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PRESSURE FILTRATION CHARACTERISTICS OF ENZYMATICALLY HYDROLYZED BIOMASS SUSPENSIONS

Thesis for the degree of Doctor of Science (Technology) to be presented with due permission for public examination and criticism in the Auditorium 1381 at Lappeenranta University of Technology, Lappeenranta, Finland on the 25th of April, 2014, at noon.

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ABSTRACT

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Pressure filtration characteristics of enzymatically hydrolyzed biomass suspensions

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Enzymatic hydrolysis of lignocellulosic polymers is likely to become one of the key technologies enabling industrial production of liquid biofuels and chemicals from lignocellulosic biomass. Certain types of enzymes are able to hydrolyze cellulose and hemicellulose polymers to shorter units and finally to sugar monomers. These monomeric sugars are environmentally acceptable carbon sources for the production of liquid biofuels, such as bioethanol, and other chemicals, such as organic acids. Liquid biofuels in particular have been shown to contribute to the reduction of net emissions of greenhouse gases.

The solid residue of enzymatic hydrolysis is composed mainly of lignin and partially degraded fibers, while the liquid phase contains the produced sugars. It is usually necessary to separate these two phases at some point after the hydrolysis stage. Pressure filtration is an efficient technique for this separation.

Solid-liquid separation of biomass suspensions is difficult, because biomass solids are able to retain high amounts of water, which cannot be readily liberated by mechanical separation techniques. Most importantly, the filter cakes formed from biomaterials are compressible, which ultimately means that the separation may not be much improved by increasing the filtration pressure. The use of filter aids can therefore facilitate the filtration significantly. On the other hand, the upstream process conditions have a major influence on the filtration process. This thesis investigates how enzymatic hydrolysis and related process conditions affect the filtration properties of a cardboard suspension. The experimental work consists of pressure filtration and characterization of hydrolysates. The study provides novel information about both issues, as the relationship between enzymatic hydrolysis conditions and subsequent filtration properties has so far not been considered in academic studies.

The results of the work reveal that the final degree of hydrolysis is an important factor in the filtration stage. High hydrolysis yield generally increases the average specific cake resistance. Mixing during the hydrolysis stage resulted in undefined changes in the physical properties of the solid residue, causing a high filtration resistance when the mixing intensity was high. Theoretical processing of the mixing data led to an interesting observation: the average specific cake resistance was observed to be linearly proportional to the mixer shear stress. Another finding worth attention is that the size distributions of the solids did not change very dramatically during enzymatic

hydrolysis. There was an observable size reduction during the first couple of hours, but after that the size reduction was minimal. Similarly, the size distribution of the suspended solids remained almost constant when the hydrolyzed suspension was subjected to intensive mixing.

It was also found that the average specific cake resistance was successfully reduced by the use of filter aids. This reduction depended on the method of how the filter aids were applied. In order to obtain high filtration capacity, it is recommended to use the body feed mode, i.e. to mix the filter aid with the slurry prior to filtration. Regarding the quality of the filtrate, precoat filtration was observed to produce a clear filtrate with negligible suspended solids content, while the body feed filtrates were turbid, irrespective of which type of filter aid was used.

Keywords: Biofuels, Bioethanol, Enzymatic hydrolysis, Cardboard, Separation, Pressure filtration

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Lappeenranta, April 2014

Teemu Kinnarinen

LIST OF PUBLICATIONS

I Kinnarinen, T., Huhtanen, M., Häkkinen, A., Louhi-Kultanen, M. 2012. Solid-liquid separation of hydrolysates obtained from enzymatic hydrolysis of cardboard waste. *Industrial Crops and Products*, 38, 72-80.

II Kinnarinen, T., Shakhanova, M., Hietanen, E., Salmimies, R., Häkkinen, A., Louhi-Kultanen, M. 2012. Effect of mixing conditions on enzymatic hydrolysis of cardboard waste: Saccharification yield and separation of the solid residue using a pressure filter. *Bioresource Technology*, 110, 405-411.

III Kinnarinen, T., Golmaei, M., Häkkinen, A. 2013. Use of filter aids to improve the filterability of enzymatically hydrolyzed biomass suspensions. *Industrial & Engineering Chemistry Research*, 52(42), 14955-14964.

IV Kinnarinen, T., Koiranen, T., Häkkinen, A., Louhi-Kultanen, M. 2014. Enzymatically hydrolyzed and agitated biomass suspensions: experimental determination of fiber size distributions and filtration characteristics. *Cellulose Chemistry and Technology* (Accepted for publication).

V Kinnarinen, T., Häkkinen, A. 2014. Influence of enzyme loading on enzymatic hydrolysis of cardboard waste and size distribution of the solid residue. *Bioresource Technology*, 159, 136-142.

Author's contribution

The author has been responsible for planning the research, performing most of the experimental work, processing the data, and writing all the journal papers listed above. In papers II and III, the co-authors have had a significant role in producing the hydrolysates and performing the filtration experiments, respectively. Chemical analysis of the raw material and characterization of solids in papers III-V, including microscopic evaluation and size measurements, have been carried out by other persons.

Related publications

I Kinnarinen, T., Häkkinen, A., Louhi-Kultanen, M. Solid-liquid separation of hydrolysates obtained from enzymatic hydrolysis of recycled fibre. AFS 24th Annual Conference, 10.5.-12.5.2011, Louisville, KY, USA.

II Kinnarinen, T., Häkkinen, A., Filtration characteristics of enzymatically hydrolyzed and fermented biomass suspensions, The 20th European Biomass Conference and Exhibition, 18.6.-22.6.2012, Milan, Italy.

III Kinnarinen, T., Häkkinen, A. The role of mixing in enzymatic hydrolysis of biomass and its influence on the filtration properties of hydrolyzed suspensions. AFS 2012 Annual Conference, 4.6.-7.6.2012, Boca Raton, FL, USA.

IV Kinnarinen, T., Golmaei, M., Li, W., Häkkinen, A. Reduction of cake resistance with filter aids: improved filterability of hydrolyzed biomass suspensions. AFS 2013 Spring Conference, 6.5.-9.5.2013, Minneapolis, MN, USA.

V Kinnarinen, T., Häkkinen, A. Reduction of fiber size during enzymatic hydrolysis of biomass and determination of filtration properties of biomass suspensions. Filtech 2013, 22.10.-24.10.2013, Wiesbaden, Germany.

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SYMBOLS

A	Filtration area	m^2
A_{Cyl}	Surface area of cylinder	m^2
A_{Rec}	Surface area of rectangular prism	m^2
A_{sp}	Surface area of sphere	m^2
a	Slope of t/V vs. V line	$s\ m^{-6}$
a_e	Length	m
b	Intersection of t/V vs. V line and y-axis	$s\ m^{-3}$
b_e	Length	m
C_c	Mass fraction of solids in cake	-
C_f	Mass fraction of solids in filtrate	-
C_{Sl}	Mass fraction of solids in slurry	-
c	Filtration concentration	$kg_{cake}\ m^{-3}{}_{filtrate}$
c_e	Length	m
d_p	Diameter of particle	m
d_S	Sauter diameter of particle	m
K	Permeability	m^2
K_0	Kozeny constant	-
k_0	Constant (particle shape)	-
L	Thickness of flow medium or cake	m
L_f	Length of fiber	m
m	Mass ratio of wet and dry cake	-
m_{Dc}	Mass of dry cake	kg
m_f	Mass of filtrate	kg
$m_{L,C}$	Mass of liquid in cake	kg
m_{Sl}	Mass of slurry	kg
m_{TDS}	Mass of total dissolved solids	kg
m_W	Mass of water	kg
m_{Wc}	Mass of wet cake	kg
n	Compressibility index	-
p	Pressure	Pa
p_L	Liquid pressure	Pa
p_S	Solid compressive stress	Pa
Δp	Pressure difference	Pa
Δp_c	Pressure difference across cake	Pa
Δp_m	Pressure difference across filter medium	Pa
p_0	Standard pressure difference	Pa
q	Superficial velocity of filtrate	ms^{-1}
Q	Flow rate of filtrate	m^3s^{-1}
R_c	Cake resistance	m^{-1}
R_m	Medium resistance	m^{-1}
r	Radius of particle	m
S_{Cyl}	Specific surface area of cylinder	$m^2\ m^{-3}$
S_{Rec}	Specific surface area of rectangular prism	$m^2\ m^{-3}$

S_{Sp}	Specific surface area of sphere	$m^2\ m^{-3}$
S_v	Specific inner surface area	$m^2\ m^{-3}$
S_0	Specific surface area	$m^2\ m^{-3}$
u	Flow velocity of liquid	$m\ s^{-1}$
t	Time	s
t_{start}	Time required to obtain constant pressure	s
V	Volume of filtrate	m^3
V_C	Volume of cake	m^3
V_{Cyl}	Volume of cylindrical object	m^3
V_L	Volume of liquid	m^3
V_{Rec}	Volume of rectangular prism	m^3
V_{Sl}	Volume of slurry	m^3
V_{Sp}	Volume of sphere	m^3
V_{start}	Volume of filtrate at the beginning of constant pressure period	m^3
V_v	Volume of void in cake	m^3
w	Mass of cake per unit area	$kg\ m^{-2}$
x	Distance from filter medium	m

α	Local specific cake resistance	$m\ kg^{-1}$
α_{av}	Average specific cake resistance	$m\ kg^{-1}$
α_0	Local specific cake resistance at unit applied pressure	$m\ kg^{-1}$
ε	Local porosity	-
ε_{av}	Average porosity	-
ε_0	Empirical constant (at $p_S = 0$)	-
β	Compressibility index (related to ε)	-
ρ_s	True density of solids	$kg\ m^{-3}$
ρ_L	Density of liquid	$kg\ m^{-3}$
ρ_{Sl}	Density of slurry	$kg\ m^{-3}$
Φ_{av}	Average solidosity	-
μ	Dynamic viscosity of liquid	Pa s

ABBREVIATIONS

AFEX	Ammonia fiber expansion
AL	Alkali
CBP	Consolidated bioprocessing
DA	Dilute acid
FD	Fungal degradation
GC	Gas chromatography
HHV	Higher heating value
HPLC	High-performance liquid chromatography
HWE	Hot water extraction
IL	Ionic liquid
NMR	Nuclear magnetic resonance
OS	Organic solvent
SE	Steam explosion
SEM	Scanning electron microscope
SU	Sulfide / sulfite
SHCF	Separate hydrolysis and co-fermentation
SHF	Separate hydrolysis and fermentation
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous saccharification and fermentation
XRD	X-ray diffraction

1 INTRODUCTION

This thesis establishes a link between two different fields of research, production of liquid biofuels from lignocellulosic biomass and solid-liquid separation using pressure filters.

1.1 Background

Due to the unavoidable depletion of fossil fuel reserves, it is of crucial importance to develop technologies for utilizing alternative carbon sources. The use of renewable energy may reduce the CO_2 emissions and dependence on fossil fuels in many countries. Plant biomass has the potential to enable the production of more sustainable liquid fuels, because the technology for biofuel production from plant biomass, instead of traditional sugar and starch materials, is eventually becoming available. Enzymatic hydrolysis, i.e. saccharification of structural carbohydrate polymers of biomass, enables the conversion of these solid carbohydrates to liquid biofuels, such as bioethanol. After the hydrolysis stage, the depolymerized carbohydrates are in a dissolved state in the liquid phase that can be converted to ethanol in a subsequent or simultaneous fermentation stage. The solid residue, consisting of lignin, non-hydrolyzed fibers, other biomass components and impurities, can be burnt after solid-liquid separation to produce heat and power. As regards the hydrolysis stage, the enzymatic approach has several benefits in comparison with the conventional acid-catalyzed hydrolysis: the reaction can be performed under mild conditions and there is no need for complicated sugar recovery and neutralization sequences. The factors affecting the rate and extent of enzymatic hydrolysis have recently been under extensive research. As a result of significant advances in the enzyme technology, the first commercial lignocellulosic bioethanol plants in Europe and the USA have been or are being opened, and a number of demonstration plants are either in operation or under development.

The operation of a lignocellulosic bioethanol plant may require various separation steps, including e.g. cake filtration, membrane filtration, sedimentation, extraction, evaporation, distillation and dehydration. The current research activity around most of these important unit operations is insufficient, taking into account the fact that the separation processes are typically the most

significant contributor to the total costs of the process (Kochergin and Miller, 2011). Deeper understanding of the behavior of biomass at different process stages is required for effective implementation of separation techniques in industrial-scale bioethanol plants in the future. The focus of this thesis is on cake filtration of enzymatically hydrolyzed biomass suspensions with pressure filters.

1.2 Aims and scope

The aim of this work is to produce new information about the pressure filtration of biomass hydrolysates. Separation of the non-degraded residue composed of lignin and fibers from the liquid containing the dissolved sugars has been performed by using two types of pressure filters. Because of the large number of different biomass materials and proposed process layouts, the experimental study has been limited to cover enzymatic hydrolysis of cardboard waste and solid-liquid separation of the hydrolyzed suspensions using pressure filters. The study focuses on investigating the hydrolysis conditions that have an influence on the filtration properties of biomass suspensions. Additionally, improvement of filtration performance with the use of filter aids is studied.

The objectives of this thesis can be summarized by three main research questions (*answered in Papers I-V*):

1) How significant are the effects of process conditions during enzymatic hydrolysis and pressure filtration on the separation performance and cake characteristics? (*Papers I & II*)

2) In particular, how does mixing affect the enzymatic hydrolysis and subsequent filtration? (*Papers II & IV*)

3) How to reduce the cake resistance with filter aids? (*Paper III*)

4) Which physical changes occur in fibers when a certain proportion of their carbohydrate content is hydrolyzed to water-soluble sugars? (*Papers IV & V*)

I LITERATURE SURVEY

The literature part of this thesis aims at introducing the most potential raw materials, production techniques, solid-liquid separation processes (especially filtration), and analysis methods for the production of liquid biofuels from biomass using the microbiological pathway. The main focus is on bioethanol production, although the process sequence applied in the experimental study is not limited to bioethanol. A detailed description of ethanol fermentation, distillation and final purification is out of the scope of the study.

- *As the content of the literature section may partially exceed the scope of the experimental study, the parts are linked to each other by short bullet point notes presented at appropriate locations in Sections 2-6.*

2 LIQUID BIOFUELS FROM LIGNOCELLULOSIC BIOMASS

The growing need for the development of alternative energy sources to reduce the use of fossil fuels is the primary contributor to the increasing use of biomass for the production of liquid biofuels. After decades of bioethanol production from easy-to-convert sugar and starch feedstocks, such as sugarcane and corn, the technology for effective conversion of lignocellulosic biomass is becoming available. Although this thesis concentrates mainly on bioethanol production, the future use of lignocellulosics is not limited to bioethanol: the variety of fuels and chemicals that can be produced from the main components of biomass is immense (Werpy et al., 2004). In spite of the recent steps taken towards economical industrial production, there are still several difficulties to overcome. Most of these difficulties are related to the physicochemical properties of biomaterials and their main components.

2.1 Composition of lignocellulosic materials

Lignocellulosic biomasses consist of three main components: cellulose, hemicellulose and lignin, which together are mainly responsible for the structure of a plant. The proportions of cellulose, hemicelluloses and lignin in dry biomass are typically 40-50, 25-30 and 15-20 w-%, respectively. The most important structural hexose sugars in biomass are D-glucose, D-galactose and D-mannose, whereas D-xylose and L-arabinose are the main pentose monosaccharides.

2.1.1 Cellulose

The most abundant biomass component on earth, cellulose, is a linear polymer consisting of β-(1-4)-linked glucopyranose (glucose) units. Cellulose is the most important structural component in a plant, providing it with a solid backbone that is necessary for mechanical strength. Cellulose consists of amorphous and crystalline phases. The proportion of the crystalline phase, crystallinity, has an influence on the stability of cellulose, for instance during enzymatic saccharification. To be exact, there is not just one strictly defined form of cellulose, since the crystalline phase can consist of different polymorphs, cellulose I_α, I_β, II, III, IV and X (Mansfield et al., 1999; Newman, 1997), and the length of the polymer chain may vary. The degree of polymerization of cellulose ranges from 10,000, which is a typical value for wood, to 15,000 of native cotton (Agbor et al., 2011).

2.1.2 Hemicelluloses

Hemicelluloses are branched, heterogeneous non-cellulosic polysaccharides, composed of different structural sugars. The polymer chains of hemicelluloses are relatively short, compared to cellulose. The structure of hemicelluloses is amorphous and they are physically attached to cellulose and to some extent to lignin. Hemicelluloses interact with cellulose and have therefore an impact on the physical properties of the plant. The composition of hemicelluloses depends essentially on the plant type and may vary significantly. Hardwood species are typically rich in xylans, for instance methylglucuronoxylans, while softwoods contain significant amounts of

mannans, in particular galactoglucomannans. Arabinoglucoronoxylans are the most typically occurring xylans in non-woody biomass. Xylans are broadly divided into four categories, namely homo-, arabino-, glucurono-, and arabinoglucuronoxylans. (Dodd and Cann, 2009; Willför et al., 2005a; Girio et al., 2010)

A specific feature of hemicelluloses is that they have acetyl groups attached to their backbone. From the technological point of view, undesirable formation of acetic acid may easily occur during pretreatment and acid-catalyzed hydrolysis, because these acetyl groups are readily released at high temperature and under acidic conditions (Sassner, 2008).

2.1.3 Lignin

Lignin is a highly branched, amorphous plant biopolymer consisting of cross-linked phenylpropanoid units (Menon and Rao, 2012; Vázquez et al., 1997). Lignins are composed of several functional groups, for instance hydroxyl (phenolic or alcoholic), methoxyl, carbonyl, and carboxyl groups (Gosselink et al., 2004). The heterogeneous composition of lignin is mainly caused by differences in the molecular composition and linkage type of its phenylpropane monomers, p-hydroxyphenyl, guaiacyl and syringyl units that are derived from three cinnamyl alcohols (monolignols), coumaryl, coniferyl and sinapyl alcohols, respectively (Gosselink et al., 2004; Boeriu et al., 2004). Due to its composition, lignin is hydrophobic and non-digestible by most microbes. Cellulose and hemicellulose are covered by lignin within a densely packed lignocellulosic structure, which protects the polysaccharides from microbial degradation and exposure to most other threats.

Cellulose, hemicelluloses and lignin together form the physical structure of plant biomass. The structure of biomass is kept together by hydrogen bonds between cellulose chains and the covalent bonds between cellulose chains and lignin. The cellulose fiber consists of long and thin (a few nanometers) microfibrils that typically comprise 30-36 cellulose chains. The biomass structure comprises also nanoscale pores, filled with water, which causes swelling and enhances its

plasticity. Like the chemical composition, the physical structure of biomass materials depends upon the plant species. Drying has a significant effect on the physical properties of biomass.

2.1.4 Other components

Pectins are complex non-cellulosic polysaccharides found most typically in biomass at low concentrations, usually 1.5-3 w-% of dry matter in wood. The most typical structural component in pectins is α-(1-4)-linked galacturonic acid. Pectins as water-soluble and gel-forming biopolymers are regarded as interesting compounds from the point of view of biorefinery, but have relatively low importance regarding the production of liquid biofuels. (Willför et al., 2005a,b)

Extractives are organic compounds with low molecular weights. They are basically a heterogeneous group of substances (waxes, resins, terpenes, phenolics, etc.), which are extractable from biomass using polar and non-polar solvents (Telmo and Lousada, 2011). The most essential functions of extractives are to protect the polysaccharides, serve as an energy reserve of a plant and to contribute to its metabolism.

There are also some inorganic constituents present in the biomass structure. The most typically occurring ones of these ash-forming compounds are metal silicates, oxides, carbonates, phosphates, chlorides, sulphates, oxyhydroxides, and nitrates. The ash-forming matter, at the atomic level, can be divided into three groups: nutrients (Ca, Mg, Na, K, P), heavy metals, and other elements (Vassilev et al., 2010).

2.2 Overview of conversion technologies

When lignocellulosic materials are converted to liquid biofuels, the main difficulties are encountered in the hydrolysis stage. Hydrolysis means conversion of polysaccharides to smaller compounds that are soluble in an aqueous solution by the breakage of the various glycosidic bonds between the monomeric units. The primary aim of hydrolysis is to cleave the cellulose and

hemicelluloses to simple monosaccharides. In practice, however, hydrolysis processes are somewhat inefficient, which finally results in heterogeneous slurry with dissolved mono-, di-, oligo- and polysaccharides and a solid polysaccharide-lignin residue. During the last decade, the technologies for the cleavage of polysaccharides to monomeric sugars have been significantly improved. Furthermore, methods for the production of novel liquid biofuels from the monomeric sugars have been developed, in addition to the conventional bioethanol fermentation.

The microbial conversion processes of lignocellulosic biomass to liquid biofuels typically include the following stages:

Pretreatment → Hydrolysis → Conversion → Product recovery and purification

The hydrolysis and conversion can be carried out either sequentially or simultaneously, in suitable conditions, in particular with respect to pH and temperature.

There have been strong efforts to enable a shift from the traditionally used strong and dilute acid hydrolysis to enzyme-catalyzed hydrolysis. Enzymatic hydrolysis can be carried out at low temperature and near-neutral pH, while acid hydrolysis, performed under high pressure, requires very high temperatures and significant pH adjustments. In this thesis, the focus is on enzymatic hydrolysis. A more detailed description of this process stage is given in Section 3.2.

The most promising end products from the process include ethanol, butanol, acetone, microbial oil (which is further processed to biodiesel), as well as various biorefinery products which are obtainable for instance from carbohydrate polymers, hexoses, pentoses, alcohols, and organic acids (Posada et al., 2013). Glucose obtained from biomass via hydrolysis can be converted to liquid biofuels as follows:

Glucose → (Fermentation by yeast) → Ethanol

Glucose → (Fermentation by bacteria) → Butanol, Acetone, Ethanol

Glucose → (Conversion by oleaginous microbes) → Microbial oil → Biodiesel

Glucose → (Fermentation by engineered microbes) → Terpenes, e.g. farnesine

In the case of conventional ethanol fermentation, specific strains of *Saccharomyces cerevisiae* can be used. The main weakness of *S. cerevisiae* is that it is unable to ferment pentose sugars (Banerjee et al., 2009). Biobutanol, acetone and ethanol can be produced from glucose by bacterial fermentation. Various *Clostridium* bacterial species are currently regarded as the most suitable ones for the purpose (García et al., 2011; Swana et al., 2011). A novel method for producing lipids for biodiesel production from carbohydrates present in biomass hydrolysates is synthesis performed by oleaginous microorganisms (Xu et al., 2012; Nigam and Singh, 2011; Meng et al., 2009).

The technology for bioethanol production is the most mature and already shown to be applicable in a large scale (Larsen et al., 2012). A simplified reaction equation for anaerobic fermentation of glucose to ethanol is:

$$C_6H_{12}O_6 + 2P_i + 2ADP \rightarrow 2C_2H_5OH + 2CO_2 + 2ATP + 2H_2O \quad (2.1)$$

Similarly, for xylose fermentation it can be written:

$$3C_5H_{10}O_5 + 3ADP + 3P_i \rightarrow 5C_2H_5OH + 5CO_2 + 3ATP + 3H_2O \quad (2.2)$$

where P_i, ADP and ATP stand for inorganic phosphate, adenosine diphosphate, and adenosine triphosphate, respectively.

Due to the stoichiometry of the reactions presented above, the maximum theoretical yield for the most important hexose sugar glucose is 0.51 $g_{ethanol}/g_{glucose}$. For the most important pentose sugar, xylose, the maximum yield in ethanol fermentation is also about 0.51 $g_{ethanol}/g_{xylose}$. In practice, 90-95 % of the maximum yields can be obtained. (Drapcho et al., 2008)

- *In the experimental part of this thesis, fermentation of biomass hydrolysates is demonstrated but not investigated in detail, due to the lack of suitable equipment and in order to keep the research as straightforward as possible.*

2.3 Potential feedstocks and their global availability

There is a large number of different plant species suitable as carbon sources for the production of liquid biofuels. The global production potential is huge: 32 % of the land area on earth is covered by forests, which are the most abundant biomass carbon sources, accounting for 89 % of the total bound carbon (Liu et al., 2012). Compared to woody biomass, agricultural residues and energy crops are typically more suitable for biofuel carbon sources, because of their structure that is less resistant to hydrolysis.

2.3.1 Agricultural residues

Agricultural residues provide a relatively readily hydrolyzable source of biomass. These lignocellulosic residues are non-eatable parts of sugar- and starch-rich crops. Straws of rice, wheat, corn, and sorghum are possibly the most easily utilizable of these residues. Rice and wheat straws definitely have the largest potential in Asia, while corn is most abundantly available in America. In Europe, wheat and corn straws are largely available. Additionally, sugarcane bagasse in South America, as well as corn and maize stovers in North America may have great potential as biofuel feedstocks. (Sarkar et al., 2012)

2.3.2 Woody biomass

Woody biomass species have mostly a closed, dense structure, making hydrolysis difficult, which may be detrimental to the economy of the process. As stated above, woody biomass is globally the most important source of renewable carbon. However, the main weakness of most wood species is

related to their slow growth rate, which can also affect the carbon neutrality, as discussed below in Section 2.4. The recycle time, i.e. the time required for wood growth before harvesting, varies from 5-25 years for tropical forests to 25-80 years for boreal forests (Liu et al., 2012). Woody biomass is divided into two sub-categories, hardwood and softwood. The lignin content of softwood is usually higher, while hardwood contains larger proportions of hemicelluloses and extractives. Woody feedstocks in general have a higher lignin content and lower hemicellulose and ash content, compared to those of agricultural residues (Limayem and Ricke, 2012). With respect to availability and convertibility, eucalyptus, birch, ash and aspen are hardwood species worth consideration. Different subspecies of pine and spruce may have the greatest potential among the softwoods widely available on the northern hemisphere.

2.3.3 Industrial and municipal residues and wastes

Cellulosic fibers are present in several industrial and municipal residues. In order to obtain a sufficient yield, it is important that the cellulose content of the material is high, preferably over 50 %. Previous research has evaluated the potential of waste papers (Wang et al., 2012b; Wang et al., 2013a; Shi et al., 2009) and cardboards (Yáñez et al., 2004), recycled fiber (Ruffell et al., 2010), and waste fiber and fiber sludge (Kemppainen et al., 2012). Waste materials may contain impurities that have an adverse influence on the conversion process, in particular, the hydrolysis and fermentation stages. The main benefits of waste materials are that they do not compete with agriculture and forestry, waste is globally available at a low price, and the contribution to CO_2 reduction can be exceptionally significant.

2.3.4 Energy crops

Some crop species do not produce sugar and starch for human consumption. Instead, these dedicated energy crops are able to produce large amounts of cellulosic polysaccharides, which make them suitable feedstocks for biofuel production. Grasses, such as switchgrass (Liu et al.,

2013) and various *Miscanthus* species (Somerville et al., 2010) will probably be of the highest interest in the future.

2.3.5 Algae

Certain types of algae have been considered for bioethanol production, due to the favorable carbohydrate content of their cellulosic layer. The applicability of algae is currently poor for technical reasons. However, their benefits, such as extremely high productivity and the ability to consume CO_2, are undisputed (Limayem and Ricke, 2012).

- *Cardboard waste was used as the raw material for the hydrolysis and filtration experiments in the experimental part of this thesis. The selection of the raw material was done according to three main criteria: 1) pretreatment with chemicals or at high temperatures was not necessary, 2) cellulose, hemicellulose and lignin were all present at relatively high amounts, and 3) the material was easy to store at room temperature.*

2.4 Sustainability of lignocellulosic bioethanol

The annual production of lignocellulosic materials by photosynthesis has been approximated to be 150 billion tons (Zhang, 2011), which is equal to approximately 30 billion tons oil equivalent (Salameh, 2003). Basically, bioethanol production from lignocellulosic materials is net neutral with respect to the most common greenhouse gas, carbon dioxide (CO_2). Burning ethanol releases carbon dioxide, which is again captured by plant photosynthesis converting it back to biomass (Chandel et al., 2007). If only net CO_2 emissions are considered, bioethanol produced from lignocellulosics has significant benefits, compared to fossil fuels produced from practically non-renewable sources. However, the global share of biofuels in transportation is only about 1 % (Heinimö and Junginger, 2009). The depleting fuels account for almost 90 % of the global energy supply currently (Metzger and Hüttermann, 2009). The recycle time required for natural renewal

of the finite fossil resources is approximately 280 million years, which makes it necessary to develop novel technology for biofuel production (Liu et al., 2012).

Sustainability as a concept generally covers, but is not limited to, environmental, social and economical aspects. When the sustainability of lignocellulosic ethanol is evaluated, it is important to take account of all the possible impacts that ethanol production may bring about. In addition to CO_2 emissions, the energy and material consumption of the production process should be considered. Further, the impacts of feedstock cultivation and harvesting on the environment have to be taken into account. Soimakallio and Koponen (2011) present three fundamental requirements for sustainable biofuel production: 1) there must be unused materials and land area available, 2) greenhouse gas impacts caused during the total biofuel lifecycle must not exceed those of corresponding fossil fuels, and 3) the use of fossil fuels should not become more ineffective as a result of biofuel production.

The Renewable Energy directive of the European Union has also defined the sustainability of biofuels with several criteria. It is clearly expressed in the directive that the greenhouse gas balance should be sufficiently positive, biofuel production should not compete with food production or with any other locally necessary use, and biofuel production should not have negative influences on the environment and biodiversity (Virkajärvi et al., 2009). Lattimore et al. (2009) have recognized several possible environmental impacts, including for instance effects on the soil, waters, forest biodiversity, and greenhouse gas balance.

Sustainable and economical production of bioethanol from biomass may require integration of the production facility with existing industrial facilities, such as pulp and paper mills. This would probably enable more economical raw material supply, which is a prerequisite for feasible operation. Solid residues, especially lignin, could be effectively utilized to recover the energy content, for instance, together with black liquor in recovery boilers. Moreover, the wastewater streams of the process could be treated more economically.

3 ENZYMATIC HYDROLYSIS

The purpose of enzymatic hydrolysis of biomass is to cleave the polysaccharides and intermediate products to fermentable sugars. In an ideal case for bioethanol production, the product is a monosaccharide-rich liquid, which contains only hexoses. Before the hydrolysis of cellulose and hemicelluloses can occur, the structural constraints of the biomass must be reduced in the pretreatment stage. Different process strategies for enzymatic hydrolysis and fermentation have been proposed, all of which with both advantages and drawbacks.

Enzymes for the hydrolysis of biomass are produced by microorganisms, typically by filamentous fungi. *Trichoderma reesei* is generally regarded as the most suitable fungus for the production of cellulolytic enzymes. The production of cellulolytic enzymes also requires a carbon source, and it is easiest to use biomass also for this purpose, which should be taken into account when evaluating the raw material consumption of the whole process.

3.1 Pretreatment methods

Pretreatment of the biomass is required, because the cellulose fibers are well protected by lignin, which makes them difficult to be accessed by the enzymes. The most widely used pretreatments are performed at a high temperature and pressure and/or consume significant quantities of chemicals. Pretreatment is, therefore, a very energy-intensive process. In lignocellulosic bioethanol production, the pretreatment stage is one of the most substantial cost factors, along with the raw material and enzymes, accounting for about 20-30 % of the total processing costs (Chiaramonti et al., 2012; Karunanithy and Muthukumarappan, 2011; Gregg and Saddler, 1996). A good pretreatment process is able to produce material which can be readily hydrolyzed, keeping the formation of inhibitory compounds at a low level (Talebnia et al., 2010). Pretreatment under modest conditions could help to achieve these goals while contributing to process economy (Zhang et al., 2007). After the pretreatment stage, at least one of the three main components of biomass, namely cellulose, lignin, or hemicellulose, is in the dissolved state and can thus be processed further separately.

Some well-investigated pretreatment methods are presented in Figure 3.1, where the dashed lines illustrate which component is mainly targeted for being removed. Some treatment methods remove more than one component at high efficiency: organic solvent (organosolv) treatment removes also most of the lignin, in addition to hemicelluloses, and the ionic liquids dissolve also hemicelluloses, along with cellulose. The pretreatment methods are introduced and discussed in closer detail in Sections 3.1.1-3.1.3.

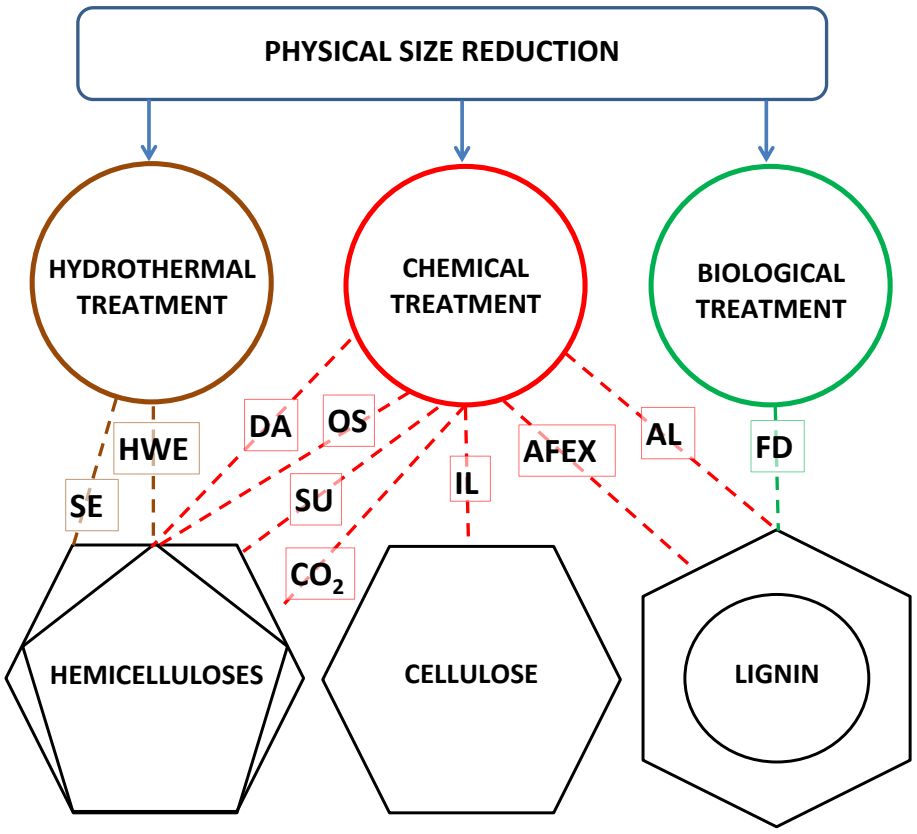

Figure 3.1 An overview of pretreatment methods. The dotted lines represent the main component removed by the treatment. SE = steam explosion, HWE = hot water extraction, DA = dilute acid, OS = organic solvent, SU = sulfide (or sulfite), CO_2 = carbon dioxide, IL = ionic liquid, AFEX = ammonia fiber expansion (or explosion), AL = alkali, FD = fungal degradation.

- *In the experimental part of this thesis, there was no need for hydrothermal, chemical or biological pretreatment, as the raw material, cardboard waste, was already pretreated in a pulping process and was therefore satisfactorily well hydrolysable. Only physical size reduction of the raw material was performed.*

3.1.1 Physical treatment

Physical treatments include mechanical processing methods, such as different milling/ grinding operations (Jin and Chen, 2006; Khullar et al., 2013; Jones et al., 2013), shredding (Zhu et al., 2009), nanofibrillation (Hoeger et al., 2013), and extrusion (Lin et al., 2012). Disc and hammer mills are suitable equipment for particle size reduction in most cases. Additionally, (vibratory) ball mills and compression mills have been used in past decades (Mosier et al., 2005). Before other pretreatment methods can be effectively used, physical treatment is applied in order to reduce the chip dimensions, particle size and fiber strength of the raw material. A significant increase in the accessible surface area for the enzymatic hydrolysis occurs as well. Novel treatment methods, such as electron beam, gamma-ray (Wang et al., 2012a) and microwave irradiation (Taherzadeh and Karimi, 2008), as well as application of pulsed electric field (Conde-Mejia et al., 2012) or ultrasound (Li et al., 2004), may be beneficial when combined with mechanical treatments. After the physical treatment, most of the lignin and a certain proportion of hemicelluloses can be removed by other pretreatment methods, including hydrothermal, chemical and biological treatments. Several different combinations of physical and other pretreatment methods, including typically a milling stage followed by hydrothermal or chemical treatment, have been recently shown to produce good results (Barros et al., 2013; Weiqi et al., 2013; Wang et al., 2013b).

Hydrothermal methods can also be classified to belong to the group of physical treatments. Hot water has characteristics that make it behave similarly to organic solvents. This is possible because the properties of water change dramatically under high temperature and pressure. The hot water treatment is performed under subcritical conditions: required temperature and pressure are significantly lower than those at the critical point of water (374 °C, 221 bar). When heated,

reduction in the permittivity, viscosity and surface tension of water takes place and the diffusivity increases. The dielectric constant is also substantially reduced (Teo et al., 2010). Two hydrothermal pretreatment processes, namely extraction in hot water and extraction followed by mechanical explosion using steam, have been shown to be especially effective. Pressurized hot water extraction is performed by heating the biomass in liquid hot water to 170-230 °C (Talebnia et al., 2010). Steam explosion involves steam treatment under high pressure and the subsequent explosion stage where the pressure is suddenly released and the biomass rapidly "exploded" out of the tank. When the lignin content of the biomass is high, other pretreatments may help to obtain better results. Steam explosion is, therefore, more effective for hardwoods than for softwoods (Mousdale, 2008). Formation of inhibitory compounds is, unfortunately, closely related to the process (Martin et al., 2002).

3.1.2 Chemical treatment

It can be stated, as a rough rule of thumb, that cellulose and hemicelluloses can be dissolved using aqueous solutions of various acids or ionic liquids, whereas dissolution of lignin requires the use of alkaline conditions or non-polar solvents.

