudo, M., (2006), Chitin and chitosan: properties and applications, *Progress in Polymer Science* 31:603-632.

Rydholm, S.A., *Pulping Processes*, John Wiley and Sons, Inc., London, 1965.

Saeed, A., Fatehi, P., Ni, Y., (2011), Chitosan as a flocculant for pre-hydrolysis liquor of kraft-based dissolving pulp production process, *Carbohydrate Polymers*, 86(4):1630-1636.

Saeed, A., Fatehi, P., Ni, Y., (2012) An integrated process for removing the inhibitors of the prehydrolysis liquor of kraft-based dissolving pulp process via cationic polymer treatment, *AIChE*, DOI: 10.1002/btpr.1563

Sanchez, O., Cardona, C., (2008), Trends in biotechnological production of fuel ethanol from different feedstocks, *Bioresource Technology*, 99(13):5270-5295.

Schorning, P., (1957), Studies of sulphite pulping of wood without base, *Faserforsch. Textiltech.*, 8:487-494.

Schwartz, T. J., Lawoko, M., (2010), Removal of acid-soluble lignin from biomass extracts using Amberlite XAD-4 Resin, *BioResources*, 5(4):2337-2347.

Shen, J., Fatehi, P., Soleimani, P., Ni, Y. (2011), Lime treatment of prehydrolysis liquor from the Kraft-based dissolving pulp production process, *Industrial and Engineering Chemistry Research*, 51:662-667.

Shen, J., Kaur, I., Baktash, M., He, Z., Ni, Y., (2013), A combined process of activated carbon adsorption, ion exchange resin treatment and membrane

concentration for recovery of dissolved organics in pre-hydrolysis liquor of the kraft-based dissolving pulp production process, *Bioresource Technology*, 127:59-65.

Sixta, H., Gütsch, J. S., (2012), Regeneration of spent activated charcoals used for lignin removal from Prehydrolysis-Kraft prehydrolyzates, *Industrial and Engineering Chemistry Research*, 51:8624-8630.

Sjolander, N. O.; Langlykke, A. F., Peterson, W. H., (1938), Butyl alcohol fermentation of wood sugar, *Industrial and Engineering Chemistry*, 30:1251-1255.

Sjöström, E., *Wood chemistry: fundamentals and applications*, Academic Press, Inc., 1993.

Sreekala, M. S., Kumaran, M. G., Thomas, S., (1997), Oil palm fibers: Morphology, chemical composition, surface modification, and mechanical properties. *Journal of Applied Polymer Science*, 66(5):821-835.

Stenius, P., *Forest Products Chemistry*, In: Chapter 1, volume 3, Fapet Oy, Helsinki, Finland, 2000.

Strom, G., Barla, P., Stenius, P., (1985), The formation of polyelectrolyte complexes between pin xylan and cationic polymers, *Colloids and Surfaces*, 13:193-207.

Survase, S., Sklavounos, E., Jurgens, G., van Heiningen, A., Granström, T., (2011), Continuous acetone-butanol-ethanol fermentation using SO<sub>2</sub> ethanol-water spent liquor from spruce. *Bioresource Technology*, 102(23):10996-11002.

Tan, L., Yu, Y., Li, X., Zhao, J., Qu, Y., Choo, Y., M., Loh, S. K., (2012), Pretreatment of empty fruit bunch from oil palm for fuel ethanol production and proposed biorefinery process, *Bioresource Technology*, 135:275-282.

Teräsvuori, A-L., 2009, Acetone-butanol ethanol fermentation with different Clostridia strains using starch as feedstock. Master's thesis. Helsinki, University of Technology, Faculty of Chemistry and Materials Sciences, Degree Programme of Chemical Technology, Espoo.

Terbojevich, M., Muzzarelli, R. A. A., Chitosan, Chapter 21, *Handbook of hydrocolloids*, Woodhead Publishing, 2000.

Van Heiningen, A., (2006), Converting a Kraft Pulp Mill into an Integrated Forest Biorefinery. *Pulp and Paper Canada*, 107(6):38-43.