The chemicals used for pretreatment include aqueous solutions of alkali (NaOH, $Ca(OH)_2$) , ammonia ($[NH_4^+][OH^-]$), organic solvents (ethanol, benzene, butanol, etc.), dilute acids, sulfite, ionic liquids, carbon dioxide, and oxidizing agents, such as ClO_2, H_2O_2 and O_3. The selection of the pretreatment technique depends on the type of biomass: ammonia-based techniques are more effective with agricultural residues than with woody materials (Mousdale, 2008). Similarly, the use of alkali pretreatment is normally justified when the lignin content is relatively high, but when, in spite of that, the physical structure is easy to open, like in the case of agricultural residues (Taherzadeh and Karimi, 2008).

The chemical pretreatment methods are rarely effective without simultaneous thermal effects. Alkali treatment is typically performed at 100-150 °C, but can be quite effective even at room temperature, provided a long reaction time is applied (Taherzadeh and Karimi, 2008). Ammonia

fiber expansion, or explosion, (AFEX), using liquid ammonia, can be done in moderate conditions at less than 30 bar, at 70-200 °C (Bals et al., 2010), most typically at 160-180 °C (Mosier et al., 2010). The AFEX process depolymerizes and removes lignin, hydrolyses hemicellulose, and recrystallizes cellulose. These effects open the fiber structure and result in an increased number of micropores in the cell wall.

Aliphatic alcohols, ethanol and butanol in particular, are suitable for organosolv pretreatment. During organosolv pretreatment, the majority of lignin is dissolved in the solvent. The liquid is then distilled to recover the solvent. After that, lignin, which is insoluble in the residual aqueous solution, is separated by filtration from the liquid, rich in hemicellulose and its structural sugars (Zhao et al., 2009).

The use of dilute acids in the pretreatment stage aims at partial prehydrolysis of hemicelluloses and cellulose to soluble sugars. The National Renewable Energy Laboratory (NREL) has developed a dilute acid prehydrolysis process, where a dilute (< 2 w-%) solution of sulphuric acid is used in a two-stage reaction configuration. The first and second steps are performed in pressurized reactors at temperatures 130-220 °C and 190-240 °C, respectively. The main drawback of the process is that alkali is needed for neutralization and detoxification. The by-products that may affect the subsequent bioprocessing negatively include furans, especially furfural obtained from pentoses and hydroxymethylfurfural produced from hexoses, carboxylic acids, and a variety of phenolic compounds. Anion exchange has also been found to be a usable technique for the detoxification. (Drapcho et al., 2008; Dehkhoda et al., 2009; Larsson et al., 2009)

3.1.3 Biological treatment

Biological treatment aims at the removal of lignin with microbes. The suitability of fungi and actinomycetes has been most widely studied for lignin removal, while most bacteria cannot degrade the high molecular weight components of lignin (Conde-Mejia et al., 2012; Gidh et al., 2006). Fungal pretreatment is performed by using wood-rotting fungi, most typically white-rot fungi, which are able to produce lignin-degrading enzymes, such as laccase, lignin peroxidase, and manganese peroxidase (Knezevic et al., 2013; Deswal et al., 2013; Taherzadeh and Karimi, 2008).

The method is most effective when it is performed after physical or chemical treatment, enabling significantly improved yields in the subsequent enzymatic hydrolysis (Wan and Li, 2012). However, the main disadvantage of biological treatment is that the energy content of lignin is more difficult to utilize after partial degradation. The released lignin derivatives may also affect the microorganisms detrimentally (Hamelinck et al., 2005). In addition to the abovementioned disadvantages, the required fungal treatment time may be weeks, and some carbohydrate loss may occur (Agbor et al., 2011). That is why fungal pretreatment alone cannot be an optimal solution to pretreating lignocellulosic materials.

3.2 Progress of enzymatic hydrolysis

Enzymatic hydrolysis is a catalytic bioprocess, where breakage of solid cellulosic biomass to water-soluble monomeric sugars and intermediate products takes place. The weak spot in cellulosic materials is the glycosidic bond between the carbohydrate units or between carbohydrates and other components (Andersen, 2007). Both cellulose and different hemicelluloses can be hydrolyzed by using existing commercial enzyme products. The biomass quality, success of pretreatment and the hydrolysis conditions, as well as the used enzymes and their interaction with the biomass, are the main factors determining the rate of enzymatic hydrolysis.

- *In the experimental part of this thesis, commercial cellulase and hemicellulase preparations were used. The effects of some selected process variables on the hydrolysis, as well as the effect of hydrolysis on the properties of the suspension, were studied.*

3.2.1 Cellulases and hemicellulases

In order to enable effective conversion of cellulose to monomeric sugars, several different cellulolytic enzymes must be added in the hydrolysis reactor. In order to hydrolyze xylan and other heteropolysaccharides effectively, hemicellulase cocktails are also used together with cellulases. These enzymes can be produced by several different microorganisms, which in turn may co-

produce several types of cellulases and hemicellulases. Potential and proven cellulase- and xylanase-producing microorganisms include, to mention just a few, *Aspergillus*, *Trichoderma*, *Streptomycetes*, *Basidiomycetes*, and *Phanerochaetae* species (Carvalho et al., 2013; Jeya et al., 2010). Mutant strains of the fungus *Trichoderma reesei* are currently the most important source of cellulolytic enzymes (Ahamed and Vermette, 2009; Gusakov, 2011). *T. reesei* is able to produce both cellulases and xylanases, amongst other enzymes and substances (Callow and Ju, 2012). Other effective fungi for cellulase production may be found in the genera of *Penicillium*, *Acremonium*, and *Chrysosporium* (Gusakov, 2011).

The classification of cellulases can be based on for instance specificity to substrates, structural properties, and reaction mechanism (Andersen, 2007). Without considering the details, it can be roughly generalized that endoglucanases cleave cellulosic polymers, mainly the amorphous regions, to shorter units, which are broken down to cellobiose by exoglucanases a.k.a. cellobiohydrolases (CBH1 and CBH2). Cellobiose is finally broken to glucose by β-glucosidases (Sukumaran et al., 2009; Zhang et al., 2006). These steps are overlapping and the enzymes work synergistically, which also improves the achievable yield: exoglucanases can also cleave intact cellulose chains to shorter fragments, and β-glucosidases may hydrolyze cellooligosaccharides to glucose (Fang and Xia, 2013; Mansfield et al., 1999).

Because of the resistant composition of wood, the use of hemicellulase, such as endoxylanase, together with cellulase can help to obtain improved yields, as shown by Alvira et al. (2009). The hydrolysis of hemicellulases differs from that of cellulose, because the hemicellulose polymers are significantly shorter, and the hydrolysis of their branches may require exposure to tailored enzyme cocktails, which may include xylanases, acetyl xylan esterases, ferulic acid esterases, arabinofuranosidases, glucuronidases, and xylosidases (Dodd and Cann, 2009). Softwood hemicelluloses may also be hydrolyzed by mannanases, mannosidases, and galactanases.

3.2.2 Enzymatic action and enzyme deactivation

In the beginning of the hydrolysis reaction, the enzymes are adsorbed on the surface of the biomass substrate, forming enzyme-substrate complexes. The adsorption is more or less irreversible: on one hand, the enzymes have been observed to remain adsorbed until the completion of hydrolysis, while on the other hand, alternating adsorption-desorption behavior has been assumed to take place (Gan et al., 2002). A typical feature of the process is that the reaction rate slows down by time. The reasons behind the decreasing reaction rate include enzyme deactivation, lack of an easily digestible substrate, and deterioration of mass transfer during the process (Al-Zuhair, 2008). Many of the kinetic models dealing with the enzymatic hydrolysis of cellulose are based on the different digestibility of the amorphous and crystalline regions, which is assumed to be the main reason causing two-stage kinetics (Gama and Mota, 1997). It is apparent that the reduction in the efficiency of enzymes is associated with end product inhibition caused primarily by cellobiose and glucose, the former having a direct effect on cellobiohydrolases and endoglucanases, and the latter interfering with β-glucosidase (Andric et al., 2010).

The changes in the concentration of free soluble enzymes in the liquid phase can be evaluated by measuring the protein concentration. Gan et al. (2003) measured the soluble protein during enzymatic hydrolysis and compared it with a relevant model simulation. They observed that the enzyme concentration in the liquid phase after the rapid initial period remained nearly constant, unlike the modeled concentration that was reduced to half of that during the reaction time of 48 h. Their main conclusion is that the catalytic power of enzymes decreased, possibly due to enzyme deactivation, even though the enzymes once adsorbed on the substrate remained attached.

Another approach for enzyme investigation is measurement of the enzyme activity. Activity measurement, discussed below in Section 6.1.4, is often used to quantify the efficacy of enzymes to degrade cellulosic materials.

3.2.3 Factors affecting enzymatic hydrolysis

Besides the fundamental properties of the substrate and enzyme type and the enzyme-specific factors, such as the temperature- and pH-dependence, the factors having an impact on the progress of hydrolysis can be divided into two main categories: quality of pretreatment, and process conditions during hydrolysis.

Pretreatment affects the particle size, which in turn can be attributed to the external surface area of the substrate (Arantes and Saddler, 2011). A large external surface area generally promotes enzymatic saccharification, because accessible sites for enzymes are abundantly available (Zhang et al., 2013). Availability of water within the fiber structure is essential for the hydrolysis. The water sorption ability, on the other hand, correlates with the internal pore volume. Large internal pore volume enables easy access of water into the fiber structure, which reduces the medium viscosity and facilitates the mass transfer, such as diffusion of enzymes to the proper binding sites (Stauner et al., 2013). Pretreatment also reduces the proportion of at least one component present in the biomass substrate through the removal of easiest-to-dissolve structural polymers, as illustrated in Figure 3.1.

The role of hemicellulose and lignin as potential barriers to enzymatic digestion has been quite widely studied but their relative importance is still under debate. The presence of lignin has been regarded as harmful almost without exception (Santos et al., 2012; Zhu et al., 2008; Yu et al., 2011), whereas the presence of hemicelluloses in the raw material has been shown to be even positive in some cases, because hemicelluloses may facilitate the swelling of fibers during pretreatment. On the other hand, removal of hemicelluloses prior to enzymatic hydrolysis has been stated to be of high or even crucial importance. (Ju et al., 2013a,b; Leu and Zhu, 2013; Mussatto et al., 2008; Zhang et al., 2012)

The composition of lignin after pretreatment has been considered an important factor behind effective pretreatment and, consequently, successful enzymatic hydrolysis. One of the essential quality characteristics is the syringyl/guaiacyl ratio. An increase in the proportion of syringyl units in lignin results in a less condense structure, which promotes the reactivity and contributes to a

high obtainable degree of delignification (Santos et al., 2012). From another point of view, the state of lignin has an influence on the rate and efficiency of hydrolysis: the non-productive adsorption of enzymes on solid lignin, together with the presence of dissolved phenolic compounds in the liquid phase may deteriorate the efficiency of hydrolysis (Tejirian and Xu, 2011; Van Dyk and Pletsche, 2012).

With respect to cellulose, the amorphous regions are relatively easy to be hydrolyzed. That is why the cellulose crystallinity should be reduced in the pretreatment stage (Chandra et al., 2007). The crystalline regions are more resistant to enzymatic attack, which results in an increase in the proportion of crystalline cellulose as the saccharification proceeds (Nazhad et al., 1995). Mandels et al. (1974) suggest cautiously that the crystallinity of cellulose could play the most important role, before particle size and available surface area. Wiman et al. (2012) regard the pore volume, representing the available surface area, as a more significant characteristic than the lignin content on the fiber surface, but do not characterize the cellulose crystallinity.

Each type of enzyme has a different optimum pH and temperature for hydrolysis. Generally, when the duration of hydrolysis is long, the optimum for cellulases is achieved at approximately T = 50 °C and pH = 5. Hemicellulases may typically prefer slightly higher temperatures. The use of elevated temperatures and thermotolerant enzymes would help to reduce the viscosity of the suspension (Modenbach and Nokes, 2013), which could improve mixing and mass transfer. Because of the large number of possible enzyme cocktails and enzyme-substrate combinations, it is impossible to discuss the topic in a more detailed manner. However, the process conditions during the hydrolysis have been observed to have some regular effects on the rate and extent of hydrolysis. First, the biomass loading is an important process parameter that should be optimized to enable economical conversion. The second factor to be considered is the flow conditions, i.e. mixing. The reactor design is actually dependent on the above parameters, because the reactor should be suitable for handling high solid concentrations and of a proper type to enable optimal mixing.

For economical reasons, to minimize the energy and water consumption, it is important to use high solid loadings in enzymatic hydrolysis, irrespective of whether saccharification is performed in the same process stage with fermentation. The concentration of fermentable sugars in the hydrolysate after separate hydrolysis should be as high as possible, preferably over 10 w-%, in order to obtain sufficiently high (> 4-5 w-%) ethanol concentration by fermentation. Han et al. (2011) investigated the effect of biomass loading (1-30 % w/v) on glucose yield at a constant enzyme dosage. When a biomass loading higher than 5 % was used, the enzymatic digestibility started to deteriorate. The glucose yield at the lowest solid loadings was over 90 %. It was reduced to 63 % and finally to about 40 %, when the solid loadings of 20 and 30 % were used, respectively. Because of this reduction in the digestibility, the final glucose concentration in the hydrolysate did not increase significantly when the biomass loading was doubled from 15 to 30 %. Similar behavior has been reported by several other researches as well, as summarized by Kristensen et al. (2009). Based on their own experiments, Kristensen et al. (2009) suggest that the most apparent reasons for weak enzymatic digestibility at high solid loadings could primarily be associated with the decreasing ability of the enzymes to adsorb on the substrate, and secondarily to end product inhibition by cellobiose and glucose. Inhibitors derived from lignin and hemicelluloses did not play an important role in this process. Unlike most other studies, Lu et al. (2010) report very little dependence of enzymatic digestibility on the solid loading. They report a digestibility of over 70 % and a glucose concentration of over 100 g/dm^3, when using high enzyme loadings of commercial acidic cellulase (20 FPU/g dry biomass) for steam-pretreated corn stover at 30 % solid loading. Zhang J. et al. (2009) show that the mixing energy consumption during enzymatic hydrolysis of corn stover was increased from 80 to about 1000 MJ/t_{slurry} when the solid loading was increased from 15 to 30 w-%.

The effect of mixing on enzymatic saccharification has been investigated in previous studies, concerning mixing in general (Palmkvist et al., 2011; Lavenson et al., 2012), rotary shaking (Ingesson et al., 2001), high-intensity mixing (Samaniuk et al., 2011), and total absence of mixing (Taneda et al., 2012). Palmkvist et al. (2011) used a pitched-blade turbine to mix suspensions of steam-pretreated spruce. They obtained about 100 % increase in the final glucose yield when the mixing rate was increased from 25 to 500 rpm. Ingesson et al. (2001) worked with commercial α-cellulose at low solid contents (2.5-7.5 %), shaking rates of 25 and 150 rpm and a specific

intermittent shaking sequence. The fast shaking rate was slightly better with respect to the glucose yield in all cases. However, some earlier studies cited by Ingesson et al. (2001) have shown enzyme deactivation resulting from excessive mixing. For instance, entrapment of air bubbles into the suspension has been recognized as one potential reason for such deactivation. Samaniuk et al. (2011) used Whatman #1 filter paper as cellulosic substrate at 20 % solid concentration in a modified torgue rheometer, which was used as a high-intensity mixing device. They also report about improved glucose yields for mixed suspensions, compared to non-mixed batches. However, their results did not show clear improvement in the yield as a result of increased mixing rates. Taneda et al. (2012) observed significant enzyme deactivation resulting from agitation. The deactivation was attributed to the low shear tolerance of the second type of cellobiohydrolase, CBH2. Zhang X. et al. (2009) recommend the use of pulping equipment for the mixing of biomass suspensions at high solid loadings during enzymatic hydrolysis.

Based on the abovementioned studies, the following conclusions can be drawn concerning the apparent positive effects of mixing on enzymatic hydrolysis of biomass: 1) the breakdown of fibers caused by mechanical shear increases the accessible surface area and 2) enhanced mass transfer reduces local inhibition and enables more productive enzyme adsorption. In cases when hydrolysis is facilitated by mixing, it should be carefully thought whether the improvement outweighs the increase in the energy consumption of the process.

- *The influence of solid loading (Paper I), enzyme dosage (Paper II) and mixing (Papers II and IV) on the hydrolysis yield and filtration characteristics was investigated in the experimental part of this work. Additionally, the influence of the initial particle size of the raw material was also roughly evaluated, in spite of the slightly different composition of the size fractions obtained by sieving. The process pH and temperature were kept constant.*

3.2.4 Changes in slurry properties

During enzymatic hydrolysis, the carbohydrate polymers in the slurry are converted to shorter, water-soluble structural units. On the macro level, the length of cellulose fibers is reduced at the same time. Lignin is less affected, although it interacts with the enzymes and carbohydrates. Carbohydrate conversion has a significant influence on the properties of the slurry. Additionally, the activities of cellulases, cellobiohydrolases in particular, decrease during the hydrolysis as the availability of the readily degradable substrate decreases (Wang et al., 2006; Zhang et al., 1999). Due to this enzyme-substrate relationship, the most dramatic changes in the properties of the slurry are likely to occur during the initial stages of hydrolysis.

The major physical and chemical effects are related to changes in the characteristics of cellulosic fibers in the solid phase and to the dissolved carbohydrates in the liquid phase. The properties of the slurry result essentially from the interaction between these phases. Perhaps the most interesting feature from the rheological aspect is that the viscosity of the liquid phase increases with the concentration of dissolved sugars, while the apparent viscosity of the suspension is substantially decreased. Not only is the volume fraction of solids responsible for the viscosity of the slurry: the particle shape, presence of particle aggregates, and many forms of interaction between the slurry components, are also important (Yoshida et al., 2013). The effect of hydrolysis on the reduction of fiber length varies, depending on the biomass substrate, enzymes and process conditions, as shown in previous studies (Clarke et al., 2011; Nguyen et al., 2013; Pere et al., 1995; Spiridon et al., 2001). Additionally, the degree of polymerization of cellulose may decrease more (Hosseini and Shah, 2011) or less (Mansfield et al., 1997).

The rheology of suspension during enzymatic hydrolysis has been recently studied by Nguyen et al. (2013), Palmkvist and Liden (2012), Carvajal et al. (2012), Jacquet et al. (2012), and Lu et al. (2010). In the study of Nguyen et al. (2013), microcrystalline cellulose, Whatman paper and extruded paper pulp were used. It was observed that shear-thinning behavior occurred in the case of all investigated suspensions. Their results also showed a sharp increase in the suspension viscosity when the volume fraction of solids was increased. Maybe the most surprising result of

the study of Nguyen et al. (2013) was that the hydrolysis of microcrystalline cellulose did not result in a very significant reduction of viscosity and particle size, unlike in the case of other substrates. Palmkvist and Liden (2012) used two types of steam-pretreated biomass, a grass *Arundo donax*, and spruce. During enzymatic hydrolysis using an anchor impeller for mixing, the viscosity of the spruce suspension did not decrease as rapidly and dramatically as that of the grass suspension. The glucose yields obtained from spruce at different solid concentrations were, however, significantly affected by the mixing power. The conclusion from this was that an optimal design of hydrolysis equipment depends on the type of biomass. Carvajal et al. (2012) also used steam-pretreated *Arundo donax* and an anchor impeller. They report shear-thickening behavior of the slurry, which gradually turned to more Newtonian as the hydrolysis proceeded. The results are somewhat contradictory with most other previous studies, which have typically reported shear-thinning behavior of the suspension.

A different approach to the changes taking place in the slurry was taken by Felby et al. (2008), who studied the state and location of water in a cellulose-fiber matrix (filter paper) using time domain NMR. Their results showed that the amount of primary bound water, i.e. the water located on the surfaces of the microfibrils, did not change significantly during enzymatic hydrolysis. However, hydrolysis allowed a better access of water to the cell wall level, where it was bound to hemicellulose, lignin and the existing bound water molecules by hydrogen bonds or by capillary forces. After the initial period of hydrolysis, the cellulose structure was so open that water was able to enter also into lumens, i.e. the inner space of the cells.

- *In the experimental study, the cleavage of carbohydrate polymers to soluble sugars was assessed by measuring sugar concentration in the hydrolysates with high-performance liquid chromatography. The fiber size distributions were also measured and changes in the fiber length and structure were visually evaluated from microscope images.*

3.3 Process consolidation and synergy

A large number of different processes have been proposed to be used for the enzymatic conversion of biomass to bioethanol. In order to simplify the process and enable better process economy, separate process steps can be performed simultaneously in the same vessel. This will, however, result in compromising with respect to the process conditions, which will not always be even close to an optimum.

3.3.1 Combination of process steps to produce lignocellulosic bioethanol

Lignocellulosic bioethanol is the most comprehensively studied second generation biofuel. Thus it is no wonder that various combinations of the most important stages of the process have been developed. After biomass pretreatment, all the necessary process stages incorporated can be performed separately, which enables the utilization of optimum conditions. The selection between separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) has an influence on the process design: the process layouts vary depending on the selected processing strategy, as discussed in detail in Section 4.2. The process integration (Figure 3.2) has gradually led to the concept of consolidated bioprocessing (CBP), which ultimately stands for simultaneous enzyme production, hydrolysis, and fermentation of hexoses and pentoses in the same reactor.

There are two important reasons for why simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP) are not always the best possible choices. First, the optimum hydrolysis temperature for most effective commercial cellulases is about 50 °C, while most ethanol-producing yeasts (e.g. *S. cerevisiae*) are not especially effective at over 35 °C. Second, the pentose sugars can rarely be fermented to ethanol by the same microbes that are effective for the fermentation of hexoses. Ideally, pentose fermentation should also be productive at about 50 °C and pentose and hexose fermentation should not interfere with each other. In practice, however, glucose represses the use of xylose in all relevant xylose utilizing yeasts, which decreases the xylose conversion under glucose-rich conditions (Erdei et al., 2013). Additionally,

most of the xylose is typically converted to xylitol by yeasts. Conversely, Kumar and Wyman (2009) observed that the presence of xylobiose and xylooligomers could have an adverse impact on cellulase-catalyzed hydrolysis of biomass. This effect could probably be mitigated by separation of the pentose-rich liquid from the fibers after the pretreatment.

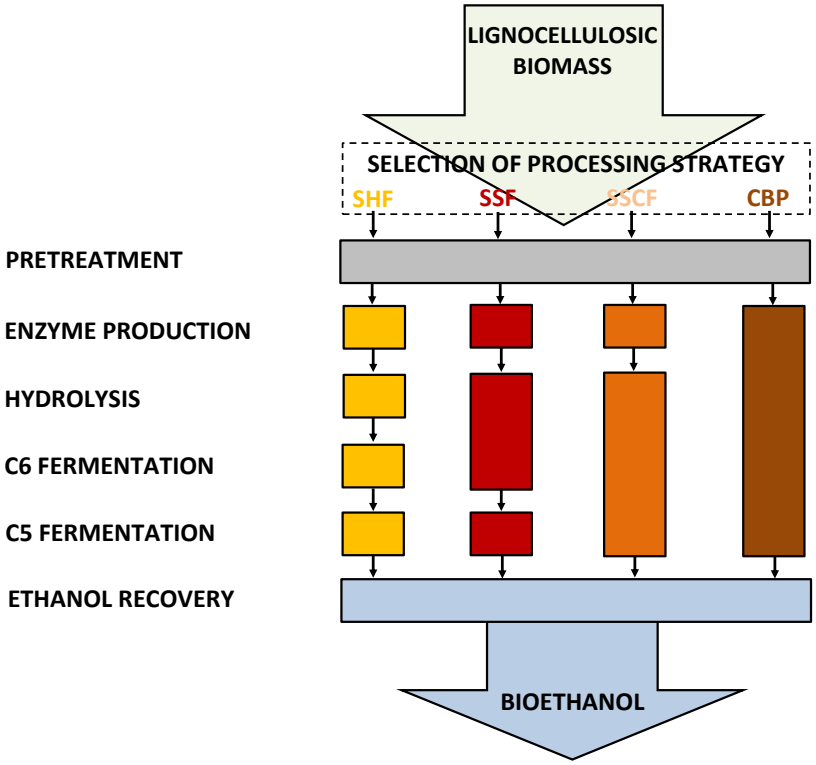

Figure 3.2 Integration of process steps in bioethanol production from biomass using pretreatment and enzymatic hydrolysis, according to Chiaramonti et al. (2012), Erdei (2013), Lynd et al. (2002). SHF = separate hydrolysis and fermentation, SSF = Simultaneous saccharification and fermentation, SSCF = simultaneous saccharification and co-fermentation, CBP = consolidated bioprocessing.

The development of a thermotolerant microbial strain capable of fermenting both C5 and C6 sugars would also be in accordance with the idea of consolidated bioprocessing. Thermophilic bacteria (Bhalla et al., 2013) and thermotolerant yeast strains (Kadar et al., 2004) have been considered for this purpose. Yeast strains, although less suitable to higher temperatures, may possibly have the most significant potential. In spite of the advances, including the development of novel recombinant strains of *S. cerevisiae* (Bettiga et al., 2009; Ha et al., 2010; Chandrakant and Bisaria, 2000; Krahulec et al., 2010), the current state of technology does not favor practical application of consolidated bioprocessing (Girio et al., 2010). Additionally, yeasts are typically poor enzyme producers and the few exceptions, however, have a poor ability to produce ethanol.

3.3.2 Utilization of the energy content of solid residues

The solid residues from lignocellulosic bioethanol production consist mainly of lignin and cellulose. In laboratory experiments aiming at improving the bioethanol yield, consideration of the utilization of these residues is usually omitted. However, in order to evaluate whether a process could be feasible in full scale, it is necessary to understand the energy production potential of these residues. Separation of the solid residues is discussed in Sections 4 and 5 below.

The higher heating values (HHV) of lignin and cellulosic polymers are approximately 23-27 and 17-19 MJ/kg, respectively (Gassner and Marechal, 2013; Demirbas, 2001). Heating values of about 19.5-21 MJ/kg have been reported for dry hardwood and softwood species and 17-19 MJ/kg for agricultural residues and dedicated energy crops (McKendry, 2002; Hamelinck et al., 2005). The higher heating value of wood extractives can be even higher, 35 to 37 MJ/kg (Telmo and Lousada, 2011), but due to the pretreatment, they do not play an important role in the residues. Correlations between the proportion of different biomass components and the higher heating value have been presented in the literature (Yin, 2011). In addition to the higher heating value, the moisture and ash content of the biomass may have a significant effect on the energy recovery by combustion. The negative effect of free moisture on the net energy production is linked to the heat of vaporization of water. The total volatile matter and fixed carbon content are also determined when the combustion potential is evaluated. Technology for effective combustion of solid biomass exists,

not least because 97 % of the bioenergy produced globally is still obtained by combustion (Vargas-Moreno et al., 2012). Among the most promising techniques, besides combustion, are gasification and production of synthetic natural gas (SNG) by gasification and methanation (Gassner and Marechal, 2013).

3.3.3 Process integration opportunities

In order to establish economical bioethanol production in the future, integration of the bioethanol process into other industrial operations may be essential. The objective of process integration is to reduce the production cost by enhancing the utilization of energy (heat and power), raw materials, by-products and waste streams. Integrated waste management, including wastewater treatment, could also bring about significant benefits.

Gonela and Zhang (2013) evaluated the performance of corn- and cellulose-based bioethanol production integrated into four other candidate plants, namely combined heat and power plant, anaerobic digestion (biogas) plant, malt plant, and cement plant. Their calculations showed that, independent of the number of integrated processes, the combined heat and power production produced the highest profit for the bioethanol plant, because of the high importance of cheap process steam. Banerjee et al. (2009) also highlight the importance of process integration with power generation to utilize the energy content of lignin. Galbe et al. (2007) mention an opportunity to integrate the process with a pulp and paper mill and to produce value-added products and chemical according to the biorefinery concept. Practically all previous studies also propose integration with a 1st generation bioethanol plant. Additionally, co-production of methanol from CO_2 produced by fermentation, production of methane by anaerobic digestion of process effluents and biohydrogen from hemicelluloses hydrolyzed in the pretreatment stage could have some potential (Banerjee et al., 2009; Kaparaju et al., 2009).

3.4 Maximizing sugar production

In the SHF process sequence, it is impossible to obtain sufficiently high sugar concentrations for fermentation without using high solid loadings in the hydrolysis stage. Basically the same applies to SSF, SSCF and CBP as well, with the exception that the fermentable sugars are rapidly converted to ethanol, which reduces end product inhibition during hydrolysis. Because of the non-optimal reaction conditions for either the enzymes or the fermenting microorganism in the SSF sequence, the use of a prehydrolysis stage could lead to significant improvement in the glucose and ethanol yields (Hoyer et al., 2013). In the case of SHF, the final glucose concentration in the hydrolysate should be high, preferably at least 8-10 w-%. Ethanol concentration of 4-5 w-% can be obtained by fermentation of such a hydrolysate, which is regarded as the minimum, because of the energy demand of ethanol distillation (Virkajärvi et al., 2009; Galbe et al., 2007). For this reason, high solid loadings are typically used, in spite of the reduced yields (Han et al., 2011; Sassner et al., 2008), mixing problems (Jorgensen et al., 2007), end product inhibition (Andric et al., 2010), and problems caused by other inhibitory compounds (Ishola et al., 2013), such as acetic acid. Reduction in the viscosity of the suspension, as well as an increase in the sugar yield under high-solid conditions, could be obtained by the addition of surfactants into the reaction mixture (Modenbach and Nokes, 2013). The addition of surfactants has been observed to enable more efficient hydrolysis through reduced unproductive binding of enzymes on lignin and increased enzyme stability (Yu et al., 2013). The substrate feeding strategy, such as fed-batch, may also have a positive effect on the final sugar concentration of the hydrolysate with less mass transfer problems and energy consumption (Yang J. et al., 2010; Yang M. et al., 2010; Jacquet et al., 2012).

The enzyme loading, besides the substrate type, concentration, and upstream processing, has a major influence on the cleavage of polysaccharides to monomeric sugars. It has been observed in several studies (Han et al., 2011; Ivetic et al., 2012) that the glucose concentration does not increase linearly with the enzyme loading: after a certain level of enzyme dosage, typically 20-30 FPU/g substrate, only marginal improvements can be achieved. Another point worth consideration is the synergistic effect between cellulases, hemicellulases, and protein additives. This effect has been shown to be of relatively high importance (Hu et al., 2011). Hemicellulases help to open up the

fiber structure to provide cellulases with an improved access to the fibers. Besides the pretreatment techniques, the specific enzyme consumption can be reduced by recovery and recycling (Moniruzzaman et al., 1997), which, however, may render the process more complicated, due to the required extraction and separation stages. The enzyme recovery can be facilitated by immobilization of enzymes onto a solid matrix, which, on the other hand, reduces the specific activity of the enzymes (Jorgensen et al., 2007).

The efficiency of enzymatic hydrolysis can also be enhanced by removing harmful, toxic and inhibitory compounds from the liquid phase. The main hydrolysis inhibitors originate from the degradation of hemicelluloses to form carboxylic acids and furfural, degradation of cellulose to glucose and HMF, and degradation of lignin to yield phenolic compounds. The separation methods of these inhibitors include, for instance, washing of solids prior to hydrolysis, precipitation with alkali, evaporation, membrane extraction, and adsorption (Gurram et al., 2011; Grzenia et al., 2008; Tejirian and Xu, 2011).

Concentration of hydrolysates by evaporation or membrane technology, including nanofiltration and reverse osmosis, is another potential way to increase the concentration of fermentable sugars in the hydrolysate in the SHF process sequence. Both abovementioned techniques are based on the removal of water from the hydrolysate, which, however, is expensive and may increase the end product inhibition if the water is removed during the hydrolysis. On the other hand, fermentation inhibitors, such as furfural and carboxylic acids, may be removed at the same time with water (Dehkhoda et al., 2009; Grzenia et al., 2008). In the case of SSF configuration, ethanol may also be separated from the liquid phase of the reaction mixture with the help of hydrophobic membranes (Huang et al., 2008).

4 SOLID-LIQUID SEPARATION TECHNIQUES

Mechanical solid-liquid separation techniques are essentially based on the physical properties of the solid and liquid phase. The most important characteristics of the two phases enabling and affecting mechanical separation are 1) the density difference between the solid and liquid, 2) the

particle size of solids, and 3) the surface properties of the solids, determining the solid-solid and solid-liquid interactions. Solid-liquid separation techniques include various filtration and screening methods, sedimentation driven by gravity or centrifugal forces, flotation, magnetic separation, and electrokinetic separation. As the production of bioethanol from lignocellulosic raw materials is gradually reaching the industrial scale, it is important to acquire deeper understanding of solid-liquid separation operations. From the engineering point of view, questions concerning the process sequence, scale-up, and selection of equipment, should most probably be taken into account (Kochergin and Miller, 2011). All the three above-mentioned areas of interest can be attributed to the fundamental separation characteristics of the biomass suspension.

- *Although the experimental part of this thesis concentrates on pressure filtration techniques, many of the general conclusions presented in the experimental part can also be applied more generally to solid-liquid separation.*

4.1 Aims and challenges

A typical production process of lignocellulosic bioethanol incorporates several stages where solid-liquid separation is either necessary or beneficial. The primary aim of separation operations in this process is to enable efficient use of the biomass raw material by maximizing the product recovery and utilization of other valuable components than cellulose, especially lignin and pentose sugars.

The separation of lignin and cellulose fibers by sedimentation methods is challenging, compared with mineral processing, where the density difference between the solid particles and the process water is significantly higher. Furthermore, when suspended in water, cellulosic fibers tend to swell and retain large amounts of water. This leads to the formation of a very thick slurry where the proportion of free water is low. The settling of fibers becomes strongly hindered as the solid concentration is increased: in many cases the solid concentration of 20 w-% is enough to disable settling and cause serious mixing problems as well. When the solids are separated by filtration techniques, the compressibility of the biomass has a negative influence on the deliquoring, even though filtration and post-treatment were performed under high pressure differences. Filtration

may also be hampered by blocking of the filter medium by lignin and lignin-fiber aggregates, which can form sticky layers that are difficult to remove without chemicals. Because of the ability of lignin and fibers to form a poorly permeable layer, the resistance of the filter cake can become exceptionally high. The separation of solid residues generated in lignocellulosic bioethanol production is, to some extent, analogous with previously studied topics, including dewatering of different wastewater treatment sludges (Stickland et al., 2008), reject streams of pulp mills (Mäkinen et al., 2013), and pulp and fiber suspensions (Wang et al., 2002; Liimatainen, 2009), which are also compressible and difficult to dewater to high dryness.

Only a few previous studies on lignocellulosic bioethanol production have focused on the topic of solid-liquid separation. Monavari et al. (2009) studied the deliquoring of dilute-acid prehydrolyzed softwood suspension by pressing between the steps of two-stage prehydrolysis. They did not evaluate the filtration characteristics, but concentrated on the sugar yields. Their results indicated that the sugar yield was not significantly affected by the separation between the prehydrolysis stages. The non-favorable degradation of sugars in the second prehydrolysis step could, however, be reduced when the solids were washed after the first prehydrolysis step.

Solid-liquid separation techniques boosted by flocculants have also been evaluated. The aim of flocculation is to make the particles gather together to form large aggregates that are easier to separate from the liquid. Centrifugal sedimentation and vacuum filtration, to produce a clear sugar-rich liquid after enzymatic hydrolysis of softwood, was studied by Burke et al. (2011). They used polymeric flocculants and obtained excellent results with respect to the sugar recovery and cake resistance. Cationic polymers were the most suitable ones, most probably because of the negative zeta potential of the slurry solids. Similarly, Duarte et al. (2010) report good results of flocculation and clarification when using a cationic polyelectrolyte for hot water extracted biomass suspension. Menkhaus et al. (2010) flocculated, centrifuged and filtered 1st generation bioethanol residue stream, corn whole stillage containing protein and oil, which are not usually present in lignocellulosic residues. They obtained the best flocculation and separation results using an anionic flocculant, in spite of the negative zeta potential of the solid residue. The exceptional behavior was explained by the unique properties of such biomass, particularly by the presence of positively

charged functional groups, which were able to bridge electrostatically with the anionic polymer. Galbe et al. (2002) separated solid residues after SSF of spruce. They report test results for a decanter centrifuge and a vibratory shear enhanced processing (VSEP) membrane unit. The decanter experiments showed that the dry matter content in the cake fraction was strongly affected by the feed rate. The solid recovery was significantly improved when a relatively small dose (100 ppm) of flocculant (Cytec superfloc C-494) was added. There was practically no difference between the final dry matter content obtained with the two different devices. However, the suspended solid concentration of the centrate was higher than that of the VSEP filtrate, which means either a loss of solids, or a need for an extra separation step.

4.2 Process flowcharts

The selection of the process flowchart and the location of solid-liquid separation operations in it depend on the conversion sequence. Separate hydrolysis and fermentation may require more separation steps than consolidated bioprocessing. In this section, some of the most apparent flowchart options comprising enzymatic hydrolysis are discussed.

Figure 4.1 presents a couple of alternative process sequences for lignocellulosic bioethanol production. Special attention should be paid to the position of solid-liquid separation operations, marked with red color. The streams that contain mainly liquid or solid can be recognized from the blue and green color, respectively, whereas the brown color represents slurry. For clarity, addition of fresh water after the first solid-liquid separation step is not shown. The solid streams to be removed from the process, separated after the hydrolysis, fermentation, and distillation, are also omitted for the sake of clarity. The location of the solid-liquid separation stage in Figure 4.1 is not fixed: the curved arrows indicate that the separation can change its location with the preceding or subsequent operation. In some cases, however, insufficiently high concentration of suspended solids in the fermentation broth can practically prevent its feeding into the distillation column (Kochergin and Miller, 2011). Burke et al. (2011) list the following benefits obtainable by performing solid-liquid separation prior to fermentation: improved mixing, reduced non-productive adsorption of the fermenting microorganism on the solids, enhanced possibilities for

the utilization of the lignin-rich residue, and easier pre-concentration of the liquid for fermentation by membranes or evaporation. The primary advantage of these improvements could be the reduced energy consumption in the distillation stage. Additionally, enzyme recovery and recycling may be facilitated.

As shown above in Figure 3.2, the production of enzymes can possibly be integrated with the conversion process. When the enzyme production is not integrated, solid-liquid separation is again required for recovering the enzymes from the growth medium, which may contain cellulosic biomass as a carbon source.

After the pretreatment stage, the slurry can be divided into liquid and solid fractions, in order to ferment the pentose sugars released from the hemicelluloses separately. The pretreatment chemicals, such as acids or organic solvents, can also be recovered in the first solid-liquid separation step, which is important to facilitate the operation of the enzymes and yeast (Galbe and Zacchi, 2012). Prior to the pentose fermentation, there may be significant amounts of dissolved hemicelluloses and their oligomers in the liquid. These intermediate compounds can be hydrolyzed to monomeric pentose sugars either separately or during the fermentation. The solids from the first separation step continue to separate or simultaneous enzymatic hydrolysis and fermentation. The separation characteristics of the solid residue vary depending especially on the type of biomass, pretreatment, and process conditions during hydrolysis and fermentation. It can be assumed that, after any process stage, short fibers and fine particles have a negative impact on the separation. Moreover, the presence of yeast in the slurry after the fermentation stage may cause significant difficulties in the solid-liquid separation step. If the solids are not separated from the liquid prior to fermentation, the recovery of yeast for further use may become difficult, because the yeast tends to attach on the solids. In addition to recirculation back to the process, the possibilities for yeast reuse and utilization include the use in animal feeds, use as additives in animal feeds, and production of biogas.

In case the first separation is not performed, the pretreated slurry can be processed to ferment pentoses and hexoses at the same time, in accordance with the simultaneous saccharification and

co-fermentation procedure (SSCF), separate hydrolysis and co-fermentation (SHCF), and consolidated bioprocessing (CBP). Steam pretreatment, for instance, enables hydrolysis and fermentation without a preceding separation. Co-fermentation can be carried out in the same reactor with the enzymatic hydrolysis, or in the subsequent stage. The solid residue, consisting of lignin and non-degraded cellulose fibers, can be separated after any stage downstream the hydrolysis.

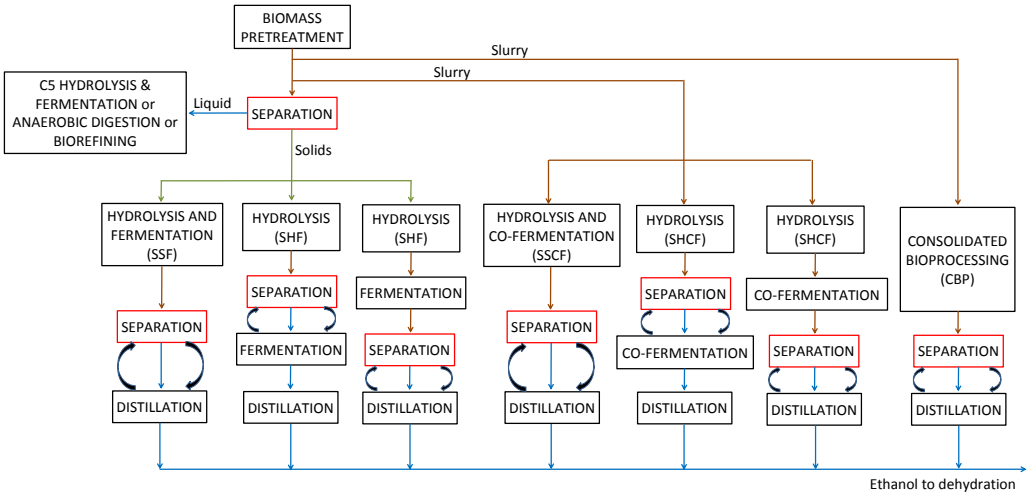

Figure 4.1 Need for solid-liquid separation steps in different process alternatives for the production of bioethanol from lignocellulosic biomass utilizing enzymatic hydrolysis.

In Figure 4.1, the first separation stage is probably of the highest importance for a biorefinery. After the pretreatment, the hemicellulose fraction is mainly in the dissolved state. The energy content of this heterogeneous pentose-rich fraction can be utilized, for instance by ethanol fermentation or biogas production. The main potential of separated hemicelluloses, however, may be associated with value-added environmentally friendly products, such as films and coatings (Mikkonen and Tenkanen, 2012). In order to maximize the hemicellulose yield in the pretreatment stage, it is essential to use an effective pretreatment technique, which does not cause extensive degradation of the polymers. Hot water extraction has been observed to enable complete hemicellulose recovery with low losses (Huang et al., 2008).

Separate hydrolysis and co-fermentation configuration has been studied by Erdei et al. (2012). They integrated the first-generation bioethanol process, using wheat meal as feedstock, with the second-generation lignocellulosic bioethanol process, where wheat straw was used as the raw material. High ethanol yields were achieved, as high as 93 % and 86 % of the theoretical yield, using water-insoluble solid contents of 7 and 18.5 %, respectively. The separation of solid residues was performed after the hydrolysis step with both raw materials. Genetically modified *S. cerevisiae* TMB 3400 was able to ferment xylose to ethanol effectively, especially after the glucose concentration was reduced as a result of its conversion to ethanol.

- *All the processing and separation alternatives presented in Figure 4.1 include the hydrolysis stage. In some process alternatives, the hemicelluloses are separated prior to hydrolysis and in many cases fermentation is performed before the solid-liquid separation. The presence of yeast, in particular, may deteriorate the filtration properties of hydrolyzed suspension.*

5 FILTRATION: THEORY AND PRACTICE

The filtration techniques are sometimes divided into four categories: screening, cake filtration, deep bed filtration, and crossflow filtration. Regarding cake filtration, there are several types of filters, which are operated either batchwise or continuously, driven by a pressure gradient. The driving force in pressure filters may be generated by various slurry pumps, compressed gas, and moving mechanical parts, such as piston or diaphragm. Vacuum filters require vacuum pumps and are more typically operated continuously. In this section, only cake filtration using pressure filters is discussed.

5.1 Description of the cake filtration process

Cake filtration starts with the cake formation period, during which the liquid starts to flow through the filter medium. At the same time, solid particles are retained above the pores of the medium surface. Fine particles may also migrate through the filter medium or get trapped within its pores. After this initial period, the structure of the cake becomes dense enough to protect the filter medium better from particles. After the cake formation, the filtrate can be practically free of suspended solids, irrespective of the pore size of the filter medium. In a pressure filter, separation takes place in a number of steps, including the necessary steps of cake formation and compression, and optional steps of cake washing, further compression and desaturation.

Two basic modes of operation, and a combination of them, are used: filtration at constant flow rate, which requires increasing filtration pressure, and filtration under constant pressure, which results in a decrease in the flow rate (Figures 5.1(1) and 5.1(2)). Constant pressure filtration is more popular in filtration science, because the processing of obtained data is easier when the driving force of the process does not change by time.

In constant pressure filtration, the filtrate flow rate decreases with the increasing cake height. In practice, the compactness of the cake is typically increased as the filtration proceeds, which results in increased resistance to the liquid flow (Tien and Bai, 2003). The volume-based proportion of solids, i.e. solidosity, is the highest at the medium-cake interface and decreases towards the top of the cake (Foley, 2006). With the void fraction, the situation is naturally the opposite.

The compressibility of a filter cake is perhaps the most important property causing difficulties in the case of filtration of biomass slurries. According to the definition of compressible cake, the average specific cake resistance increases with pressure, which may render it impossible to improve the filtration rate by increasing the pressure difference.

Figure 5.1(3) presents a typical linear relationship between filtration time and the square of filtrate volume. There are, however, several situations, illustrated in Figure 5.1(4), when the filtration behavior differs from the ordinary (A) case. These exceptions include (Ripperger et al., 2012):

(B) Partial settling of solids before filtration, causing increased medium resistance,
(C) Inaccuracy in the determination of the starting point of filtration (see Equation (5.23) for explanation) so that filtration starts before the time measurement,
(D) Complete settling of solids and increased cake growth rate,
(E) Settling of coarse particles only,
(F) Migration of fines through the cake, causing pore blocking.

In practice, the above-mentioned reasons for unexpected filtration behavior may not be an absolute truth. Some special properties of the suspension, such as presence of highly compressible gel-like or flake-shaped particles, and non-Newtonian rheology of the liquid, are possible reasons for nonlinear filtration behavior. Additionally, some highly compressible suspensions, such as activated sludge (Christensen and Keiding, 2007) and many suspensions containing soft, deformable particles (Christensen et al., 2011) have typically properties that cause nonlinearity of the t/V^2 plot.

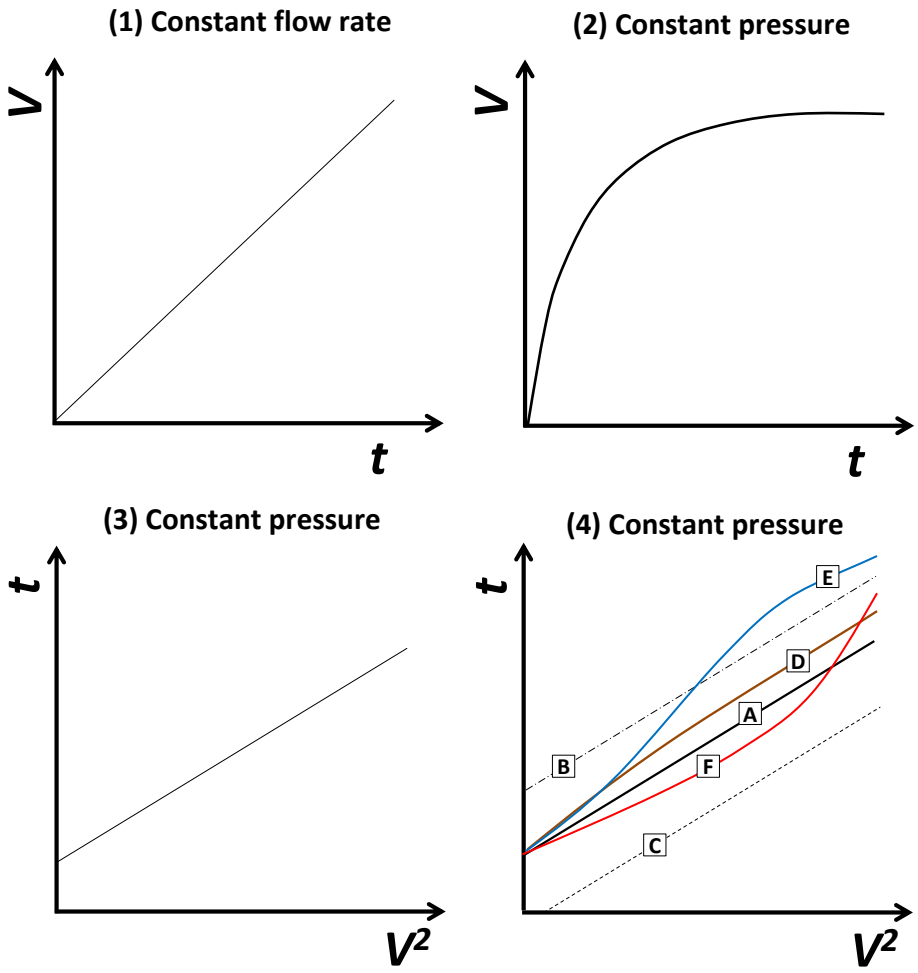

Figure 5.1 Filtrate volume against time for constant flow rate (1) and constant pressure (2) filtration. Filtration time against the square of the corresponding filtrate volume during a typical experiment under constant pressure (3) and examples of non-typical data (4) caused by different reasons.

- *In the experimental part of this thesis, only constant pressure filtration was investigated. The local cake resistances were not determined. The average cake resistances were calculated based on experimental data as described in Section 5.3.1. The compressibility and the average porosity determinations were performed as described in Section 5.3.2.*

5.2 Theory of cake filtration

A number of complicated phenomena and interactions occur in any filtration process. The conventional filtration theory aims at simplifying the investigation of filtration by taking only the most influential factors into account and making generalizing assumptions with respect to the size and arrangement of solid particles.

Combination of the mass balance equation and the momentum balance equation, the latter of which also known as Darcy's law (Darcy, 1856), was the starting point for the mathematical investigation of filtration. The conventional filtration theory was gradually developed in the 20th century, starting from the early work of Ruth (1933a,b, 1935), who proposed that the total resistance to filtration is caused by two components, namely the filter cake and the filter medium (Lee and Wang, 2000). The theory was extended to be better applicable for compressible cakes by the development work of Grace (1953), Tiller (1953), Tiller and Cooper (1960), Tiller and Shirato (1964), and others. In addition to the compression behavior of filter cakes, challenges for the theory are caused by the heterogeneity of the non-ideal slurries to be filtered in real life applications (Bürger et al., 2001; Tien and Bai, 2003; Xu et al., 2008).

5.2.1 Flow of liquid through porous media

The description of the theoretical background of cake filtration can be started with the investigation of an incompressible porous material permeable to liquid, such as a clean filter medium. At this point, there is no cake on the filter medium, through which the liquid flows, usually under laminar flow conditions.

Under the applied pressure Δp through the porous material of cross-sectional area A (later simply referred to as filtration area) and thickness L, the liquid filtrate with a viscosity μ flows at a rate Q, in accordance with Darcy's basic filtration equation (Svarovsky, 1981).

$$Q = K\frac{A\Delta p}{\mu L} \tag{5.1}$$

The permeability of the porous material is represented by constant *K*. In cake filtration, the ratio of thickness *L* and constant K is typically expressed by the medium resistance R_m. Then Equation (5.1) becomes

$$Q = \frac{\Delta pA}{\mu R_m} \tag{5.2}$$

When a suspension is filtered, a cake of solid particles is formed on the filter medium. The pressure difference Δp, is caused by two components, the cake (Δp_c) and the filter medium (Δp_m), connected in series (Tarleton and Willmer, 1997):

$$\Delta p = \Delta p_c + \Delta p_m \tag{5.3}$$

Similarly, the flow rate of the filtrate is affected by the increasing total resistance to filtration, caused by the build-up of the cake. By inserting cake resistance R_c to Equation (5.2), flow through a system formed by the cake and the filter medium can be described:

$$Q = \frac{\Delta pA}{\mu(R_m + R_c)} \tag{5.4}$$

For one-dimensional flow in a homogeneous, incompressible material, assuming a laminar flow regime under a hydraulic pressure gradient dp_L/dx, the differential form of Darcy's law is (Tiller, 2004):

$$\frac{dp_L}{dx} = \frac{\mu q}{K} \tag{5.5}$$

In the case of incompressible filter cakes, cake resistance R_c presented in Equation (5.4) is directly proportional to the mass of cake formed on the filter medium of area A, represented by w.

$$R_c = \alpha w \tag{5.6}$$

The scalable parameter α in Equation (5.6) is the specific cake resistance. By substituting R_c in Equation (5.4), the influence of the amount of cake formed on the filter medium on the filtrate flow rate can be evaluated.

$$Q = \frac{\Delta pA}{\alpha \mu w + \mu R_m} \tag{5.7}$$

From another point of view, regarding the local specific cake resistance, the Ruth modification for an incompressible cake can also be used (Tiller, 2004):

$$\frac{dp_L}{dw} = \mu \alpha q \tag{5.8}$$

where q is the superficial liquid velocity. The mass of dry solids per unit area w is related to the distance of the top of the cake from the filter medium x and local porosity ε of the cake as follows:

$$dw = \rho_S (1 - \varepsilon) dx \tag{5.9}$$

where ρ_S is the true density of solids. After integration, Equation (5.9) becomes

$$w = \rho_S (1 - \varepsilon_{av}) L \tag{5.10}$$

where ε_{av} and L are the average porosity and thickness of the cake, respectively.

The cake solidosity and specific resistance reach the maximum on the surface of the filter medium, where the permeability is at the minimum. When liquid is removed from the compacting filter cake driven by the pressure difference Δp, the frictional drag experienced by the network of solid particles increases. On the other hand, the hydraulic pressure at a certain distance from the filter medium decreases. The conventional theory of cake filtration includes certain assumptions related to the movement of solid and liquid in the cake. It is assumed that the solid phase is stationary and that the velocity of the liquid is constant across the cake. The filtrate flow rate depends only on the overall pressure drop and the average permeability of the solid bed (Chen and Hsiau, 2009). However, permeability is not constant during filtration: it is assumed that the solidosity, permeability and specific resistance of the cake depend only on the solid compressive stress p_s (Tien and Bai, 2003). When the gravity and inertial terms are neglected and the particles are assumed to be non-deformable and in point contact with each other, the change in the liquid and solid pressure gradients is equal. Thus the momentum balance between the solid and liquid phases at any time during filtration is (Lee and Wang, 2000; Tiller, 2004):

$$\frac{\partial p_S}{\partial x} + \frac{\partial p_L}{\partial x} = 0 \qquad \text{or} \qquad dp_S + dp_L = 0 \tag{5.11}$$

The pressure drop through the filter cake (Δp_c) and filter medium (Δp_m), with related symbols, is presented in Figure 5.2(A). The momentum balance (Equation (5.11)) is graphically illustrated in Figure 5.2 (B).

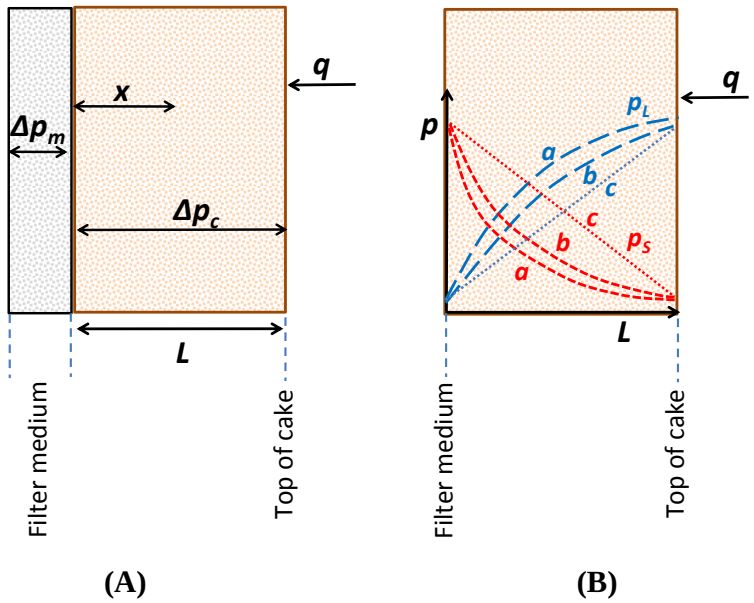

Figure 5.2 (A): Description of cake filtration, showing the pressure drop in the filter cake and the filter medium and the meaning of symbols *L*, *q*, and *x* presented in filtration equations (adapted from Tien and Bai (2003)). (B): Dependence of liquid pressure p_L and solid compressive stress p_S on the distance from the filter medium. Curves marked with *a* represent higher compressibility than curves marked with *b* and the straight lines marked with *c* represent an incompressible material (adapted from Ripperger et al. (2012)).

The local porosity ε and solidosity Φ by time, with respect to the local liquid pressure and the compressive stress of solids, can be described by (Tien and Bai, 2003; Vorobiev, 2006):

$$\frac{\partial \varepsilon}{\partial t} = \frac{\partial q_L}{\partial x} \quad \text{and} \quad \frac{\partial \Phi}{\partial t} = \frac{\partial q_S}{\partial x} \tag{5.12}$$

5.2.2 Kozeny-Carman equation

The Kozeny-Carman equation can be used to relate the specific cake resistance (or the pressure drop) to the particle size of solid particles and the porosity of a bed formed by such particles.

Assuming that the bed consists of particles of characteristic (Sauter) diameter d_s, the inner specific surface area S_v of the particle bed is (Ripperger et al., 2012; Tiller, 2004)

$$S_v = \frac{k_0}{d_s} \tag{5.13}$$

The constant k_0 depends on the particle shape and is 6 for uniform spherical particles.

Assuming an incompressible, uniform cake consisting of the above-mentioned spherical particles, the specific resistance at laminar flow conditions can be approximated using the Kozeny-Carman equation (Svarovsky, 1981):

$$\alpha = \frac{K_0(1-\varepsilon)\cdot S_0^2}{\rho_s \varepsilon^3} \tag{5.14}$$

where K_0 is the Kozeny constant and S_0 is the specific surface area of the particles. Because the specific cake resistance is inversely proportional to the square of particle size, ten-fold reduction in the particle size can be expected to cause a 100-fold increase in the specific resistance (Wakeman, 2007).

5.2.3 Mass balance aspects

The operation of a pressure filter aims at removal of liquid from the slurry. The solids are deposited on the filter medium and the liquid is collected as filtrate. The separation is in practice always incomplete, which means that the filter cake contains some residual liquid within its pore structure. On the other hand, the filtrate may contain solid material, both suspended and dissolved. A certain mass of slurry m_{Sl} fed into the filter equals the total mass of wet cake m_{Wc} and filtrate m_f as follows:

$$m_{Sl} = m_{wc} + m_f \tag{5.15}$$

The mass fraction of solids in cake C_c in the filter at any moment during a filtration cycle, including both dissolved and suspended solids, assuming zero material loss, is:

$$C_c = \frac{m_{Sl} C_{Sl} - m_f C_f}{m_{Sl} - m_f} \tag{5.16}$$

where C_{Sl} is the mass fraction of solids in slurry and C_f is the mass fraction of solids in filtrate.

The mass fraction of solids in the filter cake can be related (Tiller, 2004) to the average porosity of the cake by

$$C_c = \frac{\rho_S (1 - \varepsilon_{av})}{\rho_S (1 - \varepsilon_{av}) + \rho_L \varepsilon_{av}} \tag{5.17}$$

From another point of view, the mass fraction of solids in the slurry can be calculated backwards using the properties of the filter cake (Tien, 2002)

$$C_{Sl} = \frac{L(1 - \varepsilon_{av})\rho_s}{L(1 - \varepsilon_{av})\rho_s + L\varepsilon_{av}\rho_l + V_f \rho_l} \tag{5.18}$$

The true density of solids ρ_S can also be calculated from the mass balance. For the calculation using Equations (5.19 and 5.20), the density of the slurry ρ_{Sl} and liquid phase ρ_L, as well as the solid content of the slurry C_{Sl}, must be measured. Since the mass of slurry is the sum of the mass of solids and liquid, the mass balance can be written in the form

$$V_{Sl}\rho_{Sl} = V_S \rho_S + V_L \rho_L \tag{5.19}$$

where V_{Sl}, V_S and V_L are the volumes of slurry, solids and cake, respectively. The true density of solids is then

$$\rho_S = \frac{V_{Sl}\rho_{Sl} - V_L\rho_L}{V_S} = \frac{V_{Sl}\rho_{Sl} - V_L\rho_L}{V_{Sl} - V_L} \tag{5.20}$$

In practice, it is in most cases easiest to consider a certain volume of slurry and base the calculation on that.

5.3 Determination of cake characteristics

In constant pressure filtration, the average specific cake resistance of a filter cake can be determined from experimental data by plotting the ratio of elapsed filtration time and the cumulative filtrate volume t/V against the cumulative filtrate volume V, which typically yields a straight line, as shown in Figure 5.1 (3). Approximation of the average porosity ε_{av} and the compressibility indices n and β is also possible.

5.3.1 Average specific cake resistance

It is important to make a difference between the local specific resistance α and the average specific resistance α_{av} of a filter cake. The α values can be investigated by measuring the local compressive stress in the filter cake, while α_{av}, determined from the filtration data does not provide information about local cake characteristics. It is assumed that the specific cake resistance is constant during filtration (Oja, 1996). According to Tiller and Cooper (1960), α_{av} is defined as

$$\alpha_{av} = \frac{\Delta p}{\int_0^p \frac{dp}{\alpha}} \tag{5.21}$$

The reciprocal form of Equation (5.7) is a good starting point for the determination of the average specific cake resistance:

$$\frac{dt}{dV} = \alpha_{av}\mu c\frac{V}{A^2\Delta p} + \frac{\mu R_m}{A\Delta p} \tag{5.22}$$

which, after integration from a point (t_{start}, V_{start}) to an end point (t, V), becomes

$$\frac{t - t_{start}}{V - V_{start}} = \frac{\alpha_{av}\mu c}{2A^2\Delta p}(V + V_{start}) + \frac{\mu R_m}{A\Delta p} \tag{5.23}$$

Thus the average specific cake resistance can be calculated from

$$\alpha_{av} = \frac{2A^2\Delta pa}{\mu c} \tag{5.24}$$

where a is the slope (t/V^2) of the line shown in Figure (5.1 (3)) and the filtration concentration c, which represents the mass of dry cake obtained per volume of filtrate, is calculated from

$$c = \frac{\rho_L C_{Sl}}{1 - mC_{Sl}} \tag{5.25}$$

where m is the mass ratio of wet and dry filter cakes:

$$m = \frac{m_{Wc}}{m_{Dc}} \tag{5.26}$$

The resistance of the filter medium R_m can be calculated utilizing experimental data. The line shown in Figure (5.1 (3)) crosses the y-axis at the intersection point b.

$$R_m = \frac{A\Delta pb}{\mu} \tag{5.27}$$

The resistance of the filter medium R_m is typically much lower compared to the cake resistance R_c. The medium resistance is usually assumed to be constant during a filtration experiment. However, R_m may increase and become relatively significant in long-term operation. Regular cleaning of the filter medium is, therefore, necessary in many industrial filtration processes. The easiest and most reliable method of determining R_m is to perform filtration experiments using pure water instead of slurry.

5.3.2 Compressibility and porosity

The compressibility of a filter cake can be evaluated by performing experiments under different pressure differences. In the case of compressible cakes, the average specific cake resistance increases with the pressure difference. Biomass slurries are known to be more or less compressible, while some inorganic solids may form nearly incompressible cakes. During mechanical compression, the porosity of the filter cake decreases while the solidosity increases correspondingly.

Compressibility index n

Equation (5.28) describes the pressure dependence of the local or average specific cake resistance over a limited pressure range (Ripperger et al, 2012).

$$\alpha = \alpha_0 \left(\frac{p_S}{p_0} \right)^n \tag{5.28}$$

In Equation (5.28), α_0 is the specific cake resistance at a standard pressure difference p_0. The extent of the compressibility of the filtered material is characterized by the compressibility index n.

Additionally, other equations aiming at describing the pressure-resistance relationship have been presented in the literature (Tiller and Leu, 1980; Sorensen et al., 1996; Tien et al., 2001):

$$\alpha = \alpha_0 \left(1 + \frac{p_S}{p_0} \right)^n \tag{5.29}$$

and further (Tarleton and Willmer, 1997):

$$\alpha = (1 - n)\alpha_0 \Delta p^n \tag{5.30}$$

In practical filtration applications, Equation (5.28) is often written in the form (Svarovsky, 1981):

$$\alpha = \alpha_0 \Delta p^n \tag{5.31}$$

It is easy to determine n from a logarithmic plot of α_{av} against Δp, where the slope equals n. Another alternative for the determination of n is to fit a power-type function into the α_{av} vs. Δp plot. In the power function, the exponent indicates n.

According to the early definition of compressibility proposed by Shirato, Tiller and other pioneers of cake filtration, the compressibility index n equals zero for totally incompressible cakes, about 0.5-0.6 for moderately compressible, about 0.7-0.8 for highly compressible, and > 1 for super-compressible cakes (Du et al., 2011). According to another definition (Oja, 1996), n smaller than 0.5 indicates a slightly compressible cake, 0.5-1.0 indicates moderate compressibility, and values higher than 1 stand for high compressibility.

Average porosity ε_{av} and local porosity ε

The average porosity ε_{av} of a filter cake indicates the volume fraction of void in the cake.

$$\varepsilon_{av} = \frac{V_v}{V_C} \tag{5.32}$$

where V_v is the volume of void, i.e. the total volume of all pores, in a cake of volume V_C. Because the rest of the filter cake consists of solids, the average solidosity Φ_{av} is

$$\Phi_{av} = 1 - \varepsilon_{av} \tag{5.33}$$

Johansson (2005) has reviewed the most important methods for solidosity evaluation found in the literature. The methods include destructive dissection of cake, use of solids of different colors, measurement of electrical conductivity of cake, measurement of local hydrostatic pressure in cake, and use of modern techniques such as nuclear magnetic resonance (NMR) and γ- or x-ray attenuation.

For the case of fully saturated filter cakes, the pores of which are completely filled with liquid, Equation (5.34) applies:

$$V_v = V_L = \frac{m_{L,C}}{\rho_{L,C}} \tag{5.34}$$

where V_L is the total volume of liquid in the pores, $m_{L,C}$ is the mass of liquid and $\rho_{L,C}$ is the density of liquid.

When the liquid in the cake is pure water (of mass m_W), $m_{L,C}$ can be measured by drying the filter cake to zero moisture content. If there are any dissolved compounds in the pore liquid, $m_{L,C}$ can be calculated from

$$m_{L,C} = m_W + m_{TDS} \tag{5.35}$$

which requires that the mass of total dissolved solids m_{TDS} in the pore liquid must be known. The concentration of dissolved solids can be approximated refractometrically (Brix) or measured by drying a liquid sample, free of suspended solids, to complete dryness.

Additionally, Abe et al. (1979) present a set of equations to be used for the calculation of average porosity of cakes consisting of a binary mixture of solid particles, i.e. particles of two different sizes. The equations may have some value for the analysis of cake filtration using filter aids.

The local porosity ε in a cake depends on the solid compressive stress p_s (Sorensen et al., 1996):

$$(1-\varepsilon) = (1-\varepsilon_0)\left(1+\frac{p_S}{p_0}\right)^{\beta} \tag{5.36}$$

where ε_0 is an empirical constant describing a situation when the solid compressive stress is zero and there is a contact between the solid particles. The scaling pressure, or the standard pressure difference, is p_0 and the compressibility index related to ε is β. There is a rough correlation between n and β: when the value of either one of these indices is high, the value of the other is high as well (Sorensen et al., 1996). It has been reported (Du et al., 2011) that the compressibility index β associated with the cake porosity can be related to the compressibility index n associated with the specific resistance (Equation (5.31)) by $\beta = n/4$.

5.4 Factors affecting filtration and cake characteristics

Cake filtration is influenced by the general properties of the slurry, as well as the properties of the solids and the liquid it consists of. The solid content of the slurry has an important role, because the proportion of solids in the slurry affects the cake growth rate, which in turn has an effect on the

structure of the cake. A rapid growth rate is usually beneficial, helping to obtain a relatively open pore structure, which implies that high solid contents are favorable in most cases. Moreover, the filter medium may suffer from pore blocking caused by fine particles. This problem can sometimes be alleviated by increasing the solid concentration of the slurry. On the other hand, it can be expected that migration of fines into the filter medium may be enabled by a more open cake structure (Oja, 1996). Methods for increasing the solid content include different types of thickening (gravity thickening, use of hydrocyclones, centrifugal thickening), all of which can be improved by the use of flocculants or coagulants (Sparks, 2012).

The density difference between the solids and the liquid may have an effect on the cake formation, because high density difference results in faster sedimentation of solids in the filter.

The particle size distribution of solids is of high importance: large particles are easier to filter, as described by the Kozeny-Carman equation in Section 5.2.2. Large particles also settle faster, which has an effect on the vertical distribution of particles in the cake. In filters where the filter medium is located under the cake, fine particles may consequently have easier access to the proximity of the filter medium (Oja, 1996). The width of the size distribution is also important, narrow distribution being more favorable, because of a more porous cake structure. If the particle size distribution is wide, the voids between large particles are more effectively filled with small particles, reducing the permeability of the cake. The smallest particles may also migrate through the porous structure either into the filter medium or to the lower layers of the cake, where their relative occurrence is typically highest. The particle size distribution can be affected by reduction of the particle size by comminution, milling or pulping, followed by sieving. On the other hand, the apparent particle size can be increased by flocculation, i.e., formation of particle aggregates (Wakeman, 2007).

Particle shape has also an influence on the permeability of the cake, because it affects the volume and surface area of the particles. However, it is usually difficult to describe the particle shape using shape factors, because the particles are often of non-uniform and irregular shape. Spherical particle shape, which is assumed in the filtration theory, is not actually optimal for obtaining high

permeability. Fibrous solids have low specific surface area, and consequently, low specific cake resistance can be theoretically obtained using the Kozeny-Carman equation. On the other hand, flake-shaped particles may form nearly impermeable cakes (Wakeman, 2007).

Temperature has an effect on the filtration rate, because the viscosity of the liquid is strongly affected by the temperature. In the case of water, the reduction of dynamic viscosity with increasing temperature is not linear. For a certain type of slurry, the average specific cake resistance is theoretically independent of the temperature, because viscosity is taken into account in the calculation. In practice, other factors than viscosity can have unexpected effects. For instance, the rigidity of particles affects the flow of liquid in the cake: gum-like particles are sometimes easier to filter at low temperatures, in spite of the increased liquid viscosity (Sparks, 2012). In some applications, increasing the temperature may improve dewatering relatively more than can be expected on the basis of the viscosity reduction (Clayton et al., 2006).

The surface properties of particles at process conditions determine their interaction with each other and with the liquid. The net repulsive force between particles can be described by the zeta-potential. The net repulsive force can be reduced by the addition of a non-adsorbing electrolyte to alter the liquid properties or by the addition of adsorbing ions or charged polymers. As a result of reduced net repulsive force, the system becomes unstable and is therefore easier to be separated (Wakeman, 2007).

The factors discussed above have a significant effect on the porosity and compressibility of the filter cake. In summary, the compressibility of a filter cake is influenced by the average particle size and particle shape, shape of the particle size distribution, and surface properties of the particles (Häkkinen, 2009).

6 CHEMICAL ANALYSES AND PHYSICAL CHARACTERIZATION

All lignocellulosic biomass materials are more or less heterogeneous, consisting of a wide variety of structural components. These components are partially attached to each other, and may be

relatively difficult to separate for quantitative analysis. Although physical characterization can be challenging, the most significant amount of work is required in the chemical analysis of the solid biomass, which is enabled only after complete dissolution of some of the main components of the biomass.

6.1 Chemical analyses

During the production of lignocellulosic bioethanol, it is necessary to perform chemical analyses at several process stages. The carbohydrate content of the raw material is directly proportional to the bioethanol yield on the raw material obtainable by biochemical conversion (Sluiter, 2010). Lignin is the main barrier to prevent enzymatic hydrolysis. The activity of enzymes in the reaction mixture can be evaluated using different types of activity assays. Ethanol concentration is measured from fermented broth and from distilled ethanol.

- *In the experimental part of this thesis, the raw material was analyzed with respect to the cellulose, lignin, and ash content. The cellulase activity was approximated based on literature.*

6.1.1 Carbohydrates

The carbohydrates analyzed in bioethanol production from biomass can be roughly classified into cellulose polymers, hemicellulose polymers, intermediate products and monomeric sugars. In addition to these four main groups, non-cellulosic polysaccharides, such as starch and pectin, may be present in some types of biomass feedstock.

Cellulose content is typically measured after the removal of hemicellulose and/or lignin from the biomass. It is possible to assay depolymerized cellulose in liquid phase by chromatographic methods, such as high-performance liquid chromatography or gas chromatography (HPLC, GC) after acid hydrolysis of all polysaccharides, including cellulose, hemicelluloses, and non-cellulosic

polysaccharides (Jung and Lamb, 2004). Another method relies on sequential dissolution of hemicelluloses, non-cellulosic polysaccharides and lignin and subsequent gravimetric determination (Thygesen et al., 2005; Rivers et al., 1983). The traditional anthrone method (Updegraff, 1969) incorporates removal of hemicelluloses and lignin with an acetic acid / nitric acid reagent, and subsequent dissolution of the cellulosic residue in sulfuric acid in the presence of an anthrone reagent producing a coloured compound, which is then assayed spectrophotometrically. The removal of hemicelluloses and lignin can be performed using other reagents, such as a combination of hot dilute alkali and acid, followed by chlorination with hypochlorite (Jenkins, 1930). If there are chemically stable impurities, such as synthetic polymers, they can be quantified by gravimetric determination after the dissolution of cellulose as described above (Rivers et al., 1983).

Hemicelluloses can be readily dissolved in hot water, dilute acid, some organic solvents, and many other chemicals. After dissolution, hemicelluloses can be assayed directly or after hydrolysis to monomeric sugars. Chromatographic determination by HPLC, or by GC after derivatization, is usually preferred.

The most important monomeric sugars obtainable from biomass by pretreatment and hydrolysis include D-glucose (C6), D-xylose (C5), D-mannose (C6), D-galactose (C6) and L-arabinose (C5). In the past, glucose has been assayed as reducing sugar, using different methods requiring various reagents, as described by Miller (1959), Hulme and Narain (1931), and others. Chromatographic methods are currently utilized for the determination of these monosaccharides, their dimers and oligomers (Lefebvre et al., 2002; Jensen et al., 2010). Other possible methods found in the literature, although not widely applied for all the above-mentioned sugars, are ultraviolet-visible spectrophotometry, Raman spectroscopy (Shih and Smith, 2009), and capillary zone electrophoresis (Dahlman et al., 2000).