Van Heiningen, A., Iakovlev, M., Yamamoto, M., Sklavounos, E., Melin, K., Granström, T., (2011), Which Fractionation Process Can Overcome Techno-Economic Hurdles of a Lignocellulosic Biorefinery? AICHE Annual Meeting, Conference Proceedings, Minneapolis, MN, USA, October 19<sup>th</sup> 2011.

Van Heiningen, A., Yamamoto, M., Sklavounos, E., Survase, S., Jurgens, G., Granström, T., (2012), Biobutanol from forest residues – a process utilizing SO<sub>2</sub>-ethanol-water fractionation and ABE fermentation. 4<sup>th</sup> Nordic Wood Biorefinery Conference, October 23-25, Helsinki, Finland, 205-210.

Van Zyl, C., Prior, B. A., Du Preez, J. C., (1991), Acetic acid inhibition of D-xylose fermentation by *Pichia stipites*, *Enzyme and Microbial Technology*, 13(1):82-86.

Wang, L., Chen, H., (2011), Increased fermentability of enzymatically hydrolyzed steam-exploded corn stover for butanol production by removal of fermentation inhibitors, *Process Biochemistry*, 46(2):604–607.

Wang, S., Zhang, Y., Dong, H., Mao, S., Zhu, Y., Wang, R., Luan, G., Li, Y., (2011), Formic acid triggers the “Acid Crash” of Acetone-Butanol-Ethanol Fermentation by *Clostridium acetobutylicum*, *Applied and Environmental Microbiology*, 77(5):1674–1680.

Weizmann, C., (1915), Improvements in the bacterial fermentation of carbohydrates and in bacterial cultures for the same, British Patent No. 4845.

Werkelin, J., Lindberg, D., Bostöm, D., Skrifvars, B. J., Hupa, M., (2011), Ash-forming elements in four Scandinavian wood species part 3: Combustion of five spruce samples. *Biomass and Bioenergy*, 35:725–733.

Wilson, J. J., Deschatelets, L., Nishikawa, N. K., (1989), Comparative fermentability of enzymatic and acid hydrolysates of steam-pretreated aspenwood hemicellulose by *Pichia stipitis* CBS 5776, *Applied Microbiology and Biotechnology*, 31:592–596.

Wyman, C. E., Dale, B., E., Elander, R., T., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., (2005), Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology*, 96(18):1959-1966

Xavier, A. M. R. B., Correia, M. F., Pereira, S. R., Evtuguin, D. V., (2010), Second-generation bioethanol from eucalypt sulphite spent liquor, *Bioresource Technology* 101(8):2755-2761.

Yamamoto, M., Iakovlev, M., van Heiningen, A., (2011), Total mass balances of SO<sub>2</sub>-ethanol-water (SEW) fractionation of forest biomass, *Holzforschung*, 65(4):559–565.

Yamamoto, M., Iakovlev, M., van Heiningen, A., Enzymatic hydrolysis of hardwood and softwood biomass fibers released by SO<sub>2</sub>-ethanol-water fractionation. 12<sup>th</sup> European Workshop on Lignocellulosics and Pulp (EWLP), 27-30th August 2012, Helsinki, Finland, 66-69.

Yusoff, S., (2006), Renewable energy from palm oil-innovation on effective utilization of waste, *Journal of Cleaner Production*, 14:87-93.

Zeitsch, K. J., (2000), The chemistry and technology of furfural and its many by-products, Elsevier, Amsterdam, 75.

Zhu, J. Y., Pan, X. J., Wang, G. S., Gleisner, R., (2009), Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine, *Bioresource Technology*, 100(8):2411-2418

Zhu, J.,Y., Pan, X. J., (2010), Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation, *Bioresource Technology*, 101(13):4992-5002.

Zhu, J. Y., Pan, X. J., Zalesny, R. S. Jr., (2010), Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance, *Applied Microbiology and Biotechnology*, 87(3):847-857.

Zverlov, V. V., Berezina, O., Velikodvorskaya, G. A., Schwarz, W. H., (2006), Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery, *Applied Microbiology and Biotechnology*, 71:587-597.

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