6.1.2 Lignin

Determination of the lignin content is typically carried out by using gravimetric or disruptive and noninvasive methods. Klason lignin is the most typically gravimetrically obtained measure of the lignin content of biomass. Klason lignin is the acid insoluble solid residue, which remains after complete dissolution of carbohydrates in sulfuric acid (Thygesen et al., 2005). The total lignin content can be estimated by the total amount of the acid-insoluble residue and the (small) amount of lignin dissolved in acid (TAPPI UM-250). However, gravimetric determination does not give information about the composition of lignin, such as the proportion of different structural units. The disruptive methods are based on analysis of the degradation products of lignin, which enables approximation of the composition of the polymer. (Häkkinen, 2008; Billa et al., 1996)

In addition to the Klason method, the total lignin content can be approximated with the help of analytical pyrolysis (Alves et al., 2006), UV absorption, near infrared spectroscopy, nuclear magnetic resonance (NMR) spectroscopy (Hatfield and Fukushima, 2005), and Fourier transform infrared (FTIR) spectrometry (Dang et al., 2007; Raiskila et al., 2007). A less scientific, rough method of approximation as the Kappa number based on the consumption of potassium permanganate (Adsul et al., 2005) can possibly be used in cases when the Kappa number is low. A more detailed analysis of lignin can be performed with chromatographic methods, typically after a preceding chemical reaction step for degradation of lignin. Such reaction steps include, for instance, oxidation (Parkås et al., 2007; Billa et al., 1996), acidolysis (Lundquist, 1992), or aminolysis (El Mansouri and Salvado, 1997).

6.1.3 Extractives and ash

The low molecular weight components in biomass include extractives (terpenes, waxes, sterols, fats, phenols, etc.) and inorganic minerals (ash). As the name “extractives” suggests, these components can be extracted from biomass. Sequential leaching of size-reduced biomass in non-polar and polar solvents, followed by gravimetric analysis (Telmo and Lousada, 2011), is a relatively easy method for the approximation of the total extractive content. Extractives do not

usually play an important role in the production of bioethanol from lignocellulosic materials, because of their low initial concentration and high degree of removal in the pretreatment stage. Chromatographic methods are suitable for more detailed analysis of extractives extracted in solvents.

The total ash content is measured by burning the sample, usually at 525-575 °C, until only an inorganic residue remains. There are several standards describing the procedure, for instance, ASTM E1755-01 (575 °C), SS 187171 (550 °C), and ISO 1762:2001 (525 °C). More detailed analysis of ash-forming elements and minerals in biomass, without dissolving the ash, can be obtained by x-ray diffraction, x-ray fluorescence, and scanning electron microscopy (Xiao et al., 2011; Thy et al., 2006; Vamvuka et al., 2008). After dissolving ash in acidic liquid, the elemental composition can be analyzed, for instance, by using inductively coupled plasma mass spectrometry, atomic absorption spectrometry, or ion chromatography (Werkelin et al., 2010; Obernberger and Thek, 2004).

6.1.4 Enzyme activity

Enzyme activity is a general measure of the ability of a cellulase enzyme to hydrolyze cellulose. A typical feature of the most popular enzyme activity assays is that they do not directly measure the activity of a single component of cellulase. Among the most commonly used assays are the filter paper assay (Ghose, 1987) measuring the general performance, β-glucosidase assay evaluating the cellobiase activity, CMC'ase assay investigating the endoglucanase activity, and cellobiohydrolase assays for quantifying the activity of exoglucanase. (Singhania et al., 2010; Eveleigh et al., 2009)

The measurement of hemicellulase activities is less important in the case of bioethanol production, because hydrolysis of hemicelluloses is not technically difficult. Corresponding polymers recovered from natural sources, for instance xylans and mannans, are used as substrates in the measurement of endo-type of activities. The products measured are reducing sugars by the DNS method. Para-nitro-phenyl derivatives are often used as substrates for exo-type of activities.

Oligosaccharides may also be used. Procedures for the measurement of xylanase activity have been described by Ghose and Bisaria (1987) and Bailey et al. (1992).

6.1.5 Ethanol

Ethanol concentration after fermentation and distillation can be measured by several methods, such as potentiometry, fluorescence, capillary electrophoresis, modular Raman spectrometry, near-infrared spectroscopy, and various others (Wang et al., 2003 & 2004; Bozkurt et al., 2010; Shih and Smith, 2009). However, many of these methods have practical limitations or they are expensive. Chromatographic methods, especially gas chromatography and HPLC, are most useful for ethanol analysis (Shih and Smith, 2009; Stackler and Christensen, 1974).

6.2 Physical characterization of solids

It is important to characterize the solid particles with respect to size and shape, because these factors have an impact on the performance of solid-liquid separation. Physicochemical composition, inner structure, and surface properties of fibers are also often characterized.

- *In the experimental part, the raw material solids and the residual solids present in some hydrolyzed suspensions were characterized using size measurement techniques and microscopy.*

6.2.1 Dimensions and shape

Classical methods for particle size measurement include sieving, settling and counting of particles. Modern techniques include laser diffraction and photon correlation spectroscopy. Modern particle size measurement by image analysis, on the other hand, can be regarded as an advanced particle counter, which is able to discriminate, count and determine the dimensions of particles. (Syvitski, 2007)

Particle size distributions measured with laser diffraction analyzers are most commonly volume-based, because the finest fraction can dominate the distribution in number-based determinations. The main advantage of laser diffraction, compared with image analysis techniques, is that submicron particles can also be measured. In order to simplify the situation, fibers can be regarded as elongated cylindrical objects. It is important to understand the basis of volume-based particle size distributions and the influence of particle shape on its specific surface area. These issues are illustrated by Equations (6.1-6.9).

The surface area, volume and the specific surface area of a spherical particle depend only on its radius, according to Equations (6.1-6.3).

$$A_{Sp} = 4\pi r^2 \tag{6.1}$$

$$V_{Sp} = \frac{4}{3}\pi r^3 \tag{6.2}$$

$$S_{Sp} = \frac{3}{r} \tag{6.3}$$

A_{Sp}, V_{Sp} and S_{Sp} are the surface area, volume and specific surface area of a sphere of radius r.

The volume of a cylindrical particle V_{Cyl} is directly proportional to the square of its radius, but the surface area A_{Cyl} and specific surface area S_{Cyl} of a cylindrical particle, such as an ideal fiber with negligible surface roughness, depend also on the height of the cylinder, or the fiber length L:

$$A_{Cyl} = 2\pi r^2 + 2\pi rL \tag{6.4}$$

$$V_{Cyl} = \pi r^2 L \tag{6.5}$$

$$S_{Cyl} = \frac{2(r+L)}{rL} \tag{6.6}$$

For rectangular prism-shaped particles, the surface area A_{Rec}, volume V_{Rec}, and specific surface area S_{Rec} are calculated from:

$$A_{\mathrm{Rec}} = 2(a_e b_e + a_e c_e + b_e c_e) \tag{6.7}$$

$$V_{\mathrm{Rec}} = a_e b_e c_e \tag{6.8}$$

$$S_{\mathrm{Rec}} = \frac{2(a_e b_e + a_e c_e + b_e c_e)}{a_e b_e c_e} \tag{6.9}$$

where a_e, b_e, and c_e are the length of each edge in the three dimensions.

The morphology of particles, including fibers, can be investigated using image analysis -based instruments. Another approach is to evaluate the texture and morphology visually from microscope images. Scanning electron microscope SEM (Wang et al., 2006; Chinga-Carrasco et al., 2010), atomic force microscope AFM (Liu et al., 2009), chemical force microscope (Zhao et al., 2007), and transmission electron microscope TEM (Nasri-Nasrabadi et al., 2013) are instruments typically used for morphology investigations. Among these instruments, SEM has the lowest resolution. On the other hand, it has many advantages, such as a large scanning area and rapid operation. Sample preparation for any of these instruments may be a time-consuming procedure, which could require surface stain-coating with metals (SEM), or thinning of the sample (TEM).

6.2.2 Structure and surface properties

The physical structure of biomass, either natural or processed, can be characterized with respect to crystalline structure, degree of polymerization, accessible surface area and electrokinetic properties.

Fourier transform infrared (FTIR) spectroscopy methods can be used to monitor physicochemical changes in the fiber cell wall. Evaluation of changes in the lignin content of fibers during biomass pretreatment, or monitoring of cellulose removal during hydrolysis, can be carried out using FTIR

or attenuated total reflectance (ATR)-FTIR spectra. However, interpretation of the data can be complicated and requires the use of proper models (Foston and Ragauskas, 2012; Hansen et al., 2013; Johar et al., 2012; Nasri-Nasrabadi et al., 2013).

The crystallinity and crystalline structure of cellulose fibers are usually studied with x-ray diffraction XRD (Wang et al., 2006; Mansfield et al., 1997), FT-Raman spectroscopy (Agarwal et al., 2010), and a certain type of nuclear magnetic resonance (NMR) spectroscopy (Terinte et al., 2011), which may also be suitable for providing information about the amorphous regions of cellulose.

The average degree of polymerization of cellulose can be readily approximated on the basis of correlation with the intrinsic viscosity of fiber suspension, as described by Tunc (2003).

The accessible surface area of fibers can be measured by a number of methods, the most important of them being the Bennet-Emmit-Teller (BET) method, measuring the area accessible by the nitrogen molecule, and the solute exclusion method, measuring the inner area of pores and cavities accessible to dextran molecules of different sizes. The former method (calculation presented by Bismarck et al., 2002) requires a dry sample, while the latter method can be used in the wet state, which is likely to yield more realistic results, because the fibers are in the swollen state when well wetted. (Mansfield et al., 1999)

Zeta potential describes the electrostatic state of a solid-liquid system and depends on the pH. The combined effect of repulsive and attractive forces determines the stability of the system. Near zero zeta potential generally indicates good possibility for floc formation, while high negative or positive values indicate good stability of the suspension. The measurement of zeta potential is performed by indirect methods, requiring calculation based on, for instance, electrokinetic measurements.

II EXPERIMENTAL PART

The experimental part focuses on finding answers to the Research questions presented in Section 1.2. Papers I-V are summarized and referred to where appropriate.

7 OUTLINE OF THE EXPERIMENTAL STUDY

The experimental study, with the main focus on filtration research, provides also new information about enzymatic hydrolysis. Because of the large number of process alternatives for pretreatment (Figure 3.1) and separation (Figure 4.1), the study has been limited to the investigation of two main areas:

1) Enzymatic hydrolysis, including
   A) the effect of hydrolysis conditions on glucose output and
   B) the effect of hydrolysis conditions on cellulosic fibers

2) Constant pressure filtration of obtained suspensions, with the focus on
   A) the influence of hydrolysis and filtration conditions on filtration characteristics
   B) the impact of mixing on filtration characteristics

Papers I, II and V contain plenty of results related to enzymatic hydrolysis. Constant pressure filtration accounts for a significant proportion of the results and most of the novelty value of Papers I-IV. Enzymatic hydrolysis was performed in laboratory scale (Papers I, II, IV, V) and in bench scale (Paper III). The filtration experiments were carried out using two types of filters operating under constant pressure: the smaller Nutsche filter with the filtration area of 19 cm^2 (Papers I, II, IV) and the larger Labox 100 filter unit with the filtration area of 100 cm^2 (Paper III).

8 MATERIALS AND METHODS

Although the experimental study was designed to be as straightforward as possible, there are several issues which have to be discussed in closer detail. These issues are associated with, for instance, handling of materials, planning of experiments, description of equipment, and the experimental procedures.

8.1 Hydrolysis and mixing experiments

The original state of the raw material, shredded cardboard, consisting primarily of old corrugated cardboard (OCC) collected from Finland, is shown in Figure 8.1(A). It must be stated here that there is also a possibility to reuse low-quality cardboard for producing new cardboard products. It is, however, significantly more advantageous to reuse materials which contain fibers of better quality, and materials containing less impurities (e.g. plastics and metals). On the other hand, the low lignin content, almost total absence of extractives, and high proportion of ash reduce the interest to combust the material to generate heat and power.

(A) **(B)**

Figure 8.1 (A): The raw material as received originally; (B): the raw material after size reduction with a hammer mill.

Figure 8.1(B) presents the same material after size reduction with a hammer mill. The approximate composition of the material (Table 8.1) was determined after the size reduction. The most significant benefits of this material can be associated with the high cellulose content and ease of storage and use. Because cardboard has undergone a pulping process (some fibers perhaps a couple of times), the material is practically stable when stored as air dry in closed bags in room temperature. Another benefit of this material is that it represents a real lignocellulosic biomass, where all three main components, namely cellulose, hemicellulose and lignin, are present.
Additionally, the high cellulose content (Table 8.1) and good availability in large quantities make cardboards interesting for commercial production of liquid biofuels. The main disadvantage of this kind of material is that it contains impurities, which may have an adverse effect on enzymatic hydrolysis. Difficulties can be caused by a high proportion of inorganic minerals, which is clearly seen in the high ash content (Table 8.1), as well as the presence of plastics.

Table 8.1 Approximate chemical composition of the biomass material.

Component	Concentration* (wt. %)	Analysis method
Cellulose	63	Anthrone method (Black, 1951)
Hemicellulose	14**	-
Lignin	12	Liquid chromatography
Ash	11	Gravimetry (after burning at 525-600 °C)

* *Relative to mass of an oven-dried (105 °C) sample*

** *Calculated assuming negligible content of other components*

In the present work, the cellulose content was measured spectrophotometrically using the anthrone method (Black, 1951). The cellulose content was calculated as the average of two parallel analyses performed for the milled cardboard. For the determination of the lignin content, the carbohydrates were first dissolved in hot nitric acid in a pressurized microwave oven. The lignin present in the solid residue, containing also inorganic components, was then dissolved in a buffered alkaline solution and analyzed by liquid chromatography. The ash content was measured gravimetrically by burning the organic components at over 500 °C to obtain an inorganic ash residue. After measurement of the concentration of these three components, it was possible to approximate the

concentration of hemicelluloses by assuming total absence of other organic components, such as extractives, which, in reality, were probably present in trace amounts.

The dimensions of the hydrolysis tanks and the mixer elements, i.e. the pitched blade turbine, anchor, Rushton turbine, and two propellers, are summarized in Figure 8.2. In the case of Paper I, only one type of hydrolysis equipment (Figure 8.2 a), i.e. a glass reactor and a pitched-blade turbine, was used. For Paper II, configurations shown in Figure 8.2 b, c, and d were used. Figure 8.2 b presents the mixing assembly for the Rushton turbine, Figure 8.2 c for the propeller and double propeller, and Figure 8.2 d for the anchor. The baffles shown in Figure 8.2 b-c are detachable, which made it easy to evaluate the importance of the presence and absence of baffles in the hydrolysis and filtration stages (Paper II).

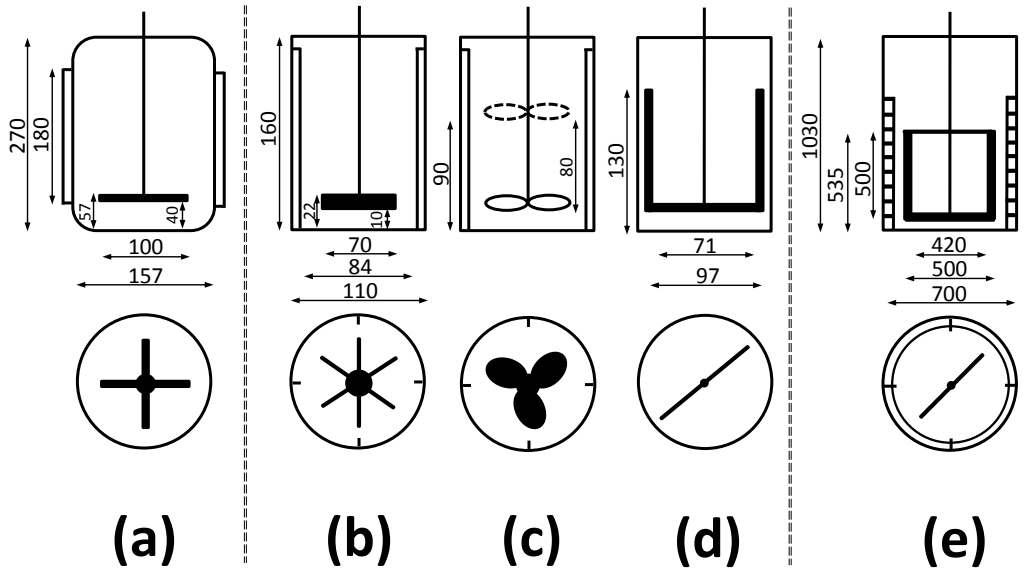

Figure 8.2 Hydrolysis tanks and impeller assemblies including their dimensions (mm). The equipment is associated with the related journal publications as shown in Table 8.1 below. The tanks shown in drawings (b), (c), and (d) are identical, with the exception that baffles were not installed in tank (d). The dashed impeller in drawing (c) describes the possibility to attach another propeller on the impeller shaft. Tank (a) was jacketed, tanks (b), (c) and (d) were kept in water bath, and tank (e) was kept at the target temperature by a heating spiral.

The largest hydrolysis tank, used for preparing the slurry for Paper III, is depicted in Figure 8.2 e. The large tank, made of plastic, was equipped with an anchor impeller in order to ensure adequate mixing and a heating spiral for controlling the temperature. The hydrolysis temperature was kept at 46 °C in all cases presented in Figure 8.2.

The hydrolysis conditions are summarized in Table 8.2. Prior to enzyme addition, the cardboard material was mixed with water and the pH was slowly adjusted to 5.0 with 2.0 M sulfuric acid. When the pH of the prepared biomass suspension had been stabilized, commercial (liquid) enzyme products, Cellic CTec, Cellic CTec2, and Cellic HTec (Novozymes, Denmark), were added into the suspension to start the hydrolysis. The mixing rate was kept constant during the time of enzymatic hydrolysis.

Table 8.2 Hydrolysis conditions in enzymatic hydrolysis (pH = 5.0, T = 46 °C in all cases). The hydrolysis time was 70-72 h.

Paper # / Assembly (Figure 8.2)	Cellulase dosage* ($mL/kg_{cellulose}$)	Hemicellulase dosage ($mL/kg_{material}$)	Solid conc. (w-%)	Particle size (µm)	Mixer type+ / mixing rate (rpm)
I / (a)	40 – 320	8 - 64	5, 7.5, 10	7 fractions**	PBT / 200
II / (b,c,d)	30	5	8, 10, 12	0 – 1600***	A, RT, P, DbP / 29 – 495
III / (e)	217	57	8	As received	A / 35
IV / (b,c,d)	150	30	10	0 – 1600***	A, RT, P / 40 -500
V / (-)	40 - 320	5 - 40	10	0 – 1600***	No mixer

* *Cellic CTec (Paper1), Cellic CTec2 (Papers II-V)*

** *Original shredded cardboard as received*

*** *Cardboard after size reduction using a hammer mill and different size fractions obtained by sieving*

\+ *PBT = pitched blade turbine (45°), A = anchor, RT = Rushton turbine, P = propeller, DbP = double propeller*

As can be observed in Table 8.2, the cellulase and hemicellulase dosages were either selected as variables (Papers I and V), or selected on the basis of experience to be sufficiently high for producing proper suspension for the filtration experiments. The solid concentration was selected as a variable in Papers I and II. The maximum solid concentration was limited to the maximum of

12 w-%, because of structural limitations of the pressure filter in question, particularly because of the small diameter of the slurry inlet.

8.2 Filtration experiments

Most of the filtration experiments for this thesis (Papers I, II, IV) were performed using the small Nutsche pressure filter of a filtration area of 19 cm^2 (Figure 8.2 A). The Labox 100 filter press, of an area of 100 cm^2 (Figure 8.2 B), was used in Paper III. There are some substantial differences between these two filter units: the Nutsche filter, with slurry feeding from the top valve by gravity and pressure obtained from a gas bottle is extremely simple. The Labox 100 filter, on the other hand, is equipped with a slurry pump, pressing diaphragm, pressure control box, and hoses for the slurry and pressurized gas. The filtration temperature was 20 °C. Because of the cake formation between these units is different, the obtained filtration results should not be directly compared.

(A) **(B)**

Figure 8.2 (A): Nutsche pressure filter. (B): Labox 100 filter press.

Table 8.3 shows the most important information related to the pressure filtration experiments (Papers I - IV). The mass of each slurry sample was 200 g in all experiments carried out using the

Nutsche filter, while the filtration time, i.e. the pumping time, was fixed in the case of Labox 100. Therefore, depending on the filter aid, the thickness of the filter cake varied. Due to the maximum structural filtration pressure of 6 bars in the Nutsche filter, the range of filtration pressure was decided to extend from 1 to 6 bars. In order to reduce the number of experiments, only the average of the above-mentioned pressures (3.5 bars) was used when the compressibility of the filter cakes was evaluated. The range of initial total solid concentration of the suspension for the filtration experiments was 5 – 12 w-%. Before the enzyme addition, practically all solids were present as suspended solids in water. As a result of enzymatic hydrolysis, the concentration of suspended solids in the hydrolysate was reduced. This was taken into account when the average specific cake resistances of the cakes were calculated using the filtration data.

Table 8.3 Filtration specifications.

Paper (#)	Mass of slurry (kg)	Filtration pressure (bar)	Total solid conc. (w-%)	Type of filter	Filter medium material
I	0.2	1, 3.5, 6	5, 7.5, 10	Nutsche	Cellulose[+]
II	0.2	1, 3.5, 6	8, 10, 12	Nutsche	Cellulose
III[*]	~1[**]	4	8	Labox 100	Polypropylene[++]
IV	0.2	1, 3.5, 6	10	Nutsche	Cellulose

[*] *Pumping / filtration time = 10 min; Pressing pressure and time = 10 bar and 4 min; No air drying*

[**] *Mass of slurry fed into the filter during the pumping time of 10 min*

[+] *T1000, Pall Corporation, with a cut-off particle size of 24 µm*

[++] *AINO T70, supplied by Outotec Oyj*

In addition to the filtration experiments included in the peer-reviewed publications, one preliminary experiment was performed to investigate the effect of subsequent fermentation on the average specific cake resistance. Pretreatment of the raw material differed from all the other experiments, because the filtration experiments were carried out already during the early stages of hydrolysis, and premilling was necessary for practical reasons. The original shredded cardboard was suspended in water to form a 10 w-% slurry, which was premilled with a Rushton turbine at 600 rpm for one

hour. The experiment was done using a low enzyme dosage: 30 mL of Cellic CTec per one kilogram of cellulose and 5 mL of Cellic HTec per one kilogram of raw material were added. In the ethanol fermentation stage, initialized after 72 hours of hydrolysis, the temperature and pH were 32 °C and 5.0, respectively. The concentration of baker's yeast (*S. cerevisiae*) was 4 g/L.

The filter medium in the Nutsche experiments was composed of cellulose and manufactured by Pall Corporation (Bad Kreuznach, Germany). The polypropylene filter cloths for the Labox tests were provided by Outotec Filters Oy, Finland.

8.3 Analysis and measurement techniques

In addition to the simple manual measurements (mass, volume and length, etc.), the hydrolyzed suspensions, cakes, and filtrates were characterized by using analysis instruments (Table 8.4).

Table 8.4 Applied analysis methods and instruments.

Paper (#)	Characteristic	Analysis method	Instrument
I-V	Sugar concentration	HPLC*	HP Agilent 1100
I-V	Particle size	Laser diffraction	Beckman Coulter LS 13320
IV,V	Fiber length	Image analysis	L&W fiber tester
I, III	Solids structure and shape	Microscopy	Olympus SZX9 microscope
IV,V	Solids structure and shape	SEM**	JEOL JSM-5800

* *High-performance liquid chromatography*

** *Scanning electron microscopy*

The solids content of the filter cakes and filtrates were measured by drying in oven at 105 °C to dryness. Measurement of the cake height may produce a relatively high variability, because the height profile can vary significantly. For this reason, the cake height was measured at five different points, and the average was then used for the porosity calculation.

8.4 Filtration calculations

The filtration characteristics were determined based on a large number of different measurements performed in the laboratory. Depending on the objective of the study and the available data, the emphasis of the calculations varied. However, the average specific cake resistances were calculated in all filtration studies (Papers I-IV). Table 8.5 summarizes the most essential calculations related to the pressure filtration experiments.

Table 8.5 Filtration calculations.

Paper (#)	Characteristic	Equation (#)
I-IV	Average specific cake resistance (m/kg)	(5.24)
III	Resistance of filter medium (1/m)	(5.27)
I, IV	Average porosity of cake (-)	(5.32)
I,II,IV	Compressibility index (-)	(5.31)

The viscosity of filtrate in Equations (5.24) and (5.27) was approximated based on the total dissolved solids in the filtrates, assuming that glucose accounted for 100 % of the dissolved solids. The dependence of the viscosity of an aqueous glucose solution on the glucose concentration and temperature is described in the literature (Converti et al., 1999). In reality, however, the proportion of glucose and xylose together was about 90 % of the total mass of all dissolved compounds, which may have caused a minor error.

In order to calculate the mixer shear pressure in Paper IV, the approximate viscosity of the hydrolyzed suspension, 0.1 Pas, was obtained from the literature (Dunaway et al., 2010).

9 RESULTS AND DISCUSSION

The presentation of the results is divided into two parts: the hydrolysis and mixing results are presented first, and pressure filtration characteristics are discussed after that.

9.1 Hydrolysis and mixing effects

The hydrolysis studies aimed at investigating the impacts of hydrolysis conditions on the production of the main sugars, glucose and xylose, and on the fiber dimensions. The most important variables were 1) enzyme dosage, 2) solid concentration, 3) initial particle size of solids, and 4) mixing conditions (mixer type, mixing rate and time).

Table 9.1 shows the observed influence of each variable on sugar production by enzymatic hydrolysis and on the dimensions of residual fibers. The idea of Table 9.1 is to evaluate the importance of the variables within the range investigated in this study. The positive and negative influences are presented for a case when the value of a variable, shown in Table 8.2, is increased from the minimum to the maximum within its range of investigation.

Table 9.1 Effect of hydrolysis conditions on the sugar production and fiber dimensions.

Property	Cellulase dosage	Hemicellulase dosage	Initial solid concentration	Initial particle size	Mixing impact
Glucose concentration	++	+	++	-	++/-
Xylose concentration	++	+	++	-	++/-
Glucose yield	++	0	+/-*	U	++/-
Fiber length	-	0	N	N	0/-
Fiber width	+	0	N	N	N

++ (strongly positive); + (slightly positive); 0 (negligible); U (unclear); - (slightly negative); N (not evaluated)

** Slightly positive over the range of 5-10 % (Paper I) and slightly negative over the range of 8-12 % (Paper II)*

It can be clearly seen in Table 9.1 that, as expected, the sugar concentrations are strongly affected by the cellulase dosage and the initial solid concentration of the hydrolyzed suspension. However, the glucose and xylose concentrations did not increase linearly with the cellulase dosage (Paper I, Fig. 4; Paper V, Fig. 2), which can be absolutely regarded as one of the main obstacles for industrial biofuel production. The same limitation applies also to other types of biomass (Ivetic et al., 2012;

Adsul et al., 2005; Han et al., 2011). The effect of hemicellulase supplementation on the obtained sugar concentrations and glucose yields was observed to be either negligible or not very significant. Although the sugar concentrations were strongly increased with the initial solid concentration of the suspension, the yield of enzymatic hydrolysis was only slightly affected. Based on the results presented in Paper I (Fig. 3), it looks that the glucose yield can be increased by increasing the initial solid concentration from 5 to 10 w-%. However, when the solid concentration is increased from 8 to 12 % (Paper II), the yield is slightly reduced. This phenomenon has been attributed to a couple of factors, such as the low volume of free water in the suspension and end product inhibition caused by glucose (Kristensen et al., 2009). On the other hand, the low yield obtained with the lowest solid concentration (Paper I) is difficult to explain by the measurement data. The presence of impurities and heterogeneity of the material could be the two most important reasons for this, because this part of Paper I, unlike Papers II-V, was performed by using non-milled raw material. Mixing effects may also have affected the conversion.

The effect of initial particle size of the biomass solids was studied after size classification by sieving. However, it was observed that the ash content of the finest size fraction was approximately 30 % higher than that of the overall milled raw material (Paper I, Table 1), which the size fractions were separated from. The high concentration of inorganic matter apparently indicates a slightly lower cellulose content of that fraction and this may have had a negative impact on the enzyme action as well. This may explain why the yield obtained from this fraction was not the highest and why the second and third finest fractions were hydrolyzed more completely. As also mentioned in the literature part, the effect of particle size on the success of enzymatic hydrolysis is quite case-dependent. It can be stated that particle size reduction must be substantial in order to achieve clearly observable improvement in the glucose yield, although there does not seem to be any universal truth in this issue (Yeh et al., 2011; Zhang et al., 2013).

The results presented in Paper II (Figs. 2 and 3) imply that the influence of mixing on the obtained glucose and xylose concentrations and glucose yield depends on the mechanical impact caused by the impeller. The glucose yield was significantly improved, compared to the static situation, up to a certain point when the suspension was mixed by avoiding high shear forces. However, the yield

started to decrease again when the mixing rates were increased. These observations can be, on one hand, associated with the improved mass transfer resulting from mixing (Samaniuk et al., 2011; Lavenson et al., 2012), and on the other hand, deactivation or non-productive adsorption of enzymes under high-shear conditions (Ingesson et al., 2001). This topic deserves to be more systematically studied in the future, with respect to the rotation speed of impeller and type of raw material.

Papers IV and V focus on changes in fiber dimensions caused by enzymatic hydrolysis and mixing. The fiber dimensions were evaluated using laser diffraction and image analysis as the analysis techniques. The effects of enzymatic hydrolysis on the particle size of the solid residue have been investigated in some previous studies (Clarke et al., 2011; Nguyen et al., 2013). It seems that the changes in the fiber dimensions depend on the process conditions, enzyme dosage, and type of biomass. The fiber length was shown to be slightly reduced, whereas the fiber width was slightly increased, when the cellulase dosage, i.e., cellulase loading, was increased. The hemicellulase loading did not have a distinct influence on the fiber dimensions. This may be due to the composition of the commercial cellulase, which in the investigated case was also effective at hemicellulase hydrolysis. It can be speculated that it would have been clearer to see the effect of hemicellulase if significantly lower cellulase dosages than 192 $cm^3/kg_{substrate}$ had been used.

The main conclusion from the fiber size analyses is that most changes occur during the first few hours of enzymatic hydrolysis. Mixing during hydrolysis does not have a notable effect on the fiber dimensions (Figure 9.1 (a-d)). The results shown in Figure 9.1 (a) and (b) are discussed in Paper IV, while the results presented in Figure 9.1 (c) and (d) have been obtained from the experimental data of Paper V. In the experiments shown in Figure 9.1, the enzyme dosage was practically equal.

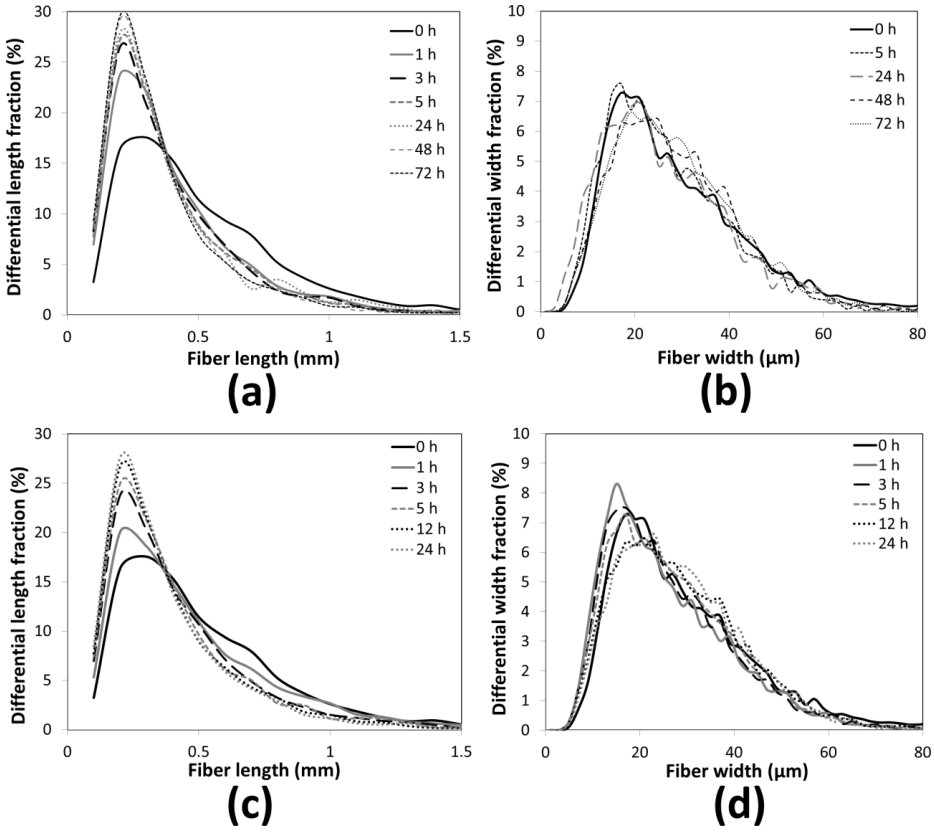

Figure 9.1 Fiber length and width distributions before and during enzymatic hydrolysis in agitated (a,b) and non-agitated (c,d) conditions, measured with the L&W fiber tester.

The particle size distribution of a hydrolyzed 10 w-% suspension was slightly affected by mixing (Paper IV). Based on this result, it is unclear if strong mixing during the initial stage of hydrolysis could cause more significant particle size reduction, because mixing was performed after the hydrolysis, to avoid the effect of mixing on the yield, which could affect the filtration properties. Based on the filtration results discussed below in Section 9.2, it is probable that fibrillation of fibers, which cannot be seen in the particle size data, occurred when the suspension was mixed.

9.2 Filtration characteristics

The most essential results of the filtration study were related to the dependence of filtration and cake properties on the final degree of hydrolysis and mixing during the process. Additionally, improvement of pressure filtration with various filter aids was investigated.

A preliminary study evaluating the filtration characteristics is presented in Paper I (Figs. 9-12), where the main focus is on the correlation of the hydrolysis conditions and the average specific cake resistance α_{av}. It is shown that an increase in the yield of enzymatic hydrolysis made filtration more difficult by increasing the average specific cake resistance and by reducing the cake porosity. A positive finding in this study was that filtration can be performed effectively immediately after hydrolysis at a temperature of about 40 °C. The data showed that heating of the suspension to 50 °C prior to filtration did not facilitate filtration any more, which is a good result regarding the energy consumption. It is probable that lignin becomes physically softer because of heating and the rigidity of the residual fibers may also decrease. All cakes still contained a significant proportion of non-hydrolyzed fiber, because of the low glucose yields, which evidently had a positive effect on the performance of the filter. A beneficial effect of fibers on dewatering has been reported in the literature (Mäkinen et al., 2013) and this was also utilized in Paper III (Fig. 6), where cellulosic filter aids were used in order to improve the filtration capacity. The filtration of hydrolysates produced from size fractions obtained by sieving, ranging from < 125 µm to 1,000-1,400 µm, was investigated in Paper I. It can be generally concluded that the values of α_{av} were more dependent on the corresponding yield and non-measured particle properties than on the size of fibers and other particles. The higher proportion of inorganic particles in the finest fraction was not observed to increase α_{av}. The differences between the size fractions became more significant when the filtration pressure was increased. The influence of solid concentration on α_{av} (Paper I) remained quite unclear because of inconsistent results, but the influence was clearer at increased filtration pressures. In the case of Paper I, it was impossible to explain the filtration results completely by the experimental variables, which was the main motivator for Paper II.

The non-published results of the preliminary hydrolysis and fermentation experiment showed that α_{av} increased during the hydrolysis time and continued to increase at a slowing rate as the fermentation stage was performed after the hydrolysis. These results are illustrated in Figure 9.2.

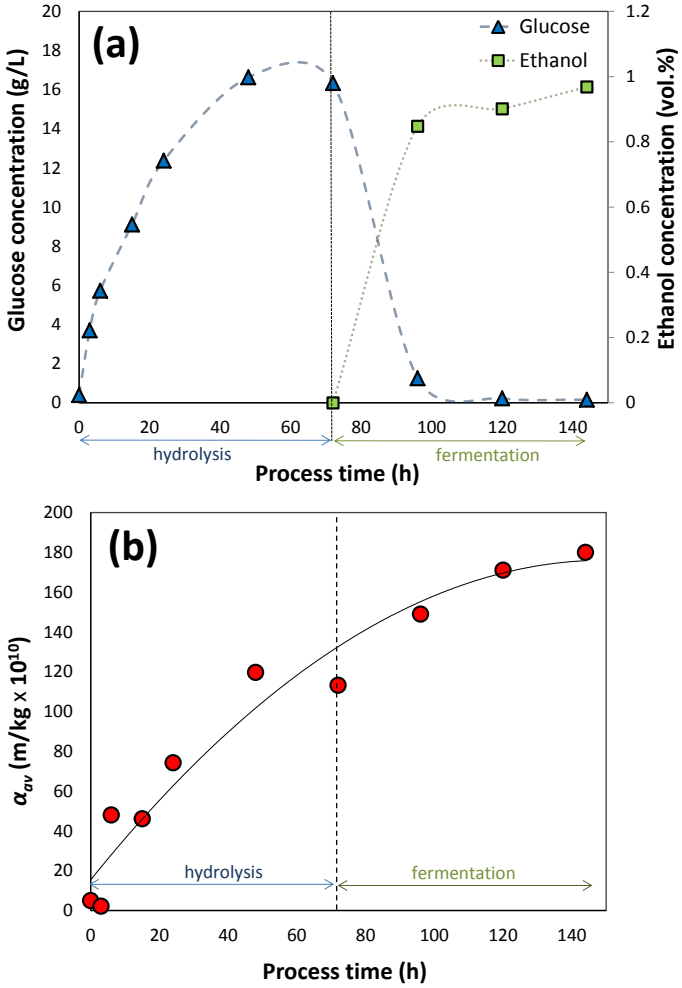

Figure 9.2 Concentration profiles of glucose and ethanol during separate hydrolysis and fermentation (a). Average specific resistances of the filter cakes during the SHF process, obtained at the filtration pressure of 1 bar (b). Note that the filtration experiment after 72 hours, shown in Fig. 9.2 (b), was performed prior to the addition of yeast.

As Figure 9.2 (a) shows, the glucose liberated into the liquid phase during the hydrolysis was rapidly consumed by the yeast after the start of the ethanol fermentation stage. In this case, the presence of yeast together with the still continuing hydrolysis increased the specific cake resistance when the fermented slurry was filtered (Figure 9.2 (b)). The effect of yeast was, however, relatively moderate, due to the low yeast dosage applied. Optimization of the filtration stage for suspensions where yeast is present could be an interesting topic for another study in the future.

The impact of mixing during hydrolysis on pressure filtration characteristics was investigated in Paper II. The main findings of Paper II are that the filtration properties, after enzymatic hydrolysis, are affected by two main factors, namely the obtained yield, which correlates with the proportion of residual cellulosic fibers, and various effects of mixing, which apparently caused changes in the physical size and structure of the suspended solids. Baffles installed in the mixing tank (Figure 8.2) did not have a positive impact on the filtration performance.

Figure 9.3 illustrates the complexity of the effect of mixing on the filtration properties of enzymatically hydrolyzed suspensions (Paper II), showing all the results obtained by using constant enzyme dosage and solid concentrations of 8 - 12 w-%. As Figure 9.3 shows, the cakes were compressible, because α_{av} increased with the filtration pressure. The compressibility indices n ranged from 0.7 to 1.1, which generally indicates either moderate or high compressibility of the cakes.

The main point in Figure 9.3 is, however, the influence of mixing on the filtration properties. Mixing did not only affect the final yield of hydrolysis, which had a positive correlation with α_{av}, but it also damaged the solids, which increased α_{av}. The type of impeller has a strong effect on the way how the suspension behaves in the filter: when the Rushton turbine was used at high mixing rates, the yield was low, and consequently, the average specific cake resistance was also low. The filtration behavior in the case of an anchor was quite the opposite, i.e. the slow mixing rate was optimal for obtaining high yields and a high mixing rate reduced the yield, but increased α_{av}. The effect of particle type, i.e. presence of inorganic fines, is unclear and could be investigated in future studies.

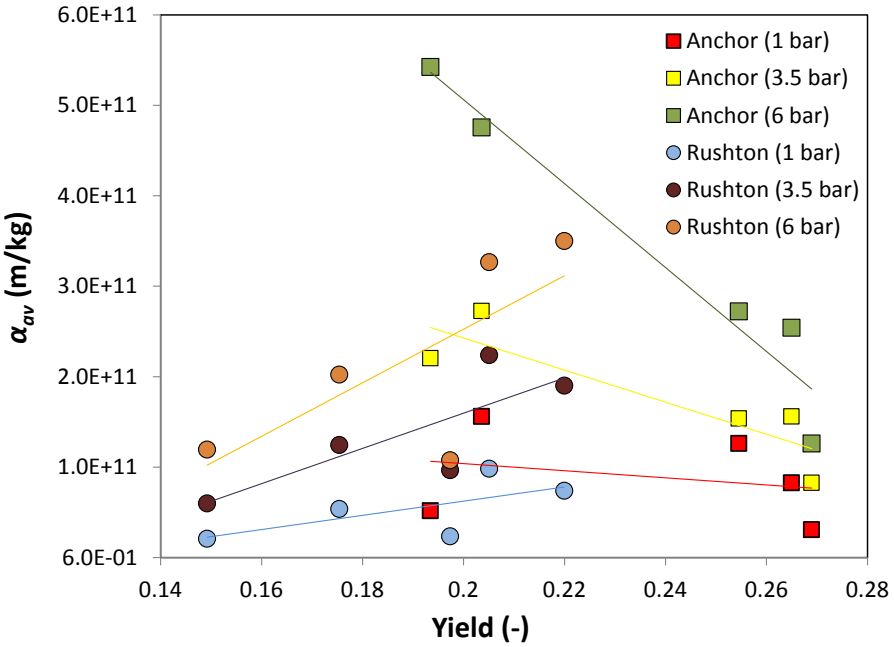

Figure 9.3 The average specific cake resistances of the filter cakes (Δp = 1, 3.5 and 6 bar) for enzymatically hydrolyzed suspensions mixed with anchor and Rushton turbine during the hydrolysis time of 72 h to obtain different glucose yields.

On the basis of the results of the mixing study (Paper II), it was still difficult to evaluate the role of mixing separately from the influence of the hydrolysis yield. It was also clear that the obtained glucose yields were too low regarding the process economy in industrial applications. Additionally, data from particle size measurements were required to understand the hydrolysis and filtration stages better. The study was, therefore, continued in Paper IV, where the type of impeller, the mixing rate and also the mixer shear pressure were related to the average specific cake resistance. In order to produce a slurry with reduced viscosity to make the slurry feed into the filter easier, and to obtain a more realistic glucose yield, an increased enzyme dosage was used. That is why the results of Paper IV may not be directly comparable with Paper II.

Figure 9.4 illustrates the influence of mixing rate and time on α_{av} (Paper IV). It can be clearly seen that both the mixing rate and time have a significant correlation with α_{av}. The Rushton turbine had a more pronounced impact on the filtration resistance, which was most probably due to its sharp edges, while the impact caused by the less sharp-edged propeller was somewhat weaker. In comparison with the situation without mixing, the maximum α_{av} obtained with the Rushton turbine was increased by almost a factor of 10, which corresponds to an approximately 3-fold filter area requirement.

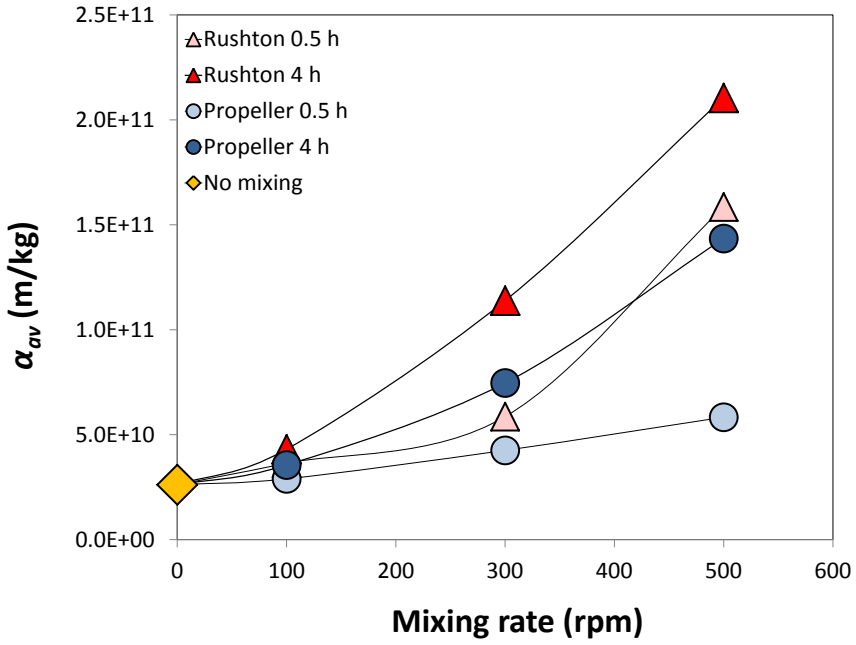

Figure 9.4 The average specific cake resistances (Δp = 1 bar) obtained after mixing with Rushton turbine and propeller for 0, 0.5 and 4.0 h using different mixing rates.

From the rheology point of view, however, the results presented above in Figure 9.4 are not directly comparable, especially with respect to the mixer types, which generate different flow conditions. In order to evaluate the results further, basic equations of mixing were applied to calculate the shear pressure (i.e. mixer head) for both impellers after 0.5 and 4.0 hours of mixing (Figure 9.5). The

final equation used for this calculation was $\Delta p_{shear} = P/Q$, where P is the mixing power (W) and Q is the volumetric flow rate caused by the mixer (m^3/s).

The results shown in Figure 9.5 (A) and (B) reveal that the mixer shear pressure can be successfully related to α_{av} by using a linear fit. The correlation coefficients for the fit of Rushton turbine, presented in Figure 9.5 (A), are slightly lower than that of the propeller (Figure 9.5 (A)). As could be expected based on the geometry of the mixer elements, the shear pressures caused by the Rushton turbine were much higher than those generated by the propeller. When the mixing rate was 100 rpm, the Rushton turbine had 52 % higher shear pressure. The difference increased to 112 % when a mixing rate of 500 rpm was used. From another point of view, the mixer shear pressure for the Rushton turbine and propeller were increased over this range by a factor of 25 and 18, respectively. The porosities of the filter cakes varied between 0.63 and 0.82. Increasing the mixing rate was generally shown to result in a reduction of cake porosity. Mixing was not observed to have an impact on the compressibility index, which was 0.84 for the mixed suspension and 0.85 for the non-mixed suspension.

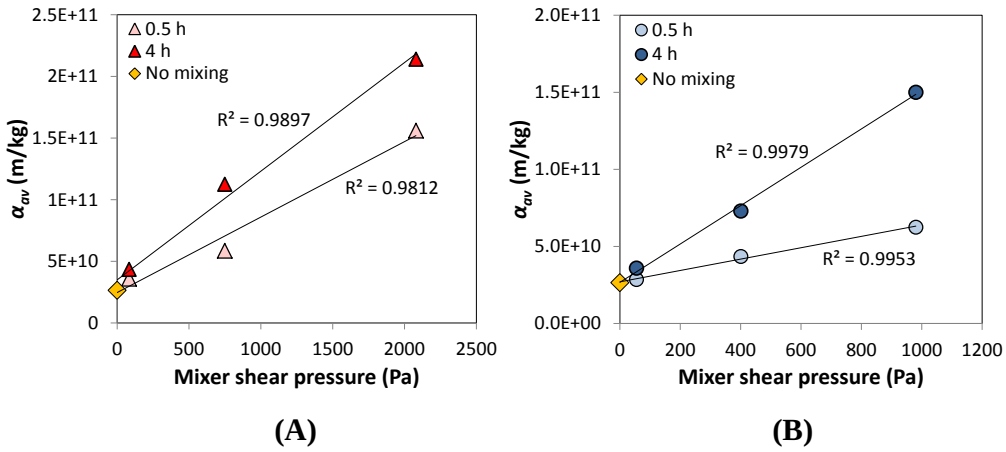

Figure 9.5 The dependence of average specific cake resistance (Δp = 1 bar) on the mixer shear pressure when hydrolyzed biomass suspension was mixed with Rushton turbine (A) and propeller (B) for 0, 0.5 and 4.0 h.

Figure 9.6 deals with the improvement of pressure filtration by the use of different filter aids, discussed in detail in Paper III. Precoat results are summarized in Figure 9.6 (A) and the body feed results in Figure 9.6 (B). It is clear that the body feed mode is more favorable with respect to the reduction of α_{av}. When using the precoat mode, the effect of filter aid dosage was of less importance, compared to the body feed experiments. The use of filter aid concentration of 20 w-%, relative to dry solids in the suspension, was beneficial almost without exception, irrespective of the method of filter aid addition. The better performance of body feed filtration can be associated with the improved cake structure: the filter aids most likely promoted a more open cake structure with higher permeability, reducing also the cake compressibility in some cases.

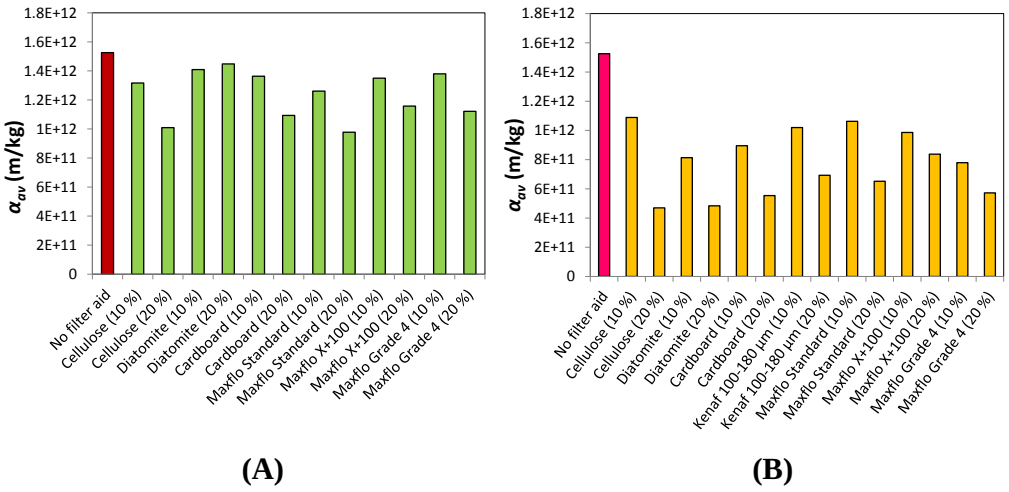

Figure 9.6 Reduction of the average specific cake resistance with filter aids. (A): Precoat filtration. (B): Body feed filtration.

Precoat filtration had an influence almost exclusively on the bottom layer of the cake, which may have prevented formation of the so called skin layer, recognized among others by Johansson (2005), at the cake-medium interface, while the inner structure of the cake remained unchanged. It can be roughly said that the precoat layer served as an additional layer of filter medium. Seen from this perspective, it is not a wonder that the quality of the precoat filtrates was excellent, whereas the body feed filtrates contained some suspended solids, which caused turbidity in these filtrates.

The importance of the selection of the filter medium is also discussed in Paper III. When good filtrate quality is the priority, it is naturally better to use a tight filter medium, which correspondingly may have significantly higher medium resistance.

Figure 9.7 presents the results of Paper III from a process engineer's point of view. The obtained filtration capacity ranged approximately from 14 to 24 $kg_{dry\ solids}m^{-2}h^{-1}$. It can be observed that precoat filtration was unable to increase the filtration capacity in many cases, but the moisture content of the filter cakes was reduced from the original in all precoat and body feed experiments. When filter aids are not used, the cake moisture content typically increases with the filtration capacity. The addition of filter aids, however, facilitates both the capacity and the cake dryness at the same time. This is why the cake moisture in Figure 9.7 can be seen to decrease as the capacity is improved.

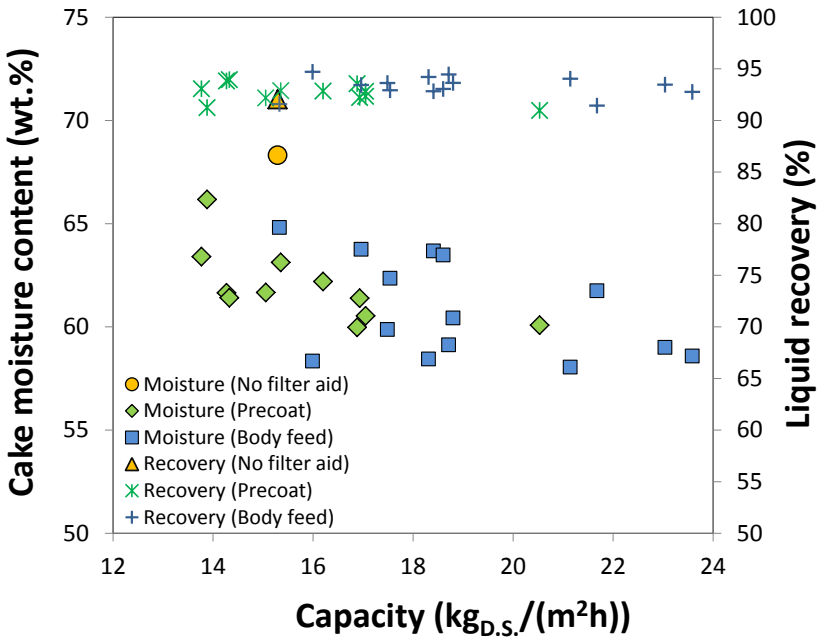

Figure 9.7 The moisture contents of filter cakes and the liquid recovery values obtained at different filtration capacities.

Liquid recovery, i.e. the proportion of liquid in the suspension that is recovered within the filtrate, was at a good level (92 %) even without the use of filter aids (Figure 9.7). There was only a very small difference between the average liquid recoveries obtained by using the precoat (92.6 %) and body feed (93.2 %) modes. In other words, the loss of monosaccharides with the cake was approximately 7 % of the total mass produced by enzymatic hydrolysis. Displacement washing of cakes in the filter unit would help to reduce this loss, but would consequently have a negative effect on the production capacity.

Recovery of the energy content of filter aids (Paper III) is also easier if the filter aids are composed of biomass materials, which are suitable for energy production by combustion. These organic filter aids can also be circulated back to enzymatic hydrolysis. When inorganic filter aids are used, the situation may be more complicated because of waste management issues. Reuse of these filter aids is possible, but requires separation from the biomass cake.

There are also many other factors which may have a significant influence on the filtration performance, but they were limited out of the scope of this study. Further studies on the topic could investigate for instance the role of yeast cells in the suspension after hydrolysis and fermentation, the effect of the quantity of inorganic fines on hydrolysis and filtration, and the influence of the microbiological state of the suspension on the separation characteristics.

10 CONCLUSIONS

Enzymatic hydrolysis and pressure filtration of obtained hydrolysates have been studied in this thesis. The results show that the process conditions in the hydrolysis and filtration stages may have significant and even surprisingly strong influences on the filtration characteristics.

The average specific cake resistance did not clearly depend on the initial particle size of the raw material in this case, probably because of differences in the composition of the size fractions, but it was observed to increase with the yield of hydrolysis. On the other hand, cake porosity decreased as a result of improved yield. Several inconsistencies in the filtration results of Paper I were the

main motivator for Papers II and IV, which finally helped to understand the reasons for the inconsistencies. It is clear that the main uncertainty in the results was caused by the hydrolysis stage, especially in Paper I, where most results were obtained without size reduction of the raw material. In the case of Papers II-V, the material was milled prior to hydrolysis. Moreover, the filtration experiments in Papers III and IV were carried out using the same suspension, when the variations in the quality of the hydrolysis batches did not affect the filtration properties.

Mixing during enzymatic hydrolysis affected the final yield of hydrolysis, which in turn had a positive correlation with the average specific cake resistance. However, the shear caused by very intensive mixing was observed to have a negative influence on the sugar yields. High shear pressure was also the main contributor to the increased average specific cake resistance, even though the fiber size was not significantly reduced. An analysis of the fiber structure, most importantly the fibrillation resulting from the mixer shear, could help understand the effect of mixing on the filtration properties better.

The effect of enzymatic hydrolysis on the fiber dimensions was relatively small. Most of the reduction of the fiber length occurred during the first hours of enzymatic hydrolysis. The changes were almost equal regardless of the presence or absence of mixing. These results can be regarded as well proven, because two different size analysis techniques (laser diffraction and image analysis) were employed and, additionally, visual evaluation of the solids was performed with scanning electron microscopy.

The compressibility of the filter cakes did not clearly depend on the yield of hydrolysis or mixing. Because the compressibility indices were determined based on experiments performed at only three filtration pressures, the uncertainty related to these results was high.

It is possible to obtain significant improvement of filterability by the use of filter aids. On the basis of the results of this study, the average specific cake resistance can be best reduced and the filtration capacity increased by the use of the body feed mode, i.e., mixing the filter aid with the biomass

suspension prior to filtration. Precoat filtration was shown to be optimal for retaining fine suspended solids, which caused turbidity of the filtrate in the case of body feed.

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Solid–liquid separation of hydrolysates obtained from enzymatic hydrolysis of cardboard waste

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ABSTRACT

Enzymatic hydrolysis provides an environmentally friendly pathway from cellulosic biomass to sugars that can be fermented to produce ethanol. The hydrolysis step has a marked influence on the overall efficiency of the bioethanol process. Since the cellulosic substrate cannot be completely cleaved to sugars, there always remains a solid residue suspended in the hydrolysate. Solid–liquid separation performed at this stage can improve subsequent membrane filtration (for recycling the enzymes, or concentrating the sugars prior to fermentation, for example). The fermentation process can also be controlled more readily if the cellulosic residue is removed after the hydrolysis. For the present study, shredded cardboard was chosen as the cellulosic raw material. Non-degraded solids were separated from enzymatically produced hydrolysates using a laboratory-scale pressure filter. The influence of process-related variables, such as enzyme dosage, the level of pre-milling, and the initial substrate concentration were investigated by determining the concentrations of glucose and xylose during the hydrolysis. Filtration tests were carried out at three different pressures immediately after each batch of hydrolysis was completed. The results show that some of the chosen variables have an unexpected effect on the filtration characteristics. The degree of conversion of the cellulosic substrate to glucose and xylose was also affected by the investigated process variables.

1. Introduction

Bioethanol produced from lignocellulosic raw materials has the potential to be one of the most important solutions for reducing the dependence upon fossil fuels in the near future. In spite of numerous technical challenges, industrial-scale production of bioethanol from lignocellulosic biomasses is becoming an option worth consideration. Cellulose is a linear biopolymer consisting of glucose units linked to each other by β-(1-4) linkages (Krässing, 1993; Balat et al., 2008). Other significant components of cellulosic biomasses include hemicelluloses and lignin (Balat et al., 2008). Extractives (for example tar and resin), proteins, organic acids and pectins (Blasi et al., 1999; Sluiter et al., 2010) are present in smaller amounts. Cellulose, hemicellulose and lignin contents of typical biomasses are 40–60, 20–40, and 10–25 wt%, respectively (Lin et al., 2010). The main reason for the attempts to use lignocellulosic biomasses to replace fossil fuels is that they are found very abundantly in nature: lignocellulose represents approximately 90% of the plant biomass on earth (Lin and Tanaka, 2006). In addition to their abundance and renewability, cellulosic materials are interesting as fuel replacements, because bioethanol can be used in existing gasoline motors (Wingren et al., 2008).

Enzymatic hydrolysis is an environmentally benign process step that can be used for cleaving the cellulose polymers to glucose, because currently available microorganisms cannot effectively convert cellulose directly to ethanol. The recalcitrance of cellulosic materials to enzymatic hydrolysis is generally regarded as the most significant obstacle to the viable conversion of cellulose to ethanol. A large variety of pretreatment methods, including, for example, steam explosion, ammonia fiber explosion and treatment with pressurized hot water at 150–200 °C, have been developed to improve the efficiency of hydrolysis (Virkajärvi et al., 2009; Kumar et al., 2009). Ease of saccharification depends on the structure of the raw material. Since cardboard and paper have already been pulped at least once, pretreatment of such feedstocks prior to hydrolysis is not necessary, and could result in improved yields (Yáñez et al., 2004).

In this experimental study, the solid–liquid separation characteristics of hydrolysates produced by enzymatic hydrolysis from cardboard waste are discussed. It is important to know the filtration behavior of the solid residue that remains after converting a large proportion of the polymeric raw material to monomeric sugars. Significant changes in the properties of the solid take place as the enzymatic hydrolysis proceeds, for example reduction of

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Table 1
Ash content of the milled raw material as the average of two determinations: all fractions (0–1.5 mm), the coarsest fraction (1–1.4 mm) and the finest fraction (<0.125 mm).

Particle size [mm]	Ash content [wt%]	Average ash content [wt%]	Relative ash content [wt%]
1–1.4	10.09 9.81	10.0	89.4
0–1.5	11.05 11.22	11.1	100
<0.125	14.19 14.62	14.4	129.4

the fiber dimensions (Clarke et al., 2011), an increase in the crystallinity of cellulose (Mansfield et al., 1999) and an increase in the proportion of lignin in the solid fraction. The residual solids, if not removed after the hydrolysis, can detrimentally affect the downstream processes, such as fermentation and product separation (Burke et al., 2011). The filter cake formed from the solid residue can be very compressible, which makes the separation more difficult. Consequently, larger filtration areas and a longer filtration times are required, which increase the separation costs. Scale-up of the filtration process is also made more complicated because of the high compressibility of the filter cakes. Taking into consideration that all separation operations together can account for more than half of the capital and operating cost of a bioethanol process (Kochergin and Miller, 2011), too little effort has been made to thoroughly understand the influence of hydrolysis conditions on the solid–liquid separation processes.

2. Materials and methods

2.1. Raw material and preliminary analyses

The raw material used in the experiments was air dry cellulosic waste consisting mainly of shredded cardboard. Besides cellulose, hemicelluloses and lignin, pieces of plastic, metals, as well as inorganic minerals were found in the raw material. The cellulose content of the raw material was 63 wt%, determined according to the method of Black (1951). Compared to the air dry material at 20 °C, the moisture content was 6.3 wt%, after drying to constant weight at 100 °C. The proportion of lignin was 11.5 wt%, measured using a liquid chromatographic method with the following specifications: HP Agilent 1050 device with Phenomenex Luna 3u C18(2) 100 mm × 2.0 mm column, 20 mM ammonium hydroxide (50 vol.%) and methanol (50 vol.%) as eluents, pH 9, injection volume 5 μL, detection by UV/vis at 254 nm and electrospray ionization/mass spectrometer (ESI–MS). The hemicellulose content was not measured in this study, but it was estimated to be about 14 wt%, assuming that no extractives were present. Because of the presence of inorganic matter, such as calcium carbonate that is used as a filler in paper and paperboard, the ash content was quite high, 11.1 wt%. The ash content was determined according to ISO 1762:2001 standard.

The cellulosic raw material was divided into two classes; (1) original untreated cardboard waste and (2) milled cardboard waste. Milling was carried out by using a specially designed machine owned by the University of Oulu. The maximum size of the particles exiting the machine was 1.5 mm. As a result of milling, the particle shape was also changed from a more-or-less crooked flake to quite spherical. Five sieves (125–1400 μm) were then utilized for dividing the milled material into six size fractions, the proportions of which are shown in Fig. 1. The ash content (Table 1) in the milled raw material and in two of the classified size fractions (<125 μm and 1000–1400 μm) were determined according to ISO 1762:2001 standard. Most experiments were performed using the non-milled

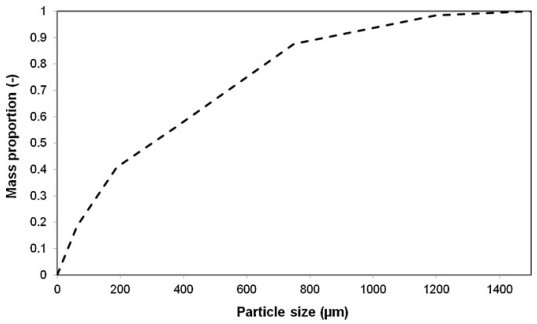

Fig. 1. Approximate particle size distribution of the milled raw material.

raw material, referred to as "original" in this paper. The original size of the pieces was about 10 mm × 20 mm × 5 mm, but it is difficult to accurately describe the particle size or shape of such a heterogeneous material. The effect of the material heterogeneity on the experiments was minimized by mixing the cardboard waste manually and by taking several sub-samples from different locations. The sub-samples were then combined, mixed again and weighed for the hydrolysis experiments.

2.2. Experimental plan

The total number of hydrolysis batches produced was 14. The experimental variables included (1) enzyme dosage, (2) particle size of the raw material, and (3) initial solid concentration of the suspension. A laboratory scale, Nutsche type, pressure filter was used for studying the influence of the variables on the filterability of the hydrolysates. Because of the filter design, in particular the small diameter of the feed pipe, the maximum solid concentration used in this study was restricted to 10%. The experimental plan is presented in Table 2.

2.3. Experimental procedure

A jacketed glass reactor with a volume of 3 dm^3 was used as the hydrolysis tank. The reactor temperature was controlled by a Lauda RK8 KP thermostat, capable of both heating and cooling. The solid–liquid suspensions were prepared from cold tap water and the cellulosic raw material. A sufficient amount of sulfuric acid (J.T. Baker, 95–97%, Netherlands) at a concentration of 20 wt% was added to adjust the pH to 5.0. The mass of pure H_2SO_4 required for the pH adjustment was approximately 8% of the mass of the raw material. It is possible that dissolution of $CaCO_3$ (a filler agent in the cardboard) and formation of $CaSO_4$ have occurred as a result of pH adjustment. Presence of fine $CaSO_4$ may have had an influence on the filtration stage as well. The hydrolysis batch was mixed with a pitched-blade turbine (mixer diameter/tank diameter = 1/2) at a constant rate of 200 rpm that corresponded to a tip speed of 0.84 m/s.

The duration of each hydrolysis was 72 h. To start the enzymatic hydrolysis, a hemicellulase preparation (Novozymes Cellic HTec) was added into the pH-adjusted suspension at an initial temperature of 40 °C. Immediately after adding the hemicellulases, the suspension was heated to 71 °C and kept at that temperature for 10 min in order to improve activation. After cooling down to 46 °C, a cellulase preparation (Novozymes Cellic CTec) was added. Since the activity of this kind of cellulase preparation can be measured in numerous ways and since single activities do not satisfactorily describe the performance of the product, cellulase activities were

Table 2
The experimental plan.

Experiment	Enzyme dosage			Solid concentration [wt%]	Particle size [μm]
	C-Tec [mL/kg cellulose]	C-Tec [FPU/g cellulose]	H-Tec [mL/kg substrate]		
1	320	38.4	64	10	Original
2	140	16.8	28	10	Original
3	140	16.8	28	10	0–125
4	140	16.8	28	10	125–250
5	140	16.8	28	10	250–500
6	140	16.8	28	10	500–1000
7	140	16.8	28	10	1000–1400
8	140	16.8	28	10	0–1400
9	140	16.8	28	5	Original
10	140	16.8	28	7.5	Original
11	140	16.8	28	10	Original
12	320	38.4	8	10	Original
13	40	4.8	64	10	Original
14	40	4.8	8	10	Original

not measured in this study. According to Alvira et al. (2011), the activity of Cellic CTec is approximately 120 FPU/mL.

The filtration experiments were conducted using a laboratory scale Nutsche pressure filter. Nitrogen was used to pressurize the filter after filling the filter chamber with the hydrolyzed suspension. The filter was kept at the required temperature with a Lauda RK8 KP thermostat, circulating water through the jacket of the filter. The filter medium, of T1000 type, supplied by Pall Corporation (Bad Kreuznach, Germany), was a disc of cellulose with an effective diameter of 49 mm, when held in its sealing. The thickness of the filter medium was approximately 3.6 mm. Assuming that the surface of the filter medium was smooth, the filtration area, therefore, was 18.9 cm^2. The cut-off particle size of the cellulosic filter medium was 24 μm. Thanks to the properties of the filter medium and the filter cake, no suspended particles were observed in the filtrates. For each test, a fresh disc of filter medium was first wetted with water and installed on the bottom grid of the filter. The mass of feed slurry was 200 g in all experiments. The applied filtration pressures were 1.0, 3.5, and 6.0 bar.

2.4. Composition of the hydrolysates

Seven samples were collected from the hydrolysis tank during each test run. The concentrations of glucose and xylose in the produced hydrolysates were determined by high performance liquid chromatography (HPLC, HP Agilent 1100). The column, a Varian Metacarb 87H, was kept at 60 °C and 0.005 M sulfuric acid was used as the eluent. Each run was performed at least twice. The injection volume was 10 μL and a precolumn was connected to the feed line to protect the column from solid particles. Prior to the analyses, the final samples (1 mL) were prepared by filtering through a syringe filter with a nominal pore size of 0.2 μm. The concentration of total dissolved solids in each sample of solid-free hydrolysate was determined by drying to constant weight in a heating chamber at 105 °C.

2.5. Calculations

The yield of glucose in the enzymatic conversion, Y, was calculated as:

$$Y = \frac{c_{glc}}{c_{cell}} \quad (1)$$

where c_{glc} (g/L) is the glucose concentration measured by HPLC and c_{cell} (g/L) is the initial cellulose concentration.

The conventional theory of constant pressure filtration was applied for the calculations. For calculating the specific cake resistances and cake compressibility indices, the filtration data were modified by plotting the filtration time per volume of filtrate (t/V) against the volume of filtrate (V). The slopes of the lines were then used to calculate the specific cake resistances. An example of this graphical presentation is shown in Fig. 2, where the slopes are determined for the experiments performed using different enzyme dosages.

Average specific resistances α_{av} (m/kg) of the filter cakes were calculated by Eq. (2):

$$\alpha_{av} = \frac{2aA^2\Delta p}{\mu c} \quad (2)$$

where a (s/m^6) is the slope obtained from, for example, Fig. 2, A (m^2) is the filtration area, Δp (Pa) is the applied pressure, μ (Pa s) is the dynamic viscosity of the liquid, and c ($kg_{solids}/m^3_{filtrate}$) is the filtration concentration – the mass of solid material in the filter cake per unit volume of filtrate collected.

Resistances R_c (1/m) of the filter cakes were calculated as

$$R_c = w\alpha_{av} \quad (3)$$

where w (kg/m^2) is the mass of solids deposited per unit area.

Resistances of the filter media R_m (1/m) were estimated using Eq. (4):

$$R_m = \frac{bA\Delta p}{\mu} \quad (4)$$

where b (s/m^3) is the interception point of the line and the y-axis as shown in Fig. 2.

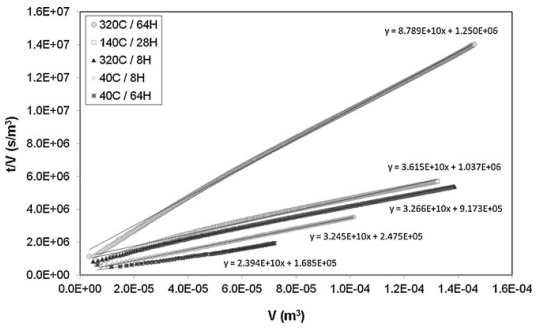

Fig. 2. Determination of slopes for calculating the average resistances of the filter cakes. Case: enzyme dosage as the variable.

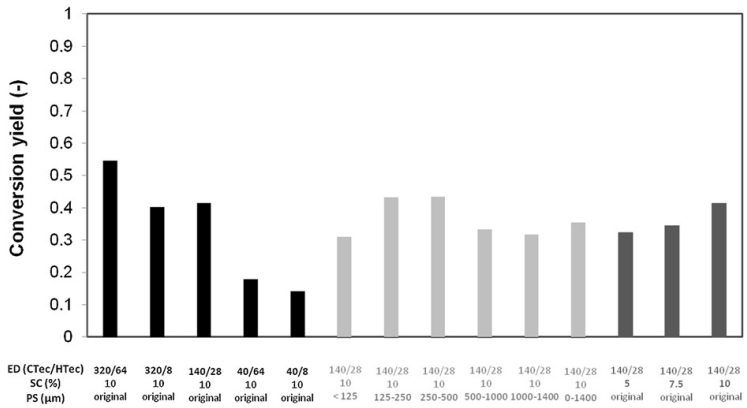

Fig. 3. Final conversion yields of the raw material to glucose at different enzyme dosages, particle sizes, and solid concentrations of the suspension. The unit of ED is $mL_{hemicellulase}/kg_{raw\ material}$ for the hemicellulase preparation and $mL_{cellulase}/kg_{cellulose}$ for the cellulase preparation.

The cake compressibility index, n, was determined according to a simple mathematical fit (Eq. (4)) presented, for example, by Wakeman and Tarleton (1999).

$$\alpha = \alpha_0 \Delta p^n \tag{5}$$

where α_0 is the specific cake resistance at unit applied pressure.

Calculations related to pressure filtration are described in more detail by Svarovsky (1981).

The average porosity ε_{av} of a filter cake was calculated based on dimensions of the cake:

$$\varepsilon_{av} = \frac{V_{pores}}{V_{cake}} \tag{6}$$

where V_{pores} is the total volume of pores in the cake with a volume V_{cake}. The pore volume was obtained by drying the cake to constant weight in a heating chamber. During the drying, water was evaporated and the evaporated volume was calculated based on the loss of weight, using a water density of 1000 kg/m^3. Dissolved solids were omitted in the calculations due to their small influence on the pore volume.

3. Results and discussion

3.1. Conversion of cellulose to glucose

Different proportions of the original raw material were converted to glucose during the hydrolysis stage. All the variables, including enzyme dosage (ED), particle size of the raw material (PS), and solid concentration (SC) of the suspension, were observed to have an influence on the conversion yield, Y, as summarized in Fig. 3. The conversion yields varied from very poor (14%) to satisfactory (55%).

Glucose concentrations in the liquid phase of the hydrolyzed suspensions, determined after 1, 3, 6, 15, 24, 48, and 72 h of enzymatic hydrolysis, are presented in Figs. 4–6.

Fig. 4 shows the effect of enzyme dosage on glucose concentration in the hydrolysate. Generally, it can be concluded that the more the enzyme is added, the greater the cellulose component of the raw material that is converted to glucose. There is a large difference between the glucose concentrations obtained at the highest enzyme dosage (320 $mL_{cellulase}/kg_{cellulose}$ and 64 $mL_{hemicellulase}/kg_{raw\ material}$) and the lowest dosage (40 $mL_{cellulase}/kg_{cellulose}$ and 8 $mL_{hemicellulase}/kg_{raw\ material}$). The volume of enzyme spent, per gram of glucose formed is, however, increasing sharply as the conversion yield is improved. An eightfold increase in the dose of both enzymes was required to increase the final glucose concentration from 10 g/L to 38 g/L.

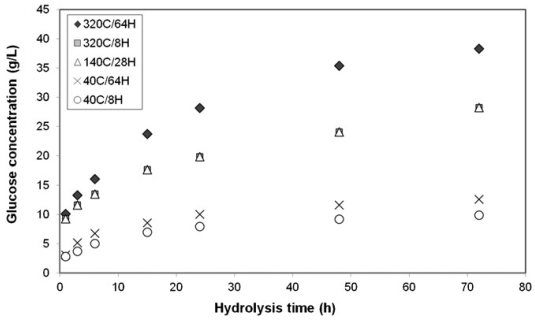

Fig. 4. Concentration of glucose in the liquid phase determined from samples taken 1, 3, 6, 15, 24, 48, and 72 h after cellulase addition. N.B. 320/64 means that there was 320 mL of cellulase/kg of cellulose and 64 mL of hemicellulase/kg of raw material. SC = 10%, PS = original.

The concentrations of dissolved solids in the filtrates ranged from 1.9 to 5.3 wt%. On average, the combined mass proportion of glucose and xylose in the dissolved solids was 81%. The proportion

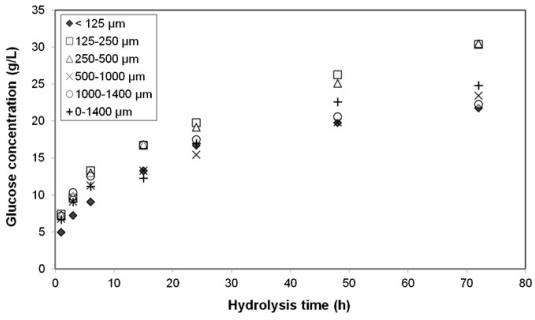

Fig. 5. Concentration of glucose in the liquid phase, determined from samples taken after 1, 3, 6, 15, 24, 48, and 72 h after cellulase addition. Effect of particle size of the raw material. SC = 10%, ED = 140 mL of cellulase/kg cellulose and 28 mL of hemicellulase/kg raw material.

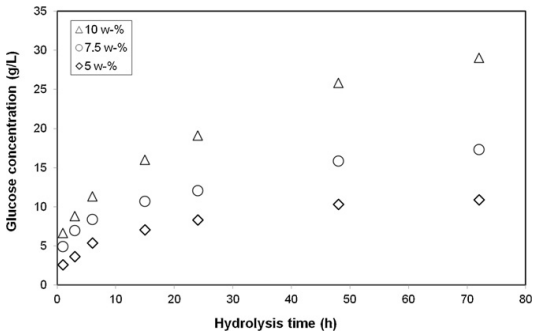

Fig. 6. Concentration of glucose in the liquid phase determined from samples taken after 1, 3, 6, 15, 24, 48, and 72 h after cellulase addition. PS = original, ED = 140 mL of cellulase/kg cellulose and 28 mL of hemicellulase/kg raw material.

of glucose and xylose was highest (93%) at the highest conversion yield (0.55).

The particle size of the raw material also had a considerable impact on glucose formation. As shown in Fig. 5, the poorest hydrolysis yield was obtained from the fraction composed of the smallest particles (under 125 μm). The fractions containing particles of 125–500 μm yielded the highest hydrolysate glucose concentration. Reduction of the particle size to submicron scale has been shown, by other workers, to reduce the cellulose crystallinity while simultaneously increasing the specific surface area. Consequently, the yield of glucose has been significantly increased (Yeh et al., 2010). The unexpected results found in this study can perhaps be explained by different cellulose content of the fractions, or by the presence of some compounds capable of disturbing the hydrolysis reaction. It is probable that the content of inorganics, such as carbonates, is highest in the finest fraction, which could explain the inadequate conversion of that fraction.

The influence of solid concentration of the suspension on the glucose formation during the hydrolysis is presented in Fig. 6. The results were obtained by applying three different solid concentrations, 5, 7.5 and 10 wt%, while keeping the other conditions constant. Glucose concentrations obtained under the above-mentioned conditions in 72 h were 10, 17, and 29 g/L, respectively.

Unlike in a typical enzymatic hydrolysis of cellulose, the conversion yield (Fig. 3) was observed to be lower as a result of lower solid concentrations. It can be seen in Fig. 6 that the conversion rate in experiments performed at a SC of 5 and 7.5% starts to slow down dramatically after 6 h of hydrolysis. One reason for that could be found in the changing mixing conditions of the suspension. Since the rotation speed of the impeller was constant, 200 rpm, the localized velocities throughout the suspension was higher at low solid concentrations.

Excessively intense mixing is generally known to decrease the conversion yield through partial deactivation of enzymes (Ingesson et al., 2001). Therefore, the mixers and rotation speeds for different suspensions should always be chosen with care. However, the effect of mixing type and intensity was not investigated in the present study.

3.2. Conversion of xylan to xylose

Xylose is the most common pentose sugar derived from the hemicellulose present in most hardwood species (Balat et al., 2008). The high xylose concentration in the hydrolysates indicated that hardwood species such as birch had been used as one component of the cardboard that was the raw material for the experiments.

Significant concentrations of xylose were detected in the hydrolysates. The relative concentrations of xylose in the experiments were fairly similar to those of glucose (Figs. 3–6). Xylose concentrations amounted to 29–46% of the glucose concentrations determined in the same samples, which implies that it could be economically interesting to utilize xylose in the subsequent ethanol fermentation as well. There are still, however, technical difficulties with the co-fermentation of pentose and hexose sugars. This is why pentose sugars, including xylose, are often removed in the pretreatment stage of the cellulosic raw material.

3.3. Filtration of hydrolysates

The filtration experiments were carried out using a laboratory scale Nutsche pressure filter at 1, 3.5 and 6 bar. The filtration temperature was 20 °C unless otherwise mentioned.

The filterability of the hydrolyzed suspension was markedly better when the enzyme dosage was low, i.e. when a smaller proportion of cellulose had been converted to glucose. This is because a high degree of hydrolysis will shorten the cellulosic fibers (Clarke et al., 2011), thus reducing the characteristic particle size of the solid. The other hydrolysis products, such as sugar oligomers and cellobiose (as well as liberated lignin) can also have a negative influence on the filtrate flow rate. During a filtration experiment, the fine fibers and lignin may be deposited in the filter medium, especially during the initial period when the cake is not yet formed. This means that if the same filter medium is used several times, the accumulated material must be removed at certain intervals. Spray washing at a high pressure is commonly applied in industrial filtration.

The results reveal interesting things about premilling of the raw material. The finest fraction (that passed through the 125 μm sieve) was easiest to filter and had the lowest filter cake resistance. The premilled raw material comprising all the fractions (0–1.5 mm) exhibited the second lowest specific filter cake resistance. The filterability of the suspensions seems to correlate quite well with the conversion yield of the fractions. The most probable explanation for that is the different composition of the fractions. If the composition of the fractions was identical, smaller particle size would result in an improved conversion yield, which should generally cause difficulties in the filtration stage. In this case, however, the finest fraction is likely to contain relatively large amounts of inorganic fillers like $CaCO_3$, and possibly contain a larger amount of metals and plastic materials as well. Another reason for the good filterability of the finest fraction could be the particle shape.

Determination of ash content (ISO 1762:2001, 525 °C) was performed for three fractions to confirm the assumption regarding the presence of inorganic materials in the fractions. The size fractions were 1–1.4 mm (coarse), 0–1.5 mm (all fractions), and <0.125 mm (fine). The results (averages of duplicates) show that the ash content in the finest fraction was almost 30% higher than that in the 0–1.5 mm material (see Fig. 1 for the size distribution). Correspondingly, the ash content in the coarse sample was 11% lower than that in the 0–1.5 mm material. These results may explain the relatively low specific cake resistance of the finest fraction and also some of the differences in the glucose yield.

The solid content of the hydrolyzed suspension had a marked effect on the filtration rate. While there was a slight difference between the filtration rates for 7.5 and 10% suspensions, the filtration rate for the 5% suspension was much higher. In addition to the higher liquid content of the 5% suspension, the relative quantity of non-converted cellulose in that suspension was largest as well. It is difficult to find a simple explanation for the poor filterability of the 7.5% hydrolysate, but the heterogeneity of the raw material combined with the influences of mixing might be the major reasons.

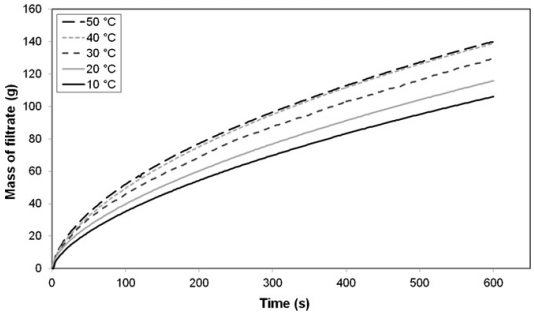

Fig. 7. Effect of filtration temperature on pressure filtration at $\Delta p = 3.5$ bar. ED = 140 mL of cellulase/kg cellulose and 28 mL of hemicellulase/kg raw material, PS = original, SC = 10%.

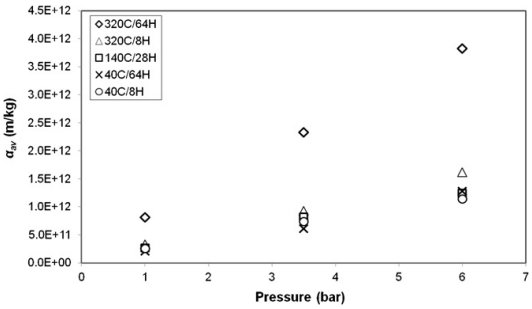

Fig. 9. Effect of enzyme dosage on average resistances of filter cakes at filtration pressures of 1, 3.5, and 6 bar. PS = original, SC = 10%.

One hydrolysis batch was produced to investigate how filtration temperature affects filterability. The applied filtration temperatures were 10, 20, 30, 40, and 50 °C and the filtration pressure was 3.5 bar. As expected based on viscosity, the filtration resistance was highest at 10 °C and lowest at 50 °C (Fig. 7). Filterability at 40 °C was almost as good as at 50 °C, because the structure of the filter cake started to change, i.e. the cake started to collapse at 50 °C. The cake thicknesses after filtration at 10, 40 and 50 °C were 27.4, 25.1 mm and 23.1 mm, respectively. The average porosity of the filter cake formed at 50 °C was therefore reduced in comparison with the cakes formed at lower filtration temperatures. It is apparent that the cake collapse plays an important role in the process as the temperature is increased, but it is unclear if the filterability could be much improved by heating the suspension closer to 100 °C. Moreover, heating can cause degradation of the fermentable sugars and would inevitably increase the energy consumption of the process.

The results presented in Fig. 7 do not differ from each other as much as could have been expected, based on charge in viscosity of the filtrate. Based on laboratory measurements done by using a capillary viscosimeter, the viscosity of solid-free hydrolysate is only slightly higher than that of water. Although the viscosity of water decreases by almost 60% as the temperature is increased from 10 to 50 °C, the observed improvement in the average flow rate of the filtrate is smaller, 33%. The main reason for this is the exceptionally high compressibility of the filter cakes at 50 °C.

3.4. Filter cake characteristics

The average resistances of the filter cakes obtained at filtration pressures of 1, 3.5, and 6 bar are presented in Figs. 9–11. Mainly because of the high compressibility of the hydrolyzed solid residue, the specific cake resistances were high, ranging from 1.2×10^{11} m/kg to 3.8×10^{12} m/kg. The fibrous structure of the cardboard waste and the filter cakes can be seen in Fig. 8. The difference in the color of the raw material and the solid residual is caused by lignin that is not significantly dissolved or degraded in the hydrolysis. Therefore, as cellulose is converted to glucose, the proportion of lignin in the solid fraction is increased. The physicohemical properties of lignin (adsorptivity on cellulose, hydrophobicity, low water permeability, etc.) cause high filtration resistances. Based on the experimental results, this applied in the present study as well. The challenging separation of lignin from hydrolysates has been earlier studied by Johansson (2005). As shown in Fig. 8B, there are still fibers that have not been converted to glucose. Pretreatment of the raw material would help to hydrolyze this part better.

Fig. 9 shows the average specific cake resistances, obtained at different enzyme dosages. The resistances of the filter cakes obtained at the highest enzyme dosage (320 $mL_{cellulase}/kg_{cellulose}$ and 64 $mL_{hemicellulase}/kg_{raw\ material}$) were clearly the highest. Unfortunately, the conversion yields were highest when filtration was most difficult. The specific cake resistances increased significantly during the hydrolysis; after the pH adjustment, at zero conversion, at 1 bar, the average specific cake resistance was 2×10^{10} m/kg.

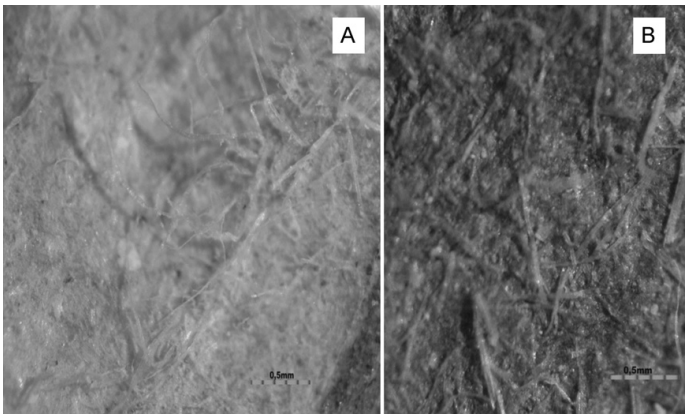

Fig. 8. Stereomicroscope photos (100×) of the raw material (A) and the filter cake of test 1 (B).

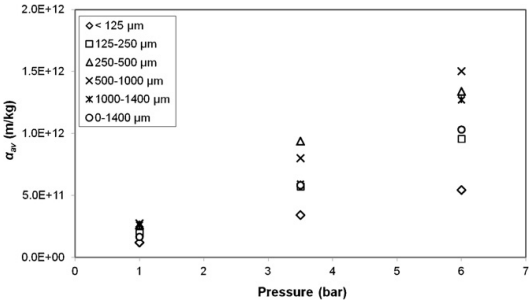

Fig. 10. Effect of particle size of the raw material on average resistances of filter cakes at filtration pressures of 1, 3.5, and 6 bar. ED = 140 mL of cellulase/kg cellulose and 28 mL of hemicellulase/kg raw material, SC = 10%.

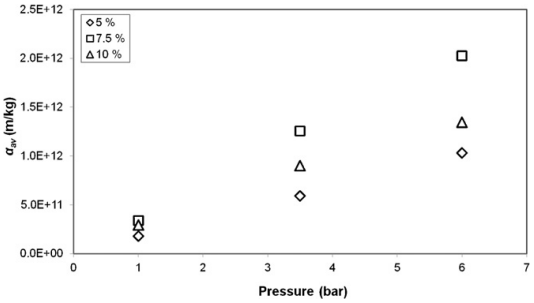

Fig. 11. Effect of solid concentration of the suspension on average resistances of filter cakes at filtration pressures of 1, 3.5, and 6 bar. ED = 140 mL of cellulase/kg cellulose and 28 mL of hemicellulase/kg raw material, PS = original.

The filterability of well-hydrolyzed suspensions could be perhaps improved, for instance, by using flocculants, such as commercial polyelectrolytes, or filter aids, such as sawdust. The overall sugar yields could be further increased by cake washing. In industrial filters, high filtration capacities and sugar yields are required, so the use of filter aids and cake washing should always be considered.

The medium resistances R_m were practically negligible in comparison with the cake resistances R_c calculated from Eq. (3). This is a typical case in cake filtration processes. A fresh piece of high-quality filter medium was used for each test, so the initial properties of the filter media were comparable between the tests. In the experimental determination of R_m, the approximated values of R_m increased with the filtration pressure, ranging from 2×10^{11} 1/m at 1 bar to 6×10^{11} 1/m at 6 bar. R_m also correlated with the yield, which implies that the fines may cause partial clogging of the filter medium. Lignin is likely to have an important role in this phenomenon.

The effect of the initial raw material particle size on the average specific resistance of the filter cake is shown in Fig. 10. The finest fraction containing particles passing through 125 μm sieve can be seen to produce the lowest specific cake resistance at all applied pressure differences. The differences in the filtration behavior are likely to be caused by the differences in the composition and characteristics of the size fractions.

Compared to the original, non-milled raw material (Fig. 9, 140C/28H), the specific cake resistances (Fig. 10) were generally lower. The specific cake resistances of the milled raw material (0–1400 μm) at filtration pressures of 1, 3.5, and 6 bar were 41, 29, and 17% lower, respectively, than those of the original material. For some reason, the conversion yield of the milled raw material was about 15% lower.

Fig. 11 shows the influence of solid concentration on the average resistance of the filter cakes. The highest specific cake resistances were found for the suspension containing 7.5% solids. Especially in experiments where the pressure difference was 3.5 and 6 bar, specific cake resistances for the 7.5% suspension were high. The compressibility index for the 7.5% suspension was over 1.0, which means (am Ende, 2010; Oja, 1996) that the filtered suspension can be regarded as highly compressible.

There was a large difference between the lowest and highest specific cake resistance: the lowest value, 1.8×10^{11} m/kg, was obtained for 5% suspension at 1 bar, whereas the highest value, 2.0×10^{12} m/kg, was obtained for 7.5% suspension at 6 bar. As discussed earlier, mixing conditions and the heterogeneity of the raw material are probable factors behind these peculiar results.

3.5. Porosity and compressibility of the filter cakes

The average porosity of the filter cakes, i.e. the volume fraction of pores in the cake, depended on the conversion yield as presented in Fig. 12. Generally, higher conversion yields correlated with reduced porosity of the filter cakes. Enzyme dosage had the largest impact on the conversion yield and thus on the average porosity. Consequently, the two experimental batches in which the enzyme dosages were the lowest, have the highest porosities (0.73–0.78) and are separately located on the left hand side of the chart in Fig. 12. The highest conversion yield (0.55) obtained at the highest enzyme dosage was probably the main reason for the lowest porosities (0.69–0.70) of these filter cakes.

The conversion yield did not have such a clear correlation with the compressibility index n. The measured compressibility indices (Table 3) varied from 0.82 to 1.03, indicating that the cakes were either compressible or highly compressible. While the suspension produced using the lowest enzyme dosage (Test 14) had the lowest compressibility index ($n = 0.82$), the suspension produced from the milled raw material (Test 8) was the most compressible, $n = 1.03$.

3.6. Repeatability of the experiments

Since each filtration test was carried out twice, it was possible to evaluate the repeatability of the experiments. The repeatability was evaluated based on the average volumetric flow rate of the filtrate during each test. Average flow rate of the parallel tests was calculated and the relative differences (%) from the average were used for repeatability evaluation. Generally, the differences were small, 1–2% from the mean flow rates.

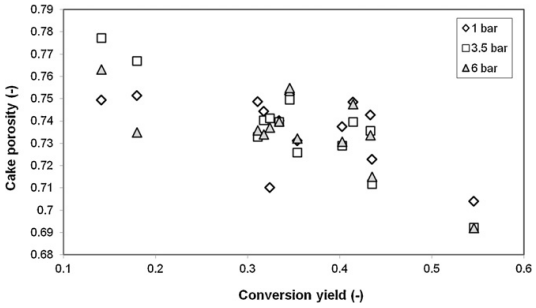

Fig. 12. Cake porosity versus conversion yield. Pressure difference = 1.0, 3.5, and 6.0 bar.

Table 3
Compressibility indices, *n*, obtained as a result of various hydrolysis conditions.

Experiment	Enzyme dosage			Solid concentration [wt%]	Particle size [μm]	*n*
	C-Tec [mL/kg cellulose]	C-Tec [FPU/g cellulose]	H-Tec [mL/kg substrate]			
1	320	38.4	64	10	Original	0.86
2	140	16.8	28	10	Original	0.85
3	140	16.8	28	10	<125	0.84
4	140	16.8	28	10	125–250	0.88
5	140	16.8	28	10	250–500	0.94
6	140	16.8	28	10	500–1000	0.94
7	140	16.8	28	10	1000–1400	0.84
8	140	16.8	28	10	0–1400	1.03
9	140	16.8	28	5	Original	0.97
10	140	16.8	28	7.5	Original	1.01
12	320	38.4	8	10	Original	0.87
13	40	4.8	64	10	Original	0.97
14	40	4.8	8	10	Original	0.82

4. Conclusions

The objective of this experimental study was to investigate how the solid–liquid separation of hydrolysates produced by enzymatic hydrolysis from cardboard waste is influenced by the enzyme dosage, premilling of the raw material, solid concentration, and temperature of filtration.

The degree of conversion of the cellulosic raw material to simple sugars (glucose and xylose) was influenced by a number of factors. The raw material was observed to be very heterogeneous, which may have caused uncertainty about the accuracy of the results. Enzyme dosage had the biggest impact on the conversion yield, but the economy of the process places limits on the use of enzymes in industrial applications. Quite surprisingly, premilling of the raw material did not increase the conversion yield and did not have a significant effect on the hydrolysis kinetics. Different amounts of sugars were produced from the separated size fractions of the premilled raw material, which implies that the material composition of the fractions was not exactly uniform. Conversion of cellulose to glucose was most efficient at the highest solid concentration of 10%. The typical concentration of xylose in the hydrolysates was approximately 30% of the corresponding glucose concentration. It is apparent that proper pretreatment of the raw material and operation at higher solid concentrations would be required to make the process economically feasible.

A low conversion yield, as a result of low enzyme dosage, corresponded with improved filterability of the hydrolysate. A high conversion yield, on the contrary, was the most important factor causing poor filterability. Unlike in mineral engineering applications, the smallest size fraction was, surprisingly, the easiest one to filter. The reason for that may be the composition of the fraction, i.e. a higher ash content and therefore a lower conversion yield and a relatively low compressibility of the cake.

Differences in particle shape and composition of the size fractions were not analyzed in this study, but these factors are likely to have a very important role in the filtration process. Filtration rate was higher at lower solid concentration of the suspension, possibly because of lower conversion yield. In industrial applications, however, the solid concentrations would need to be significantly higher than the concentrations used in this study. The average resistance of the filter cake was exceptionally high in case of the 7.5% suspension. Increasing the filtration temperature from 10 to 40 °C resulted in moderately improved filtration rates, but the filter cakes become more compressible. Filtration at 40 °C was almost as easy as at 50 °C, which indicates that the optimum filtration temperature, taking into account the economy and the filter performance, might be about 50 °C. Fortunately, the enzymatic hydrolysis is optimally performed at the same temperature. The structure of the filter cake can change dramatically, the cake can collapse at higher temperatures.

Average specific resistances of the filter cakes were high, 1.2×10^{11} m/kg to 3.8×10^{12} m/kg. High enzyme dosage was the main factor for increased specific cake resistances. Porosities of the cakes ranged from 0.69 to 0.78. High porosities were obtained together with poor conversion yield. The cakes were either compressible or highly compressible: the compressibility indices varied from 0.82 to 1.03, but did not clearly correlate with the conversion yield.

The results obtained in this study can be applied in pressure filtration operations when the slurry is of a similar type, i.e. processed cellulose, such as paper, cardboard or pulp. Especially the enzyme dosage and the filtration temperature are likely to have similar effects on filtration of other biomass slurries. The results are not directly applicable to hydrolysates containing higher proportions of lignin. Additionally, pretreatment of the raw material has also an influence on the enzymatic conversion and the subsequent filtration.

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Effect of mixing on enzymatic hydrolysis of cardboard waste: Saccharification yield and subsequent separation of the solid residue using a pressure filter

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ABSTRACT

Cellulosic wastes, from sources such as low-quality cardboard and paper, are regarded as potential feedstocks for bioethanol production. One pathway from these cellulosic materials to ethanol is saccharification (hydrolysis) followed by fermentation. Saccharification is commonly performed using enzymes that are able to cleave the cellulosic structure to smaller units, preferably to glucose monomers. During the hydrolysis, mixing conditions have a considerable impact on the performance of the enzymes. Thus mixing conditions in the hydrolysis tank can also influence the downstream operations and, consequently, the overall economy of the bioethanol process. In this experimental study, four types of impeller, at different hydrolysis conditions were used. The effect of mixing on the glucose yield and on the filtration characteristics of the hydrolysate was evaluated. It was shown that not only the sugar yield depended on the mixing conditions: the effect on the solid–liquid separation step was even more significant.

1. Introduction

In order to mitigate the looming problems caused by the accelerating use of fossil fuels, research and development activity for the production of ethanol from cellulosic biomasses is being increased (Abushammala and Hashaikeh, 2011; Balat et al., 2008). Bioethanol is one of the most viable candidate renewable fuels to be used as a substitute of gasoline in transportation (Talebnia et al., 2010; Várnai et al., 2010). In the best case, bioethanol could contribute to a reduction in carbon dioxide emissions. The overall effects of biofuels, however, should always be investigated in order to evaluate their sustainability (Soimakallio and Koponen, 2011). Real, net, benefits are most probably achieved when ethanol is produced from non-food raw materials such as biomass wastes, including agricultural, industrial, and municipal wastes and residues.

Corrugated cardboard is a common packaging material, available in large quantities all over the world. Cardboard waste typically contains at least 50% cellulose, but the value of such low-quality fiber lies mainly in its energy content. The physical structure of cardboard is quite favourable for bioethanol production, because the recalcitrant structure of wood is already disrupted in the pulping process. (Kádár et al., 2004; Yáñez et al., 2004; Yu et al., 2011).

Cellulose is a linear biopolymer, which consists of glucose units linked to each other by β-(1–4) linkages (Krässig, 1993). Other main components of cellulosic biomasses include hemicelluloses and lignin (Wickramasinghe and Grzenia, 2008). Starch is also found in many industrial biomass wastes, including cardboard. The polymeric structure of cellulosic biomasses can be converted to sugars, the most important of which is glucose. Conversion by enzymatic hydrolysis is regarded favourably, compared to the traditional acid hydrolysis, since fewer fermentation inhibitors are formed and milder conditions are required (Ingesson et al., 2001). Pretreatment of biomass (mechanical, chemical, thermal, etc.) is often necessary to facilitate the hydrolysis (Balat et al., 2008). The recalcitrance of lignocelluloses restricts the saccharification, principally due to low accessibility of crystalline cellulose and the barrier, formed by lignin and hemicelluloses, on the cellulose surface (Mooney et al., 1998; Zhang et al., 2007). From a technical point of view, it is not difficult to convert cellulose to bioethanol by means of enzymatic hydrolysis and fermentation. However, the main obstacles to the economic viability of the process are the huge demands of energy, chemicals, and enzymes (which require energy for their manufacture). Therefore it is important to pay attention to finding the best practicable conditions for each process step. In the present study, the impact of mixing on enzymatic hydrolysis and then subsequent solid–liquid (S/L) separation are investigated.

Mixing is one of the most important operations in the microbial conversion of cellulose to bioethanol. Adequate mixing under

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proper process conditions improves the rate of enzymatic hydrolysis (Ingesson et al., 2001; Samaniuk et al., 2011). Some earlier studies imply that intense mixing conditions may cause deactivation of the saccharifying enzymes, which leads to reduced conversion yields (Ingesson et al., 2001). On the other hand, a large enough surface area must be available for the enzymes as they attach the cellulosic surfaces (Taherzadeh and Karimi, 2008). Good mixing results at high solids contents have been obtained, for example, by utilizing pulping equipment designed for fibrous materials (Zhang et al., 2009), or a high-solids bioreactor designed by NREL (Roche et al., 2009). The impact of mixing of the slurry being hydrolyzed extends to downstream processes, in particular the solid–liquid separation. To the best of our knowledge, this is the first scientific paper showing the influence of mixing conditions in the hydrolysis stage on the subsequent S/L separation.

Process layouts encompassing separate hydrolysis and fermentation (SHF) vary greatly. S/L separations can be performed after pretreatment (Balat et al., 2008), after hydrolysis (Burke et al., 2011; Virkajärvi et al., 2009), after fermentation or after the distillation (Hahn-Hägerdal et al., 2006; Sassner et al., 2008). However, residual solids in the hydrolysate can have a negative influence on the fermentation and downstream separations (Burke et al., 2011). The main reason why the S/L separation in this study is performed immediately after the enzymatic hydrolysis is the poor controllability of the growth of the yeast cell mass and formation of side products during the fermentation, which could deteriorate the scientific value of the results. This study shows that proper mixing in the hydrolysis stage improves the glucose yield as well as enabling effective S/L separation.

2. Methods

2.1. Characterization of the feedstock

Shredded cardboard waste was selected as the model biomass for the experiments. Several impurities were observed in the material, e.g. plastic, staples of copper and aluminium, and inorganic particles (filler agents and coating pigments). The method of Black (1951) was utilized to determine the cellulose content of the oven-dry cardboard. The quantitative assay for lignin was conducted using a tailored liquid chromatographic method: HP Agilent 1050 device with Phenomenex Luna 3u C18(2) 100 × 2.0 mm column and 20 mM ammonium hydroxide and methanol (50/50 vol.%) as eluents, pH 9, injection volume 5 μL. Detection of the analytes was performed using UV/VIS at 254 nm and electrospray ionization/mass spectrometer (ESI–MS). Relative to the dry weight, the proportions of cellulose and lignin were 63 wt.% and 11.5 wt.%, respectively. The ash content, 9.1 wt.%, was determined according to ASTM D3516-89(2006) standard. Thus it was deduced that the hemicellulose content was about 15 wt.%. The results presented later in Chapter 3.1 show that the raw material was likely to be moderately heterogeneous with respect to the cellulose content. Therefore it can be assumed that the approximate relative deviation of the results, caused by the heterogeneity, was smaller than 2.5%.

The particle size of the original cardboard waste was reduced by dry milling. Over 10 kg of the material was milled for the experiments. Five vibrating sieves were used to divide the material into six fractions in order to determine an approximate particle size distribution of the material (Table 1).

Table 1
Particle size distribution of the raw material.

Particle size (μm)	Mass fraction (%)	Cumulative mass fraction (%)
<125	18.5	18.5
125–250	22.3	40.8
250–500	15.2	56.0
500–1000	31.6	87.6
1000–1400	10.8	98.4
>1400	1.6	100

2.2. Selection of impellers and design of experiments

The aim of the study was to compare the suitability of commonly-used impellers for mixing the slurry during enzymatic hydrolysis. Four different impellers were selected and an identical series of experiments were performed using each impeller.

The four different types of impellers: an anchor, a blade turbine (i.e. Rushton turbine), a propeller, and a double propeller are shown in Fig. 1A. Direction of rotation was clockwise in all experiments. The diameters of rotation of the impellers were 97 mm (anchor) and 70 mm (all other impellers). 10 mm

Prior to creating the experimental plan, it was necessary to determine ranges for the following variables: (1) rotation speed of the impeller and (2) solid concentration of the slurry. Minimum and maximum rotation speeds for the impellers were first determined using an 8 wt.% (total solids) slurry that was prepared in one mixing tank (V = 1.6 L). Enzymes were not used in these preliminary experiments. The minimum speed was just enough to keep the slurry (V = 1.1 L) surface in motion, whereas the maximum speed was the practical upper limit due to splashing and flooding. The range for the slurry solid content was selected based on knowl-

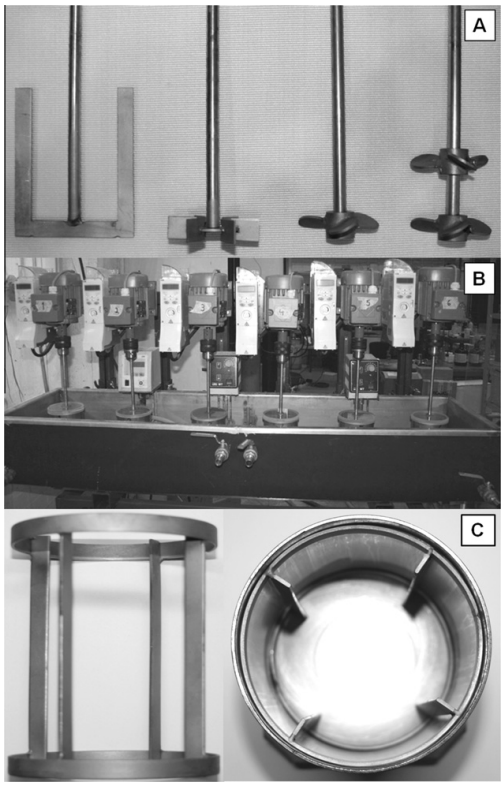

Fig. 1. The impellers left to right (A): Anchor, Rushton turbine, propeller, double propeller. The mixing device (B). The baffles and a mixing tank with the baffles (C).

edge gained from previous pressure filtration tests with a similar kind of slurry. Practical considerations limited the concentration of the slurry: reliable measurements of cake properties required relatively thick cake and thus solid concentration high enough, whereas a narrow feed pipe and valve in the filter equipment restricted the solid content.

Disposal of the solid residue is not favorable due to its relatively high energy content. More beneficial options for its utilization include, for instance, (1) recirculation to the bioethanol process, (2) combustion, and (3) biogas production.

Five tests were carried out with each impeller, according to 2^2 experimental design. Such a design comprises two levels (a minimum and maximum) for two variables, supplemented by the center point of the variable ranges. The experimental plan is presented in Table 2. In addition to the variables (rotation speed and solid concentration) the corresponding tip speeds of the impellers are shown. Compared with Rushton turbine and the propellers, the dimensions of anchor are markedly larger and the mixing effect, especially near the surface, is stronger. Therefore, it was necessary to use reduced rotation speeds for the anchor to avoid flooding. In addition to the tests shown in Table 2, four experiments were performed without baffles in the mixing tanks to investigate the influence of shear stress on the conversion yield and subsequent filterability.

2.3. Experimental set-up

The hydrolysis reactor was a specially designed mixing device (Fig. 1B), comprising six mixing tanks placed in a thermostatically controlled water bath. The tanks (height = 170 mm) were made of acid-proof steel to ensure negligible corrosion, as well as rapid temperature control due to high thermal conductivity. Each tank was equipped with four detachable baffles (Fig. 1C). The height of the baffles was 150 mm and the width of each baffle was 13 mm. The inner diameter of the tanks was 110 mm. The temperature in the tanks was controlled by three heating devices to an accuracy of 0.1 °C. The impellers were rotated by electric motors that were controlled by frequency converters (0–50 Hz, up to 1000 rpm). Furthermore, the distance of the impellers from the tank bottom was adjustable. In this study, the distance was constant (15 mm).

2.4. Batch preparation

The enzymes used in the experiments were manufactured by Novozymes A/S, Denmark. Two kinds of saccharifying enzymes were used to convert the model biomass to sugars: a hemicellulase product, Cellic HTec, and a cellulase product, Cellic CTec2.

The slurry was prepared by mixing a calculated amount of the milled biomass with one liter of water at 40 °C. As already shown in the experimental plan, the final solids content was 8, 10, or 12%. Dilute (20 wt.%) sulfuric acid (J.T. Baker, 95–97%, NL) was then added slowly until the pH was stabilized at 5.0. The hemicellulase dosage was 5 mL/kg biomass. To enhance the effectiveness of the hemicellulase, the slurry was heated up to 71 °C for 10 min. The cellulase (30 mL/kg cellulose) was added after rapidly cooling down to 46 °C. Slurry samples were taken at specific time intervals during the hydrolysis (72 h). The samples were cooled down to ambient temperature and centrifuged. Finally the supernatant was frozen for chemical assays.

Table 2
The experimental plan.

Test	Rotation speed (rpm)	Tip speed (m/s)	Solid concentration (wt.%)
1	29[A], 197[B]	0.15[A], 0.72[B]	8
2	29[A], 197[B]	0.15[A], 0.72[B]	12
3	134[A], 348[B]	0.68[A], 1.28[B]	10
4	240[A], 495[B]	1.22[A], 1.81[B]	8
5	240[A], 495[B]	1.22[A], 1.81[B]	12

[A] Anchor.
[B] Other impellers.

2.5. Separation of solid residues

In this study, the solid residues that remained after saccharification were separated from the hydrolysates by means of pressure filtration. The laboratory scale filter (Nutsche) was operated batchwise. A slurry sample of 200 g, at 30 °C, was poured into the filter chamber, which was then pressurized with nitrogen. The solids were separated from the liquid using gas pressures of 1, 3.5 and 6 bar. A filter cake formed on the filter medium was manually discharged after releasing the pressure out of the filter chamber. Regardless of the slurry quality, suspended solids were not detected in the filtrates. The proportion of solids in the filter cakes was about 30%. A cellulosic disc (T1000, Pall Corporation) with a cut-off particle size of 24 μm was used as the filter medium and each experiment was performed with a fresh filter medium to make sure that the initial medium resistance would be practically constant. The thickness of the filter medium was 3.6 mm. The effective filtration area was 18.9 cm^2 and the filter was temperature controlled (using a water-jacket) at 30.0 °C.

2.6. Chemical and physical analyses

Samples collected from the hydrolysates were stored in a freezer until all of the experiments were completed. After thawing and agitating the samples, they were filtered through a syringe filter with a pore size of 0.2 μm. Determination of sugars (glucose and xylose) and acetic acid was conducted by High Performance Liquid Chromatography, or HPLC, (HP Agilent 1100 equipped with an applicable column Varian Metacarb 87H). The column temperature was 60.0 °C and 0.005 M sulfuric acid was used as the eluent. The pumping rate and the injection volume were 0.6 mL/min and 10 μL, respectively. Determination of dissolved solids in each sample was performed by drying in a heating chamber at 105 °C for at least 48 h. Moisture contents of the filter cakes were determined using the same procedure.

2.7. Basic equation of constant pressure filtration

Solid particles form a filter cake (a porous bed of solids) when the slurry is filtered. The liquid passes through this fixed array of particles that, together with the filter medium, cause a specific resistance. In cake filtration, Darcy's basic filtration equation is supplemented with the cake resistance R_c as described by Svarovsky (1981):

$$Q = \frac{A\Delta p}{\mu(R_m + R_c)} \tag{1}$$

where Q (m^3/s) is the flow rate of a filtrate, viscosity μ (Pa s), through a filter cake with a resistance R_c (1/m) and a filter medium with a resistance R_m (1/m). The driving pressure Δp (Pa) is used to push the filtrate through the solids (filtration area A (m^2)).

Specific resistances of filter cakes α are usually determined based on a more complete, reciprocal form of Eq. (1):

$$\frac{dt}{dV} = \frac{\alpha\mu c}{A^2\Delta p}V + \frac{\mu R}{A\Delta p} \tag{2}$$

where t is time, V is the filtrate volume and α (m/kg) is the specific resistance of the filter cake and c ($kg_{solids}/m^3{}_{filtrate}$) is the filtration concentration that is defined as the mass of solid material in the filter cake per unit volume of filtrate collected.

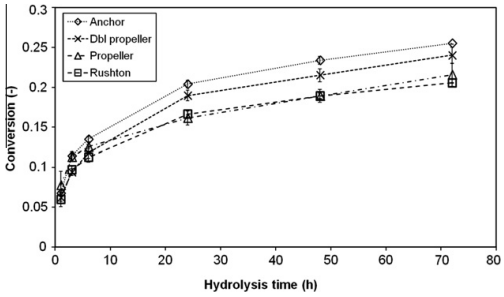

Fig. 2. Performance of the investigated impellers in enzymatic hydrolysis of the model biomass at 10% solids content, medium rotation speed.

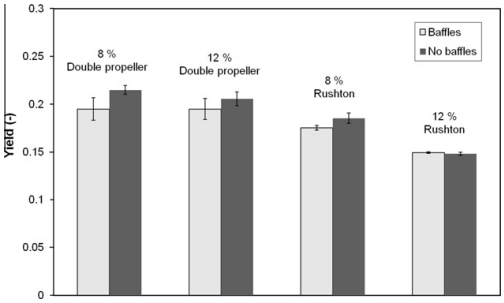

Fig. 4. Glucose yields obtained at different slurry solids content (8% and 12%) by double propeller and Rushton turbine with and without baffles. The rotation speed was constant, 495 rpm.

The specific cake resistance depends on the properties of the solid particles as well as on the properties of the liquid. Particle size and shape, including the distributions of those, are typically assumed to be the most important particle-related properties.

The degree of cake compressibility under pressure can be evaluated by determining the compressibility index n (Wakeman and Tarleton, 1999):

$$\alpha = \alpha_0 \Delta p^n \tag{3}$$

where α_0 is the specific cake resistance at unit applied pressure.

3. Results and discussion

3.1. Effect of mixing on the conversion yield

Mixing was observed to have an important role in the enzymatic conversion of the biomass to sugars. Both the impeller type and the mixing conditions were shown to have an influence on the glucose yield. To briefly summarize the results, the yields of glucose were generally low (0.15–0.28), as expected due to the low enzyme dosage and lack of pretreatment. There was only a comparatively small variation between the parallel tests, i.e. runs 1 and 2. The median of the relative standard deviations between the duplicate runs was 2.5%. In five tests, the relative standard deviations were smaller than 0.5%. The differences in the yields may be caused by the combined effect of the biomass heterogeneity and experimental error.

The principal task of the mixing system used in enzymatic hydrolysis is to ensure adequate mass and heat transfer to accelerate the reaction while avoiding shear forces that are too strong and could deactivate the enzymes. However, cellulosic biomasses have a tendency to swell by absorbing free water. This is a problem,

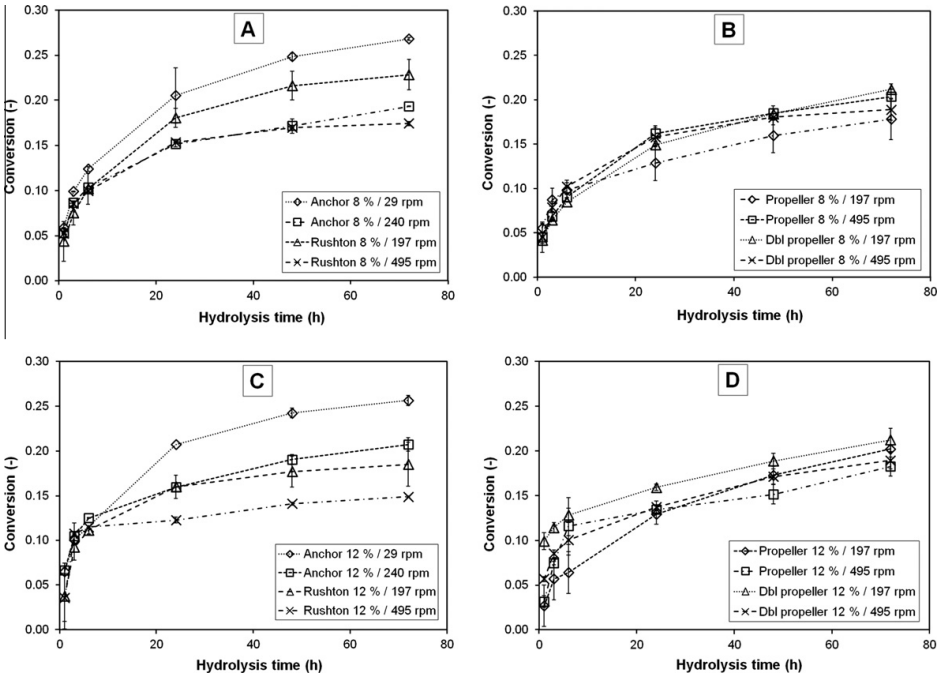

Fig. 3. Effect of rotation speed of the impeller and solids content of the slurry on the enzymatic conversion. Solids content of 8% (A and B) and 12% (C and D).

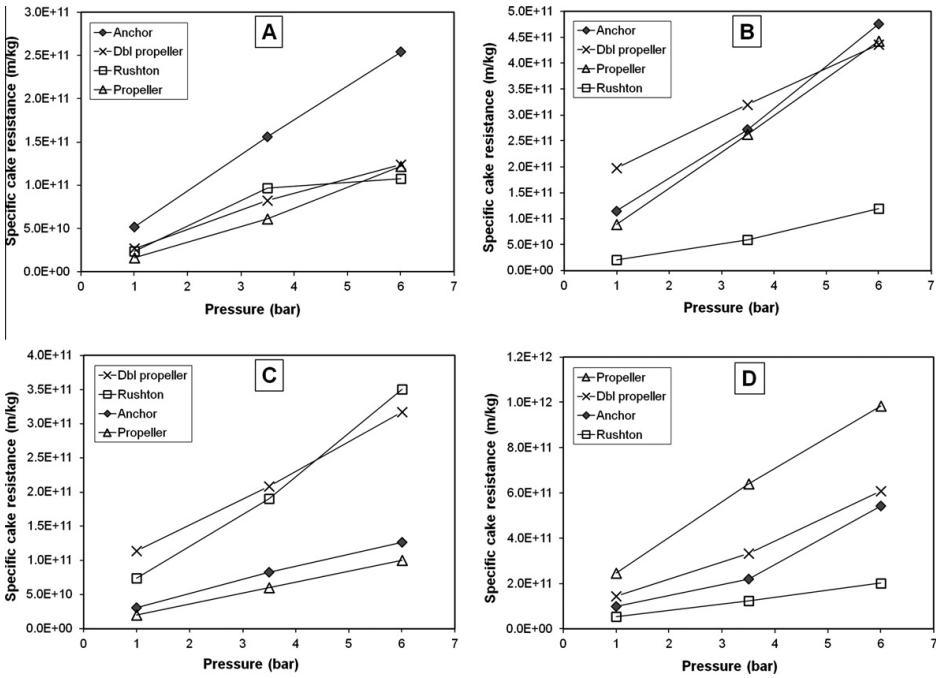

Fig. 5. Average specific cake resistances against applied filtration pressure. Slurry solids contents of 12 wt.% (A and B) and 8 wt.% (C and D). Slow (A and C) and fast (B and D) rotation speed of the impellers.

especially in the initial phase of hydrolysis, as the slurry becomes difficult to mix. High rotation speeds are often required to keep the whole batch in motion if the impeller type is not suitable for the application. A comparison of the investigated impellers is presented in Fig. 2. The differences can be clearly seen first after 24 h, by which time the quickest part of saccharification is over. Prior to paying attention to the figures that follow, it must be emphasized that selection of the impeller is of major importance regarding the conversion yield of cellulose to glucose.

The conversions obtained at different rotation speeds (slow, fast) and slurry solids content (8%, 12%) are presented in Fig. 3A–D, in which the effect of rotation speed on the conversion is shown in each figure. Comparison of Fig. 3A and B with Fig. 3C and D reveals the role of slurry solids content in the process. The contrasting performance of the impellers can also be evaluated from Fig. 3.

It was shown that the performance of anchor was the best with regard to the yield of enzymatic conversion (Fig. 3A and C). The Rushton turbine was observed to decrease the performance of the enzymes, possibly due to its strong mechanical impact (Fig. 3A and C). The double propeller outperformed the propeller and the yields were highest when slow rotation speeds were used. When propellers are employed, it is recommended to install at least two of them on the same impeller shaft, provided that they are both submerged. It was also observed that the diameter of the impeller should preferably be over two thirds of the corresponding tank diameter, because the mixing should be comprehensive in the initial stage of hydrolysis as well. The yield was generally higher at 8% solids content.

Two parallel control experiments were performed at 8 wt.% and 12 wt.% solid contents without mixing. The final yield (after 72 h) without mixing, as the average of two parallel tests, was 0.136 (8 wt.%) and 0.118 (12 wt.%). Both of these yields are lower than any of the yields obtained with mixing. A more general conclusion drawn from this is that there is an optimum mixing intensity for each biomass suspension, and it is very likely to be within the laminar flow regime.

In this study, it has been shown that there is no reason to waste energy in order to overcome the recalcitrance of cellulose by strong mixing during enzymatic conversion. Reduction of the particle size of the raw material to submicron scale has been shown to be beneficial for the glucose yield, provided it is carried out as a preliminary treatment prior to the start of the hydrolysis (Yeh et al., 2010). The high energy consumption, however, would be the main obstacle to utilizing preliminary grinding in an industrial scale. Therefore, thermally assisted pretreatment techniques, such as steam explosion, are considered more useful.

Baffles are commonly installed into the stirred tanks to prevent the formation of a large vortex around the impeller axis and to make mixing more complete. In the case of enzymatic hydrolysis, however, the baffles can cause unnecessarily strong shear forces to occur, which leads to declined yields (up to 10%) as shown in Fig. 4. The influence of baffles on the yield was investigated using two of the impellers, the double propeller and the Rushton turbine. The adverse effect of baffles on the conversion is clear, especially at 8% solids content. This is probably because of the better ability of the lower solids (8%) slurry to flow. Although the shear stress in the 12% slurry is increased by baffles, the change compared to that of the 8% slurry (with and without baffles) is relatively small, as is the effect on yield.

Flow regulation with baffles did not improve the enzymatic hydrolysis. On the contrary, the baffles probably had a slightly negative influence on the saccharification. The differences, however, are so small that further experiments should be performed to confirm the statistical significance of these results.

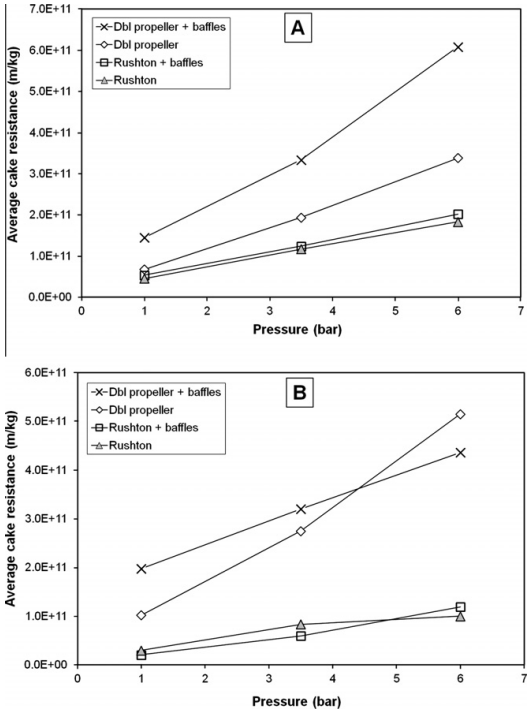

Fig. 6. Influence of baffles installed in the hydrolysis tank on average specific cake resistances at different filtration pressures. Rotation speed of the impellers (double propeller and Rushton turbine) 495 rpm, slurry solid contents 8 wt.% (A) and 12 wt.% (B).

3.2. Filtration and cake characteristics

Selection of the most suitable impeller for the enzymatic hydrolysis is of crucial importance, since the mixing conditions also affect the downstream processes. The purpose of this study was not to design novel impellers but to compare the performance of the most common types. It was observed that pressure filtration of the hydrolyzed slurry is a good option for separating the residual solids from the hydrolysate containing the dissolved sugars. The average specific cake resistance is a common measure of the filterability of the slurry. In the case of biomasses and other compressible materials, the average specific cake resistance can increase as the filtration pressure is increased.

A comparison of filter cakes from suspensions produced using different mixing conditions (impellers and speeds) is presented in Fig. 5. Differences in the average specific cake resistances α_{av} were found to be larger when faster rotational speeds of impellers were used. The absolute values of α_{av} were larger as well. Although an anchor impeller is not mechanically as rough as the other impellers employed, it was shown to cause considerably higher specific cake resistances at 12% slurry solids content (Fig. 5A and B). The likely reason for this is that the higher conversion decreases the fiber length (Lu et al., 2011), changes its morphology (Clarke et al., 2011) and increases the proportion of lignin in the solids. Hence it can be concluded that conversion of the biomass polymers to sugar oligomers and monomers has the most significant influence on the filtration of slurries with a high solids content. However, the combined effect of a relatively high conversion yield and mechanically intense conditions were probably responsible for the high specific cake resistances obtained with the propellers (Fig. 5B and C). As Fig. 5 shows, the specific cake resistances resulting from mixing conditions cannot easily be predicted. That is because the operation of each mixer is differently influenced by the conditions, i.e. the mixers have different pumping properties, besides their varying impacts on fibers and enzymes resulting from shear. The role of lignin in the filtration process is not absolutely clear, because its proportion varies as a function of the conversion yield, but the filtration resistance of lignin cakes has been reported to be high by Johansson (2005).

In fact the saccharification itself causes reduction of fiber dimensions and broken cellulosic fibers should be easier for the enzymes to digest. On the contrary, rough mixing at a tip speed of 1.8 m/s (able to reduce the fiber dimensions as well) is a major cause for low yields. That could explain why the Rushton turbine, unlike the other impellers, produced lower specific cake resistances at high rotation speeds. The enzyme dose was constant in this study. However, it has been previously observed that a more complete conversion, as a result of higher enzyme dosage, generally increases the specific cake resistance.

Generally, the specific cake resistance increased with lower slurry solids content.

It was shown that the use of baffles decreases the yield of enzymatic hydrolysis. In this case the benefits of mixing without baffles were especially notable, because improved yields were obtained simultaneously with reduced specific cake resistances (Fig. 6A and B). This effect is particularly notable with the double propeller impeller. The effect of baffles on specific cake resistances, after employing the Rushton turbine in the hydrolysis, was almost negligible both at 8% and 12% solids content. One reason for this is likely to be the radial flow pattern of the impeller. The high rotation speed (495 rpm) was the reason for the low conversion yield, which consequently was the explanation for the low specific cake resistances. Even though high rotation speed may facilitate the mass transfer and the availability of cellulose to the enzymes, deactivation and desorption of enzymes also occur, thus reducing the obtainable yield.

Filtration of the hydrolysates at three different pressures enabled the evaluation of the cake compressibility. The determined compressibility indices n as defined by Eq. (3) ranged from 0.7 to 1.1, which means that the cakes were moderately ($0.5 < n < 1$) or highly ($n > 1$) compressible (Oja, 1996). There was no clear correlation between the experimental variables and n.

4. Conclusions

The objectives of this study were to investigate the effect of mixing conditions on the yield of enzymatic hydrolysis and the subsequent S/L separation. The results show that mixing conditions (impeller-type, baffles and speed) do indeed have a significant effect. Gentle mixing conditions proved most suitable for enzymatic hydrolysis yield, under the conditions studied. Experiments performed with a laboratory-scale pressure filter showed that the filterability is also affected by mixing conditions, as a result of conversion yield (which affects particle size and properties) and shearing of the suspension (which affects particle size). In general, high intensity mixing should be avoided.

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III

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Use of Filter Aids to Improve the Filterability of Enzymatically Hydrolyzed Biomass Suspensions

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ABSTRACT: Filter aids are used in challenging filtration applications, such as the deliquoring of biomass suspensions and separation of fine particles from liquids. Organic filter aids, typically composed of cellulose, may be the preferred option in the case of bioprocesses because their energy content can be recovered together with the primary filter cake. In this study, a laboratory-scale pressure filter was used in order to investigate the effect of various filter aids on the filtration of an enzymatically hydrolyzed biomass suspension. Various organic and inorganic filter aids were used in two modes: as a body feed and as a precoat. According to the results, the average specific resistance of the cakes could be best reduced using the body feed mode. Filter aids also helped to reduce the moisture content of the cakes and to increase the corresponding filtration capacities. Additionally, a process scheme enabling utilization of the solids was proposed.

1. INTRODUCTION

Efficient separation operations are one of the prerequisites for the industrial production of liquid biofuels. Separations are typically necessary at several process steps and they are a major contributor to the capital and operating cost, as well as to the environmental impact. In the case of bioethanol production, enzymatic hydrolysis of lignocellulosic materials to fermentable sugars is regarded as a potential technology for utilizing the energy content of plant biomass.[1] Industrial production of lignocellulosic bioethanol is close to successful implementation,[2] although a number of limitations[3] still remain. Depending upon the selected process layout, the number and type of physical separation stages may vary. Forms of separation include screening, filtration, centrifugation, and membrane separation. Different processing alternatives have been introduced and studied by, among others, Balat,[4] Cardona and Sanchez,[5] Hamelinck et al.,[6] Huang,[7] and Kochergin and Miller.[8] The nature of the biomass suspension, either after hydrolysis, fermentation, or a combination, makes efficient separation difficult. Solid−liquid separation, and filtration in particular, has been observed to be challenging. This is usually caused by fine particles, lignin, and partially hydrolyzed, but highly compactible, cellulose that do not settle easily in centrifuges but also form a relatively impermeable cake in a filter. In cake filtration, the average specific resistance of a filter cake is influenced by several factors, including the particle size and shape, porosity, tortuosity of the flow channels, and chemical phenomena taking place between the phases.[9] In the present study, the solid content of the biomass suspension is high enough to rapidly form a filter cake on a filter cloth in a pressure filter. It is, therefore, important to distinguish between depth filtration, which is typically used for adsorptive removal of trace amounts of solids, and cake filtration, which is investigated in this paper.

Filter aids can be used to improve the performance of challenging cake filtration operations if other techniques, such as the proper selection of a filter cloth, an increased driving force, pretreatments, and preclassifications, are not enough to provide a sufficient filtration rate. In some cases, polymeric flocculants may also facilitate the separation.[10] The most important objective of application of filter aids is to make the cake more permeable by increasing the porosity and reducing the compressibility of the cake,[11−13] which together help to obtain a higher filtration capacity. In many cases, these improvements in the cake properties also have a positive effect on the residual moisture content of the cakes. The clarity of filtrate can also be enhanced along with the filtration capacity compared to the situation without filter aids. Because of the factors listed above, which relate to the cake properties and interactions between the filter aid and the original suspension, the selection of a filter aid usually requires experimental work.

Filter aids have traditionally been used in order to improve filtration of beer and food products[14−17] and other suspensions where fine or compactible particles are present.[18] The type and dosage of a filter aid is determined by the aim of the filtration process; the ideal product can be, for instance, a clear liquid or a dry solid fraction. Additionally, simultaneous removal of impurities (e.g., heavy metals) from the liquid by adsorption can also be considered.[19] For cake filtration, several materials have been recognized to be suitable as filter aids. Forms of diatomaceous earth (diatomite), together with perlite, are the most frequently applied inorganic filter aids in the chemical and process industries. Organic filter aids, suitable for the filtration of bulk products, are mainly composed of cellulose. The inorganic waste fractions, ashes, which are produced when organic materials are burnt, can also be utilized as filter aids.[20] In addition to these established filter aids, coal preparations, talc, plastics, and synthetic silicates may also be considered.[11]

The objective of this study was to improve the filterability of a cardboard waste suspension after enzymatic hydrolysis

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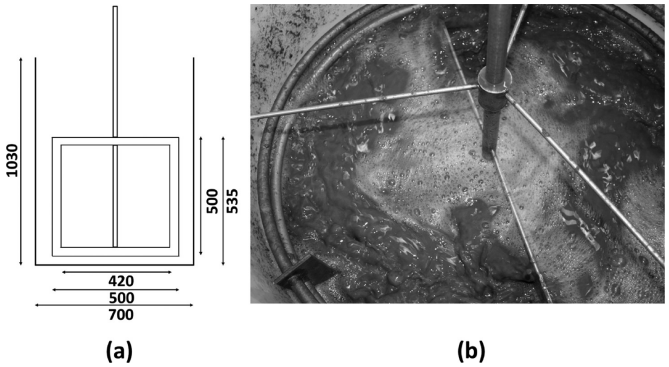

Figure 1. Dimensions (mm) of the hydrolysis vessel and the mixing element (a). The suspension after 70 h of enzymatic hydrolysis (b).

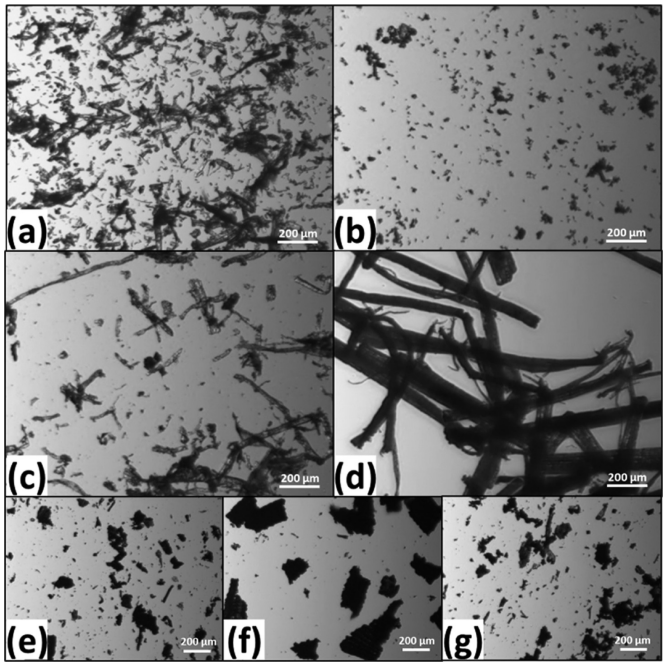

Figure 2. Stereomicroscope images of the filter aids: cellulose (a), diatomite (b), milled cardboard (c), kenaf fibers (d), and three grades of rice hull ash, standard (e), X+100 (f), and grade 4 (g).

performed for bioethanol production. Enzymatic hydrolysis of recycled paper and residual cardboard to produce monomeric sugars have previously been studied by Nazhad et al.,[21] Wang et al.,[22] and Yanez et al.[23] In this study, a laboratory-scale pressure filter was used in order to investigate the effect of filter aid type, dosage, and method of use on the filtration rate and characteristics of the filter cakes. Both organic and inorganic filter aids were used as either precoat or body feed. The filter aid dosage was varied from 10 to 20 wt % relative to the corresponding mass of solids present in the hydrolyzed suspension.

2. Materials and Methods. 2.1. Enzymatic Hydrolysis of Cardboard Waste. The enzymatic hydrolysis of shredded cardboard was performed in an agitated vessel with a volume of 300 dm^3. The cardboard sample mainly consisted of old corrugated cardboard collected from Finland. An approximate composition of the cardboard was determined as described by Kinnarinen et al.[24] The cellulose, hemicellulose, lignin, and ash contents were 63, 14, 12, and 11 wt %, respectively. Only one large batch of hydrolysate (170 dm^3) was produced in order to provide the best possible comparability for the subsequent filtration experiments.

The following materials were used for the batch preparation: (1) shredded cardboard, (2) water, (3) sulfuric acid, (4) cellulase preparation, and (5) hemicellulase preparation. The cardboard was first mixed with water to form a suspension with a total solid (TS) content of 8 wt %. Two types of commercially available enzyme preparations were used for

converting the solid cellulose and hemicellulose to soluble sugars at a constant temperature of 46 °C. Both of these products, Cellic CTec2 and Cellic HTec, were manufactured by Novozymes, Denmark. There was a need for pH adjustment, because a pH of 5 was optimal for the enzymes. Sulfuric acid (2 mol/L) was slowly added into the mixture to lower the pH from greater than 7 to 5.0. The volumes of the cellulase and hemicellulase products added were 1.92 dm^3 and 0.80 dm^3, respectively. These volumes correspond to 137 and 57 mL_{enzyme}/kg_{solids}.

During hydrolysis, the mixing rate was relatively slow, 35 rpm, because the aim was to avoid deactivation of enzymes and mixing of air bubbles into the suspension. The dimensions of the vessel and the mixing element are shown in Figure 1a. Initially, the suspension was difficult to mix. However, during the first hour after the enzyme addition, its viscosity was significantly reduced and mixing became easier. The final suspension (Figure 1b) after the hydrolysis was very easy to mix and pump, with the exception of the largest impurities. These impurities, comprising mainly metals and plastics, were removed from the suspension by screening, using a sieve with a mesh size of 4 mm.

After screening, the suspension was again mixed well and divided into several small vessels of 10 dm^3 that were then stored in a freezer. Prior to each series of experiments, a sufficient amount of slurry was slowly brought to 20−22 °C.

2.2. Filter Aids. Seven different types of filter aids were investigated. These materials, listed below, varied from cellulosic to inorganic and from traditional to novel.

1. Cellulose EFC-900
2. Diatomaceous earth/diatomite
3. Milled cardboard waste/the hydrolyzed material itself
4. Kenaf fibers
5. Rice hull ash (standard)
6. Rice hull ash (X+100)
7. Rice hull ash (grade 4)

An Olympus SZX9 optical microscope (50× magnification) equipped with a Leica DFC 450 camera was used for physical characterization of the filter aids. Stereomicroscope images of all filter aids are shown in Figure 2a−g.

The organic filter aids, including cellulose, milled cardboard waste and kenaf, were mainly composed of (broken and intact) cellulosic fibers, and diatomite and the rice hull ashes (RHA) represented particulate inorganic solids of irregular particle shape. The approximate volumetric particle size distributions of all filter aids, measured using a Beckman Coulter LS 13320 particle size analyzer, are presented in Table 1. A detailed description of the particle size measurement is presented in Section 2.4.

Diatomite, produced by Dicalite/Dicaperl Minerals (Bala Cynwyd, PA, U. S. A.), contained the smallest particles, whereas the rice hull ashes consisted of larger particles, which were of relatively equal size in all three grades used. The particle size of the milled cardboard and, especially, the kenaf fibers were significantly larger than in the case of cellulose. It is important to note that the size distribution determined for fibers is not directly comparable with particulate solids, due to the needle-like shape and totally different structure of the fibers. The analysis technique used for the particle size analysis was based on laser diffraction, and the device used assumes the particles to be spherical.

Table 1. Approximate Particle Size Distributions of the Filter Aids

	particle diameter (μm)				
filter aid	D_{10}	D_{25}	D_{50}	D_{75}	D_{90}
diatomite	2.8	8.3	18.1	32.3	49.5
cellulose	18.4	51.9	118.5	207.6	325.7
cardboard	29.4	74.1	220.3	654.5	1222.0
kenaf	104.7	167.1	385.6	951.7	1503.0
RHA (standard)	9.3	24.7	52.7	91.4	144.4
RHA (X+100)	10.7	30.5	69.1	132.8	188.2
RHA (grade 4)	9.0	22.9	46.7	77.7	114.4

In this case, shredded cardboard was milled in order to further reduce the particle size. After milling, the maximum particle size of the material was 1.5 mm. The EFC-900 cellulose, a commercial extract-free, nonbleached product, was manufactured by J. Rettenmaier & Söhne Gmbh (Rosenberg, Germany). The kenaf (*Hibiscus cannabinus*) sample was supplied by Kenaf U. S. A. (St. Augustine, FL, U. S. A.). The kenaf sample was sieved using a pack of several sieves with mesh sizes between 50 and 355 μm. From this, one of the most abundant fractions, 100−180 μm, was selected as a filter aid for the experiments. Filter aids consisting of cellulosic fibers have some special benefits, such as low density, favorable structure with rough surfaces and high porosity, easy cleaning of filter cloth, and good possibilities for disposal or energy production by combustion.[25]

Among the investigated materials, diatomaceous earth (diatomite) is perhaps the most used in the chemical and processing industries. The high porosity and low compressibility of diatomite make it an excellent filter aid for several applications, including related processes, such as beer filtration.[14] However, there are also some major challenges associated to diatomite, including health hazards, long-distance transportation, regeneration after use, and final disposal. The other inorganic filter aids, different types of rice hull ash, were provided by Agrielectric Research (Lake Charles, LA, U. S. A.). The ash grades were obtained from size classification of the same original RHA. Besides particle size, there were variations in particle shape and pH. The largest RHA particles typically had the highest pH and ζ potential. The smallest particles, on the other hand, had a more irregular shape, which increased the permeability. Rice hull ash is the final waste produced when rice hulls, the nonfood part covering rice grains, are burned. It is high in silica content and provides a high porosity when used as a filter aid. Rice hull ash, as with many other types of ashes, could also be used for concrete and brick manufacture, soil amelioration, or as an adsorbent, to mention just a few potential applications.[26] There are several factors that affect the performance of rice hull ash in filtration applications, including the chemical composition, physical structure and properties, and characteristics related to the particle size and shape.[20]

2.3. Filtration Experiments. In the filtration experiments, a laboratory-scale pressure filter was used. The filter unit, Labox 100, was designed by Outotec Oy, Finland. The main parts of the filter are shown in Figure 3. The pump attached to the equipment can be seen behind the pressure gauge next to the slurry inlet. Based on preliminary tests, AINO T70 cloth, also supplied by Outotec Oy, was selected as the filter cloth. The cloth material was polypropylene, and the air permeability of the cloth was 15 $m^3\ m^{-2}\ min^{-1}$. The height of the filter chamber was adjustable but was kept constant (at 33 mm)

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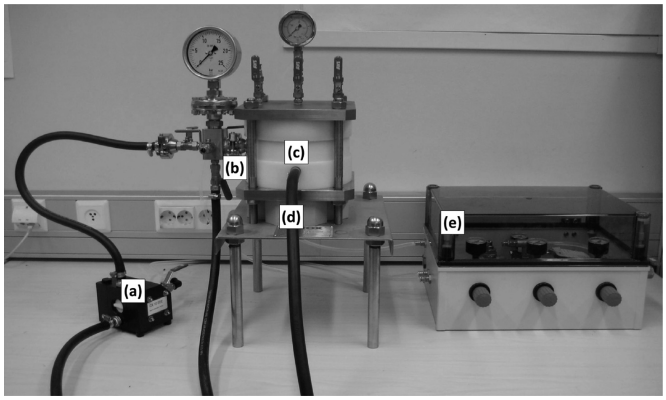

Figure 3. Labox 100 filter press. Feed pump (a), slurry inlet (b), filter chamber (c), filtrate outlet (d), and the pressure control unit (e).

throughout the study. In the top of the filter chamber, there was an elastic diaphragm that was inflated with compressed air to perform cake squeezing. Additionally, the experimental arrangement included a mixing tank (V = 20 dm^3) for the slurry and a small (3 dm^3) mixing tank for mixing the suspensions consisting of water and the precoat filter aids. The small mixing tank was also used when the predetermined amount of slurry was mixed with a certain mass of filter aids for the body feed tests. The filtration parameters were selected based on the results of a preliminary series of experiments. A simple filtration cycle was selected, comprising only pumping at 4 bar for 10 min and cake pressing at 10 bar for 4 min, in order to produce completely saturated filter cakes. All filtration experiments were carried out at room temperature (22 °C).

The experimental study consisted of two parts, where the selected filter aids were used for improving the filterability of the biomass hydrolysate, using (i) precoat and (ii) body feed modes. In addition to the method of using filter aids, the relative amount of the filter aids was varied. Concentrations of 10 and 20 wt %, relative to the amount of total solids (TS) in the slurry, were used. When the filter aids were applied using the precoat mode, the filter aid was first slurried with water and this suspension was then filtered at low pressure (1 bar) to form the precoat cake on the filter cloth. In this case, excess water was removed from the cake by pressing it at 2 bar for 2 min.

2.4. Measurements and Calculations. The final concentrations of the main sugars, glucose and xylose, were determined by high-performance liquid chromatography (HPLC). An HP Agilent 1100 was used, equipped with a Varian Metacarb 87H column that was kept at 60.0 °C. The eluent was 0.005 M sulfuric acid. The pumping rate and the injection volume were 0.6 mL/min and 10 μL, respectively.

Determination of total solids (TS) in the slurry and filtrate was performed by drying in a heating chamber at 105 °C until a constant weight was reached. The moisture content of the filter cakes was determined using the same procedure.

Particle size distributions (PSD) of the filter aids were measured using a Beckman Coulter LS 13320 laser diffraction analyzer with the Fraunhofer optical model and an aqueous liquid module. After careful sampling, a small amount of each filter aid was suspended in water and a small sample from this suspension taken for the PSD analysis. Each sample was analyzed six times and provided none of the PSDs significantly differed from the others, the distributions were averaged. If significant differences were observed, another sample of the same filter aid was analyzed six times and then averaged.

The filtration capacities (kg/(m^2 h)), with respect to dry solids, were calculated as gross capacities, omitting the technical time that is associated with opening, closing, cake discharge, cloth washing, and other such necessary operations in a full-scale filter. Calculation of the average specific cake resistance (m/kg) is presented in Section 3.

3. THEORY

The theory of cake filtration is derived from Darcy's law, which deals with flow of fluid through a porous medium. The driving force in cake filtration is the pressure drop Δp (Pa) through the medium, which in this case includes both the cake and the filter cloth. As a result of the driving force, the liquid is forced to flow in the direction of the decreasing hydraulic pressure gradient. Regarding the packing of solid particles in the cake, the minimum porosity (the volume ratio of the nonsolid to the total) is found at the filter medium and the maximum on the top of the cake.[27] For constant pressure filtration, the filtrate flow rate Q (m^3/s) through the cake and the filter medium can be calculated from eq 1

$$Q = \frac{A\Delta p}{\mu(R + R_c)} \tag{1}$$

Where A (m^2) is the filtration area, μ (Pa s) is the viscosity of the filtrate, and the resistances of the medium and the cake are R and R_c (1/m), respectively. Usually, the medium resistance, caused by the filter medium and the solid particles that interact with it, is assumed to be constant, whereas the cake resistance increases with cake growth. Therefore, the amount of cake formed per filtration area from slurry with a solid concentration c ($kg_{solids}/m_{filtrate}^3$) when a filtrate volume V (m^3) is collected must be taken into account. Then eq 1 becomes

$$Q = \frac{A\Delta p}{\alpha\mu c\frac{V}{A} + \mu R} \tag{2}$$

where α (m/kg) is the specific cake resistance.

The reciprocal form of eq 2 presents the separate terms for the specific cake resistance and the resistance of the filter medium

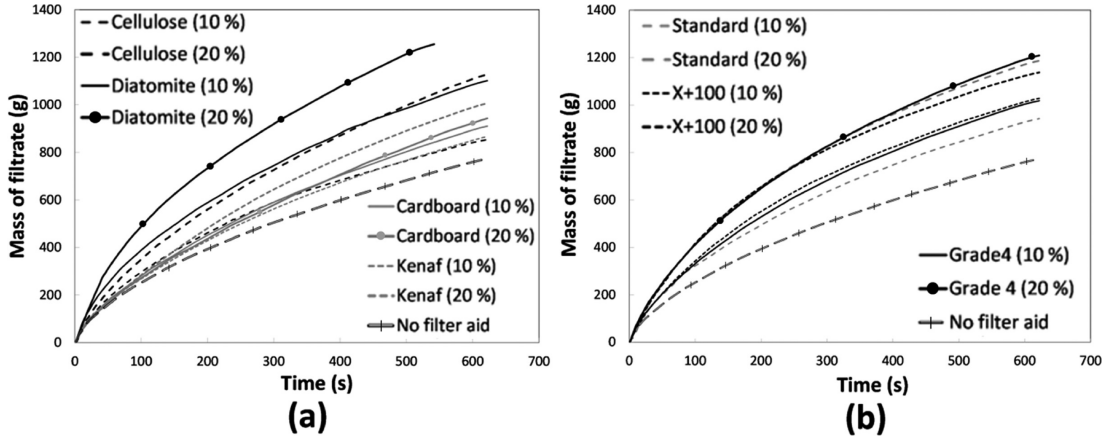

Figure 4. Accumulation of filtrate during pressure filtration at 4 bar. Body feed mode using cellulose, diatomite, cardboard and kenaf (a) and body feed mode using various types of rice hull ash (b).

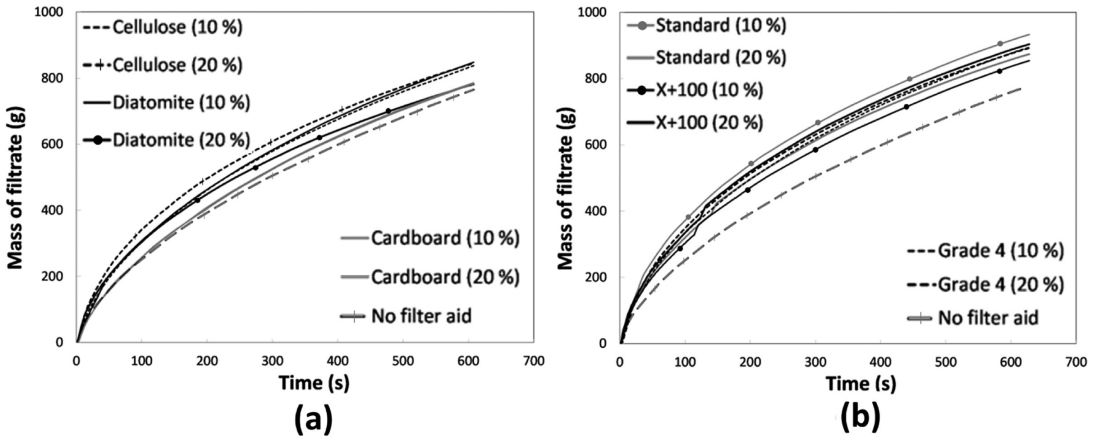

Figure 5. Accumulation of filtrate during pressure filtration at 4 bar: precoat mode using cellulose, diatomite and cardboard (a); precoat mode using various types of rice hull ash (b).

$$\frac{dt}{dV} = \frac{\alpha\mu c}{A^2\Delta p}V + \frac{\mu R}{A\Delta p} \tag{3}$$

After integration, starting from the beginning of the constant pressure period (t_s, V_s), eq 3 finally becomes

$$\frac{t - t_s}{V - V_s} = \frac{\alpha\mu c}{2A^2\Delta p}(V + V_s) + \frac{\mu R}{A\Delta p} \tag{4}$$

In experimental filtration studies, the average specific cake resistance α_{av} (m/kg) and the medium resistance R can be calculated from the collected data of constant pressure filtration using the slopes a (s/m^6) and b (s/m) obtained from t/V against V plots.[24] These plots are created based on the filtrate volume collected in time t. The final equations for constant pressure filtration are then

$$\alpha_{av} = \frac{2aA^2\Delta p}{\mu c} \tag{5}$$

$$R = \frac{A\Delta p b}{\mu} \tag{6}$$

The equations presented above can be used to evaluate cake filtration in applications where local differences in the cake structure are not considered. The above equations and a precise introduction on the theory of constant pressure filtration have been presented, among others, by Svarovsky[28] and Gösele.[29] The motion of solids in the cake is assumed to be negligible, and the specific cake resistance for certain slurry is assumed to depend only on the compressive stress.[30] In practice, determination of average specific cake resistance can be very time-consuming. Therefore, methods to simplify the determination of α_{av} have been proposed, for instance, by Teoh et al.,[31] who used instantaneous filtration rates for the calculation of α_{av}. The main drawback of this method is that accurate estimation of the instantaneous filtration velocities is difficult and may lead to poor repeatability. In this study, all moments of filtration were considered when the average specific cake resistances were calculated.

An important measure of the success of separation, the liquid recovery as filtrate, R_l, was calculated from eq 7

$$R_l = \frac{m_{\text{liquid,filtrate}}}{m_{\text{liquid,filtrate}} + m_{\text{liquid,cake}}} \quad (7)$$

4. RESULTS AND DISCUSSION

The results obtained using filter aids were compared with those of the case without filter aids. The filterability was evaluated with the help of filtrate accumulation curves and the average specific cake resistances obtained.

4.1. Filtration Curves. The body feed experiments were performed using all available filter aids. Because of technical problems with pumping the long and high-frictional kenaf fibers, these were excluded from the precoat experiments.

The performance of the filter aids in body feed and precoat filtration of the biomass hydrolysate can be evaluated based on Figures 4 and 5. As illustrated in Figure 4, all filter aids helped to enhance filterability when the body feed mode was used. In this case, organic filter aids (Figure 4a) were observed to be less effective than diatomite, which increased the average filtration rate during the pumping stage by 75% compared to that of the case without filter aid. However, the milled cardboard waste would possibly be the most economical option for the studied process because it is always available from the raw material. The superior performance of diatomite at 20 wt % concentration could partially result from the higher compressibility of the organic filter aid materials. Moreover, the dosing of the filter aids was mass-based, so the volume of organic filter aids pumped into the filter was significantly larger. This may have caused an excessively dense structure of the filter cake already during the filtration stage.

The differences in the performance of the rice hull ashes (Figure 4 b) were smaller. During the filtration time of 600 s, the amount of filtrate collected was increased by 57% in the best case (RHA grade 4). As shown above in Table 1, the particle size distribution implies that this RHA is composed of the finest particles, in comparison with the other RHA products. Irrespective of the filter aid, the increase in the accumulation rate of filtrate was clear when the filter aid consumption was increased from 10 to 20 wt %.

There was only a slight improvement in the filterability as a result of precoat addition (Figure 5 a-b). The maximum increase in the quantity of filtrate collected was only 10% for cellulose and diatomite, and 10−21% for the different grades of RHAs. It can be assumed that the precoat layer forms an independent filter medium, on which the filter cake is formed. The liquid permeability of the precoat layer varies depending on the type of filter aid and the precoating conditions. These factors may also affect the ability of the precoat layer to retain solid particles. It is apparent that the filter aid dosage should not be too high, because, after a certain point, the resistance caused by the increasing cake thickness surpasses the benefits.

The differences between the precoat results were smaller than those in the case of body feed. Further, the filter aid dosage did not have a clear influence on the filtrate flow rate. These results are most likely due to the high filtration resistance of the biomass suspension, which is, in all cases, clearly higher than the resistance caused by any of the filter aids. Based on observations made during the experiments, flocculation of solids did not occur during the process.

With respect to the retention of suspended solids, precoating was excellent in all cases. The average total solid (TS) contents in the body feed and precoat filtrates were 4.9 and 4.6 wt %, respectively. The precoat filtrates were practically free of suspended solids, whereas the body feed filtrates were turbid, containing both colloidal and settleable matter. The clarity of filtrate did not depend on the type of filter aid used.

However, it may be possible to improve the separation performance by selecting a filter aid that has an opposite surface charge to the particles present in the hydrolysate under process conditions in order to reduce the electrical repulsion between particles and fibers. The surface charge of cellulose fibers and most inorganic minerals, including the silica-based diatomite and rice hull ash, is net negative at the pH of the process. When the surface charge of the solids is low enough, attractive van der Waals forces dominate the particle−particle interactions and flocculation can take place. Flocculation of solids, and in particular colloids, occurring as a result of reduced electrical repulsion can be assumed to improve the separation process. This is with respect to not only enhanced clarity of the filtrate but also cake production capacity.

Varghese and Cleveland[32] used kenaf as a body feed filter aid. They compared the performance of kenaf with commercial filter aids (diatomite, perlite, Solkafloc) by filtering a dilute (1%) kaolin suspension and obtained a significant improvement in the filtration rate in all cases. They concluded that the filter area requirement using kenaf was about 25−30% larger than that required when commercial filter aids were used. In the case of kenaf, the filtrate turbidities were also at a high level compared to those of diatomite and perlite. In addition to kenaf fibers, kenaf core has also been successfully used as a filter aid.[33] Carman[13] investigated the suitability of diatomite as a filter aid for fine metal hydroxides. He concluded that the mechanical action of the filter aid was mainly responsible for the improved permeability of the filter cakes. Li et al.[17] observed that filtration rate of wastewater sludge could be greatly increased by the addition of rice hull ash (RHA). Depending on the origin of the sludge, the relative increase in the dewatering rate varied from 7 to 45% with a 50% RHA dosage and from 2 to 14% with a 10% RHA dosage. The filtrate quality (color) and the cake dryness were also significantly improved. With respect to diatomite, Carman[13] concluded that the positive influence of diatomite addition is not only due to the increased porosity: the rigid structure of the cake and the surface properties of diatomite particles contribute to the filtration rate and the quality of the filtrate. The benefits of organic filter aids, such as refined cellulose products, have been earlier discussed by Gerdes.[25] Regarding the disposal, combustion or reuse in certain situations, organic filter aids may have an advantage, as discussed later in Section 4.4.

It is possible that the filter aid dosages used in this study were higher or lower than the optimum dosage of the filter aids. Therefore, the performance of a single type of filter aid should not be generalized. Further, the results are process specific, which means that small changes in the pH, temperature, solid contents, and so forth may either improve or decrease the performance of a filter aid.

4.2. Specific Cake Resistance, Cake Moisture, and Filtration Capacity. The average specific cake resistances for the slurry without filter aid and for all precoat experiments are illustrated in Figure 6a. In most cases, the reduction in α_{av} is quite poor. It is apparent that precoat is not the most favorable

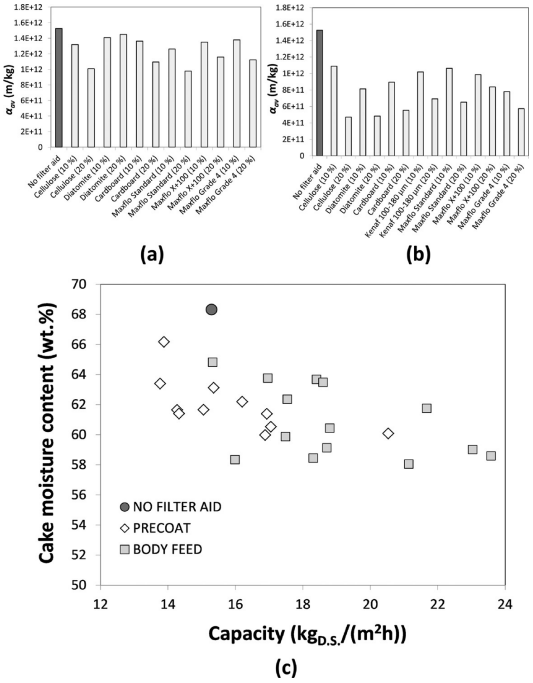

Figure 6. Average specific cake resistance α_{av} obtained using the precoat (a) and body feed (b) modes. Moisture contents of the filter cakes plotted against filtration capacities obtained using the precoat and body feed modes (c).

feeding strategy for a pressure filter because none of the filter aids worked especially well.

As Figure 6b shows, the same amounts of filter aids that were used in the precoat experiments are enough to produce a significantly reduced α_{av} when the body feed mode is applied. Unlike in the case of the precoat mode, the amount of the filter aid had a clear influence on α_{av}. This influence was of different magnitude for each filter aid, but α_{av} was, without exception, reduced as the filter aid concentration was increased. There are a number of reasons for the good performance of the body feed mode: (1) open cake structure that reduced the flow resistance, (2) rigid cake structure that reduced the compressibility, and (3) the inclusion of all filter aid particles in the cake, not below the cake or in the pores of the filter cloth. After all, when the aspect of the complete filtration cycle is considered, body feed clearly seems to be the recommended feeding strategy for pressure filters. This filter aid feeding strategy reduces the total cycle time and simplifies the process.

The repeatability of the filtration experiments was evaluated by performing two parallel trials for selected body feed and precoat experiments. Cardboard and Maxflo Standard were used, at a concentration of 20 wt %. The mass of filtrate collected during the pumping stage did not vary dramatically: the minimum variation from the average was ±0.5% in the case of cardboard/body feed and the maximum was ±2.2% in the case of Maxflo Standard/precoat. In the case of cardboard/precoat and Maxflo Standard/body feed, the variations were ±1.0 and ±1.2%, respectively. Because the pressure control was performed manually, it is apparent that the inaccuracy of pressure control in the pumping stage was the most important source of this variation.

These results also imply that the performance of the filter medium was successfully restored by washing the filter medium with water after each experiment. In other words, there was no serious pore blockage. The relatively open pore structure of the filter medium apparently facilitated the effectiveness of the cloth washing.

Figure 6c presents the obtained filtration capacities and the corresponding moisture contents of the cakes after the filtration and cake pressing stages. It can be observed in Figure 6c that the successful use of filter aids, irrespective of the mode of addition, helps to increase the filtration capacity and to reduce the cake moisture content. The moisture content was reduced in all cases, but the capacity could not be improved in a few precoat experiments. The recovery of liquid within the filtrate (eq 7) varied from 91.4 to 94.7%, in the case of body feed, and from 91.0 to 94.0 in the case of precoat (Table 2).

Table 2. Liquid Recovery within the Filtrate

	liquid recovery (%)			standard deviation	
filtration mode	average	min.	max.	absolute (%-units)	relative (%)
body feed (n = 14)	93.2	91.4	94.7	1.0	1.1
precoat (n = 12)	92.6	91.0	94.0	0.9	1.0

Modeling of the average specific cake resistance for a binary mixture containing filter aids and the material to be filtered has been carried out by Abe et al.[34] They used Kozeny-Carman's equation as a basis for two models, which in turn were based on the assumption of complete mixing and segregation of particles. The assumption of complete mixing was shown to be valid for nonspherical particles, whereas segregation phenomena had an important role in the case of spherical particles of significantly different sizes.

The compressibility of filter cakes under pressure can also be reduced using filter aids. In the case of compressible cakes, specific cake resistance increases with filtration pressure. The compressibility index n (not determined in this study) can be determined using an empirical relationship $\alpha = \alpha_0 \Delta p^n$, as presented by Svarovsky.[28] During the pressing stage, consolidation of the filter cake is sometimes divided into two different types: (1) primary, which involves removal of liquid from the pores and the resulting reduction in the height of the cake, and (2) secondary, which stands for rearrangement of particles and creep of the solid structure. In the present study, both types of consolidation probably occurred, although precise evaluation of the consolidation was not possible, due to the configuration of the filter unit. Li et al.[35] mixed rice hull ash with supercompactible wastewater sludge and obtained a significant reduction in the cake compressibility. The compressibility index n was reduced from the supercompactible 1.13 to a moderately compactible 0.4. The filtration rates and the solid contents of the cakes were consequently increased.

In the case of very compressible cakes that have exceptionally high average specific resistances, it is possible that the addition of filter aids alone cannot reduce the resistance to filtration sufficiently to enable feasible operation. Moreover, the specific filter aid requirement can have a significant effect on the process economy. There are, fortunately, other methods that can be used together with filter aids. Polymeric flocculation, preconcentration of the feed suspension, and filtration at

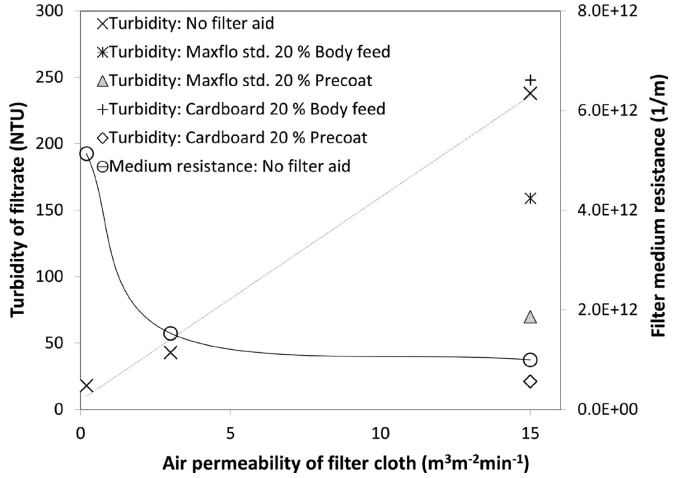

Figure 7. Turbidity of filtrate obtained using three different polypropylene filter cloths, having air permeabilities of 0.2, 3, and 15 $m^3\ m^{-2}\ min^{-1}$, according to the manufacturer (Outotec Oy, Finland). The experimentally determined resistances of the filter media (eq 6) are also shown.

elevated temperature, for instance, could help to increase the productivity.

4.3. Effect of Filter Cloth on the Quality of Filtrate. The filter cloth is usually regarded as the most important component of a filter. The purpose of the filter cloth is to capture the suspended particles, to provide a support layer for cake growth and to permit a high flow of filtrate. In this study, three polypropylene filter cloths (AINO K10, T32, and T70) were evaluated with respect to the filtration resistance and the turbidity of filtrates, as shown in Figure 7. The main difference between the selected filter cloths was the pore size distribution and, consequently, permeability.

As illustrated in Figure 7, the filter cloth has a significant influence on the turbidity of the filtrate. However, the resistance of the least permeable filter cloth is relatively high. It is, therefore, important to select a filter cloth, which is able to fulfill the requirements regarding both permeability and the degree of filtrate turbidity. It is also clear from Figure 7 that the filtrate turbidity cannot be substantially reduced using body feed mode. The difference between the average total solid concentrations of the body feed and precoat filtrates, 4.9 and 4.6 wt % (Section 4.1), represent the amount of fine solids that cause this increase in the turbidity.

4.4. Alternatives for Filter Aid Reuse and Utilization. The material efficiency of a filtration process can be improved by recycling the digestible fiber back to the enzymatic hydrolysis and by reusing the nondigestible inorganic matter as a filter aid. The process sketch presented in Figure 8 is quite simplified and may require some additional process units to be effective in practice. In Figure 8, the hydrolysate is filtered using a pressure filter and the filtrate (a) is recovered for subsequent processing, to be converted to bioethanol. A suggestion for the utilization of the cake, in the case of organic (cellulosic) filter aids, is presented on the left in Figure 8. On the other hand, the reuse of inorganic filter aids is illustrated on the right. The incoming fresh materials in the former case include the materials required in the hydrolysis stage and the fresh filter aid. In the latter case, the addition of fresh water, as well as the above-mentioned materials, is necessary for the filter aid reuse in the same process.

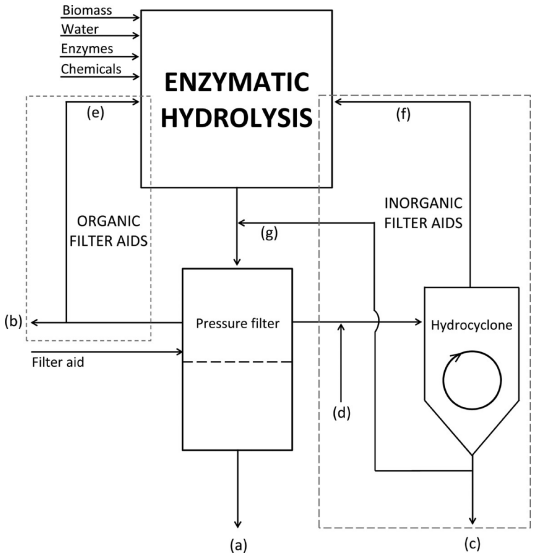

Figure 8. Alternatives for the recycling and utilization of organic and inorganic filter aids: (a) filtrate to the next process stage, (b) cake fraction to combustion, (c) spent inorganic filter aids to landfill, (d) fresh water, (e) filter cake recirculated to hydrolysis, (f) cake and water recirculated to hydrolysis, and (g) inorganic filter aids recirculated to the filter.

It is clear to see from Figure 8 that organic filter aids can be more readily reused. However, it is probable that the nondigestible constituents of lignocellulosic biomass (i.e. lignin and minerals) will concentrate in the process over time and have a negative effect on the hydrolysis and the subsequent filtration. Therefore, these constituents should be either separated or removed (b) from the circuit with the associated digestible fiber. The fiber-rich fraction, or part of the cake, can then be recirculated (e) to the process.

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The reuse of inorganic filter aids requires at least one separation stage. This separation stage requires a certain amount of fresh water (d), but is likely to be easy to perform due to the large density difference between the biomass residue (ρ < 1100 kg/m^3) and the filter aid (ρ > 2000 kg/m^3). Hydrocyclones, centrifuges, and perhaps also sedimentation tanks could be applied. However, a prerequisite for successful separation is that the cakes can be readily dispersed in water. The filter aids should be returned to the filter at a relatively low moisture content, which means that water should be removed from stream g. This could be accomplished by a gravity thickener, the water overflow of which could be utilized in the hydrolysis stage, together with stream f. Part of the used filter aid (c) can also be removed from the process and sent to landfill. If the reuse of filter aid is not of interest, then the filter cake, including the inorganic filter aid, can be landfilled or considered for combustion, although the high mineral content may impair the combustibility in practice.

5. CONCLUSIONS

The purpose of this study was to improve the filtration of enzymatically hydrolyzed biomass suspensions using inorganic and organic filter aids. It was shown that various filter aids can be successfully applied to improve the filterability of a hydrolyzed lignocellulosic biomass suspension using a pressure filter. However, further research is needed to optimize the filter aid dosage. The use of the body feed strategy simplifies the process and should be used with pressure filtration when some turbidity in the filtrate can be accepted. Based on the findings, the precoat strategy is not recommended because the poorly permeable structure of the biomass cake remains unchanged. It is clear that filter aids not only increase the production rate but also help to reduce the cake moisture. These effects are closely related to the increased porosity and rigidity of the filter cake. The selection between organic and inorganic filter aids depends upon the reuse and utilization of the filter aids and the cake. In order to minimize unnecessary waste disposal, future studies could investigate the utilization of these solids from technical and economical points of view.

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Notes

The authors declare no competing financial interest.

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IV

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ENZYMATICALLY HYDROLYZED AND AGITATED BIOMASS SUSPENSIONS: EXPERIMENTAL DETERMINATION OF FIBER SIZE DISTRIBUTIONS AND FILTRATION CHARACTERISTICS

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Separation processes in general present a significant challenge in the production of bioethanol from lignocellulosic materials. Solid-liquid separation, prior to the concentration of ethanol (for instance, by distillation), is often essential and upstream process conditions may determine how effectively this separation can be performed. In this experimental study, properties of a lignocellulosic solid residue, generated through the enzymatic hydrolysis of biomass, and solid-liquid separations after the hydrolysis stage were studied, focusing on the fiber and particle size distribution (FSD and PSD) of the solids. During the course of enzymatic hydrolysis, fiber and particle size distributions of the biomass during and after enzymatic hydrolysis were measured using a fiber tester and a laser diffraction analyzer, respectively, in order to quantify the effect of enzymatic saccharification on the size distribution of the suspended solids. The main target, however, was to investigate the filtration properties of hydrolyzed and agitated suspensions using a pressure filter. The particle size distributions of the filtered samples were measured with the laser diffraction analyzer. Even though the filtration properties were strongly influenced by agitation, the effect on particle size distributions was found to be much smaller. During enzymatic hydrolysis, the most significant reduction in the size of the solids took place rapidly after the cellulase addition. The width of the fibers was not observed to decrease during the hydrolysis stage.

Keywords: Enzymatic hydrolysis, Fiber size, Particle size, Mixing, Agitation, Filtration

INTRODUCTION

Enzymatic hydrolysis for cleaving cellulose to sugars may be one of the key enabling technologies for the production of bioethanol from lignocellulosic raw materials that are sustainably available. Such raw materials include the waste fractions of agriculture, forestry, the pulp and paper industry, as well as waste paper and cardboard collected from municipal and industrial sources. After six decades of development, accelerated by the energy crisis in the 1970s, commercially feasible enzymatic degradation of biomass is gradually becoming a reality.[1] A number of large pilot plants for producing ethanol from lignocellulosic biomass have recently been established, for instance, in Europe, Canada and the United States.[2] Lignocellulosic biomass is, however, typically highly resistant to enzymatic saccharification, which results in a high enzyme requirement and, consequently, limited applicability to many potential raw materials.

Regardless of the current technological problems related to the hydrolysis stage, paper and cardboard products have the potential to become an important source of fermentable sugars for the production of lignocellulosic ethanol. The use of waste fibre and papers has been recently studied by, amongst others, Kemppainen et al.[3] and Wang et al.[4] and, earlier, by Mandels et al.[5] and Walpot[6]. Cardboard waste is globally available and its structure is suitable for further processing. Recycling and incineration are the two most common ways of utilizing the material and energy content of cardboard waste. Unfortunately, the enzymatic hydrolysis of lignocellulose, including products of paper and pulp industry is challenging,[7,8] especially because of fiber hornification.[9] The effect of enzymatic hydrolysis on the fiber properties is not completely understood. Solid-liquid separation of enzymatically hydrolyzed lignocellulosic suspensions is another process step in which changes in the process can bring about unpredictable results.[10] As proposed in many previous studies on separate hydrolysis and fermentation (SHF), solid-liquid separation may be essential at various stages of the bioethanol process: after either pretreatment, hydrolysis, fermentation or after the ethanol recovery by distillation.[11-15] In any event, non-

degraded fibers in the hydrolysate could have an adverse effect on fermentation and downstream separations.[12] In the case of lignocellulosic ethanol, the hydrolysis stage has been studied more extensively than the downstream separation processes. More attention has been previously paid to the solid-liquid separation of other difficult-to-filter suspensions, such as wastewater sludges. There are some similarities between biomass hydrolysates and wastewater sludges, including high cake compressibility[16], difficult deliquoring of cakes with low fiber content,[17] and limited possibilities to increase the solids content of the cakes.

Several factors, either separately or together, determine the success of enzymatic hydrolysis. These factors include, for instance, crystallinity of cellulose,[18-20] and particle size of biomass.[21-24] The quality of the cellulosic biomass itself has an influence on enzymatic hydrolysis and, conversely, hydrolysis alters the physical properties and the chemical composition of the biomass. During enzymatic hydrolysis, properties such as the pore volume[20] and the average length[23] of cellulosic fibers may be reduced. Pulping, as well as enzymatic treatment,[25] may drastically change the morphology and properties of cellulose fibers.[26,27,9] The effect of mixing on enzymatic saccharification has been recently evaluated[28,29], but its effect on separation performance is still unclear.

The topic of this article, the influence of enzymatic hydrolysis and mixing conditions on the fiber and particle size of a lignocellulosic biomass and the filtration characteristic of the resulting suspensions, is a continuation of the authors' previous work[30]. In this previous paper, it was stated that both the extent of hydrolysis and mixing conditions affected solid-liquid separation characteristics. The influence of mixing conditions on the yield of hydrolysis has been investigated in several studies, but the results vary, depending on factors such as the substrate quality and quantity, enzymes used, as well as reaction time.[31] The purpose of this article is to show how important a role mixing plays with respect to the solid-liquid separation of lignocellulosic hydrolysates and how it affects the particle size. The effect of the degree of conversion is eliminated by using the same hydrolyzed suspension in all experiments. Furthermore, measurement of fiber and particle size distributions, carried out with two different instruments, a fiber tester and a laser diffraction particle size analyzer, during the enzymatic hydrolysis,

will help to understand better how the fiber size is reduced during enzymatic saccharification and how it correlates with the resulting filtration properties.

EXPERIMENTAL

Description of the process

A sample of air dry old corrugated cardboard, collected from Finland and shredded prior to transportation to the laboratory, was first milled, using a hammer mill. The aim of milling was to reduce the initial particle size to a range measurable with the particle and fiber size analyzers and to enable rapid wetting of fibers. The device was equipped with a screening system, in which a screen with a mesh size of 2 mm was used. After forming the solid-liquid suspensions for the experiments, and thus after complete wetting of the material, the size distributions were measured.

Enzymatic hydrolysis, of duration 72 hours, was performed using the milled cardboard waste, described above, as the model biomass. The enzymes were commercial cellulase and hemicellullase products, Cellic CTec2 and Cellic HTec (Novozymes, Denmark). The enzyme dosages were 150 mL of CTec per kilogram of cellulose and 30 mL of HTec per kilogram of dry raw material. The FPU activity for the cellulase product has been reported to be approximately 120-150 FPU/mL.[32,33] The cellulose and lignin contents of the cardboard waste, previously described by Kinnarinen et al.[10] were 63 wt.% and 11.5 wt.%, respectively. The ash content of the raw material was 9.1 wt.%, obtained by dry oxidation at 575 °C (ASTM D3516–89(2006) standard). The hemicellulose content was therefore estimated to be approximately 15 wt.%.

After hydrolysis, there was a certain amount of non-hydrolyzed solid residue (cellulose, lignin, minerals, pigments and other impurities) in the suspension. The aim was to separate this residue from the monosaccharide-containing liquid using a laboratory-scale pressure filter. Filtration tests were performed both after the hydrolysis and after additional mixing. In most tests, the filtration pressure was

100 kPa (1 bar). In addition, in order to determine the compression properties of the filter cake produced, filtration tests were also performed at two further pressures (3.5 and 6 bar).

Equipment and conditions

The hydrolysis experiments were carried out in a mixing device, which consisted of six mixing tanks (D_t = 110 mm, h_t = 170 mm), mixing elements with electric motors, and a temperature-controlled bath. The volume of the suspension prepared in each tank was 1.3 L. Because pH has an important role in the hydrolysis, the pH of each batch of suspension was slowly adjusted to 5.0 with 2.0 M sulfuric acid. The water bath was kept at a constant 46 °C (±0.2 °C) for 72 h, and the mixing speed was held constant during the hydrolysis. The solid content in all slurry batches was 10.0 wt.% and the rotation speed of each impeller was 40 rpm (0.67 1/s). Identical anchor-shaped (Fig. 1(a)) impellers (d_i = 97 mm, h_i = 120 mm) without baffles were employed in each batch to avoid mechanical reduction of particle size.

In this part of the study, any reduction of particle size of the model biomass during the hydrolysis stage was investigated and the suspension samples for the mixing experiments produced. The initial fiber and particle size distributions of the biomass are presented with the results in the Results and Discussion Section.

Determination of particle sizes and sugar concentrations

For the fiber and particle size measurements and the determination of monosaccharides, nine samples were taken from each of the six tanks during the hydrolysis (after 1, 2, 3, 5, 8.5, 12, 24, 48 and 72 hours) and one sample prior to adding the enzymes. There was no reason for more frequent sampling intervals during the first hours of hydrolysis, because the kinetics of enzymatic saccharification were out of the scope for this study. Six samples of equal volume (7 mL), intended for sugar determination, were combined, vacuum filtered through Whatman #42 filter paper to separate most solids, and finally filtered

through a syringe filter (0.2 μm). For the fiber and particle size measurements, the samples were kept in a water bath at 100 °C for 10 minutes in order to stop the progress of hydrolysis.

The fiber size distributions were determined using a Lorentzen & Wettre fiber tester (Kista, Sweden). This instrument was capable of measuring both the length and width distribution of the fibers using an image analysis technique. The ranges for the measurable fiber length and width were 0.2-7.5 mm and 10-100 μm, respectively. Objects smaller than 0.2 mm in length were regarded as fines and therefore excluded from the fiber size distributions. Premilling of the raw material was probably the main reason for the abundance of fibers shorter than 1 mm also in the original suspension. Fiber size measurement was performed 2-3 times for each sample.

The volumetric particle size distributions (PSD) were measured with a Beckman Coulter LS13320 laser diffraction analyzer. The Fraunhofer model of light scattering was selected as the calculation basis for the measurements. The size range of the particles that could be analyzed using this device was from 0.04 to approx. 2,000 μm. Within this range, the particle size analyzer measured all solids, without the ability to distinguish between spherical and elongated particles. Each measurement was performed at least 6 times for each sample and the results were averaged. While it is probable that the particle size analysis, which is most suitable for spherical particles, cannot completely detect changes in the fiber size during the hydrolysis stage, it can be used for acquiring comparative data about the relative size of solid particles in the suspension that may affect both the hydrolysis rate and the subsequent solid-liquid separation processes. Only this technique was used for measuring the size of the solids after the mixing experiments, because the cut fibers and other fine solids in particular were expected to be responsible for the resistance to filtration.

A JEOL JSM-5800 scanning electron microscope (15 kV, 100 x magnification) was used for visual characterization of the fibers. The samples were first diluted with 10-fold volume of Millipore water, filtered through Whatman 42 filter paper to recover the fibers, and finally dried slowly at room temperature.

The two main monosaccharides, liberated from the polysaccharide matrix during hydrolysis, glucose and xylose, were determined by high performance liquid chromatography, HPLC (HP Agilent 1100), using a Varian Metacarb 87H column. The temperature during the elution was 60 °C and the eluent was 5 mM H_2SO_4, with a flow rate of 0.6 mL/min and an injection volume of 10 µL. It is possible that small amounts of other monomeric sugars and cellobiose were also present in the hydrolysates, although they were not assayed in this case.

Mixing experiments

After the hydrolysis, the slurry was mixed with two impellers: a Rushton blade turbine and a conventional propeller (Fig. 1(b-c)). The rotational diameter of both impellers was 70 mm. The distance of the impellers from the bottom of the mixing tanks was 15 mm. Baffles were installed in the tanks to increase turbulence and the potential for size reduction of the particles during mixing.

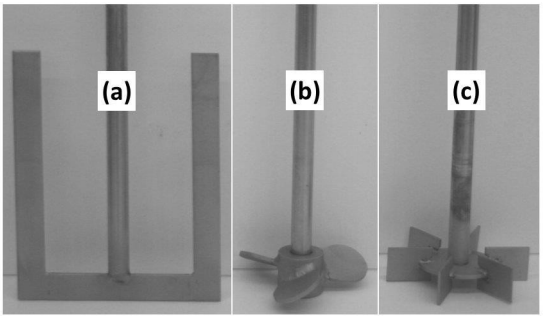

Figure 1: The mixing elements: The anchor used for agitation during the hydrolysis stage (a). The propeller (b) and the Rushton turbine (c) used in the filtration part of the study

In order to investigate the effect of mixing on the particle size and filtration properties of the slurry, three rotation speeds (100, 300, 500 rpm) were used. Filtration tests were performed prior to the additional mixing, after 0.5 hours, and finally after 4 h of mixing. The samples for the PSD measurement were taken at the same time as those for the filtration, and additionally after 2 hours of mixing. The experimental plan is shown in Table 1.

Table 1
Mixing conditions for filtration experiments and particle size analysis

Test	Mixer type	Rotation speed (rpm)	Mixing time, filtration (h)	Mixing time, PSD measurement (h)
1	Rushton	100	0.5; 4	0.5; 2; 4
2	Rushton	300	0.5; 4	0.5; 2; 4
3	Rushton	500	0.5; 4	0.5; 2; 4
4	Propeller	100	0.5; 4	0.5; 2; 4
5	Propeller	300	0.5; 4	0.5; 2; 4
6	Propeller	500	0.5; 4	0.5; 2; 4

Filtration experiments

Immediately after sampling, the solids were separated from the fresh and mixed hydrolysates by pressure filtration. Batches of 200 g were filtered at 23 °C using a laboratory-scale filter (Nutsche). The filter chamber was pressurized with nitrogen: the applied gas pressures were 1, 3.5 and 6 bar. A cellulosic disc (T1000, Pall Corporation) with a cut-off particle size of 24 μm was used as the filter medium. After 72 hours of hydrolysis, the ability of the cellulase present in the suspension to degrade cellulose was very limited, mainly due to the temperature which was far below the optimum, so there was no significant risk of degradation of the filter medium during the relatively short (2-15 min) filtration experiments. Additionally, each disc of filter medium was used only once. The thickness and the effective filtration area of the filter medium were 3.6 mm and 18.9 cm^2, respectively. The estimated concentration of total suspended solids in the slurry was 5.8 wt.%. The total solid contents in the filter cakes were typically approximately 30 wt.%, consisting of suspended (about 85 wt.% of the total) and dissolved solids (about 15 wt.% of the total).

All cakes were analyzed for the total solids (TS) concentration by drying them in a heating chamber at 105 °C until the weight was constant. The total dissolved solids (TDS) content in the filtrate was approximated using a Brix refractometer after filtering the filtrate sample through a syringe filter (0.2 μm nominal pore size). The TDS content in the filtrate was typically approximately 60 g/L.

Calculations

The specific resistance of a filter cake, α, can be determined from Eq. (1), derived from Darcy's basic filtration equation:

$$\frac{dt}{dV} = \frac{\alpha \mu c}{A^2 \Delta p} V + \frac{\mu R}{A \Delta p} \quad (1)$$

where t (s) is time, V (m^3) is the filtrate volume, α (m/kg) is the specific resistance of the filter cake, μ (Pa s) is the dynamic viscosity and c ($kg_{solids}/m^3_{filtrate}$) is the filtration concentration that is defined as the mass of solid material in the filter cake per unit volume of filtrate collected (and is in turn related to the solidosity of the cake and the solids content of the slurry). The filtration pressure is Δp (Pa), applied on the filtration area A (m^2). R (1/m) is the average resistance of the filter medium.

However, in practice, neither the solidosity nor the specific cake resistance is constant inside the filter cake so typically an average value, α_{av}, is determined.

The compressibility index, n, is a commonly-used indicator of the susceptibility of the cake to compression. If n >0, the average specific cake resistance increases with the applied pressure. A convenient method for determining n is to plot α_{av} against Δp and to add a power-type trendline. The function is of the form (Eq. (2))

$$\alpha_{av} = \alpha_0 \Delta p^n \quad (2)$$

where α_0 is the average specific cake resistance at unit applied pressure.

The average porosity of a filter cake, ε_{av}, is calculated from

$$\varepsilon_{av} = \frac{V_{pores}}{V_{cake}} = 1 - \frac{V_{solids}}{V_{cake}} \quad (3)$$

where V is the volume. In this study, the volume of each filter cake was calculated based on the height (average of 5 points) and the cross-sectional area of the cake. The volumes of solids were calculated using the dry cake weights and solid densities determined earlier.

The cake compressibility has also an influence on the average porosity ε_{av}:

$$\varepsilon_{av} = \varepsilon_0 \Delta p^{-\lambda} \quad (4)$$

where ε_0 is the average porosity of a filter cake at unit applied pressure and λ is the cake compressibility index.

The effect of mixing on the average specific cake resistance was evaluated with respect to 1) the mixing rate and 2) the mixer head, i.e. the shear pressure Δp_{shear}, which was calculated[34] from:

$$\Delta p_{shear} = \frac{P}{Q} \quad (5)$$

where P is the mixing power (W) and Q is the volumetric flow rate (m^3/s).

The mixing power was estimated based on impeller power number, impeller diameter and impeller speed. The volumetric flow rate by mixer was estimated based on impeller flow number, impeller diameter and impeller speed.[34]

RESULTS AND DISCUSSION

Fiber and particle size during enzymatic hydrolysis

Enzymatic hydrolysis of cellulose and hemicellulose cleaves the polymeric structure to smaller structural units (mono-, di- and oligosaccharides). At mild conditions, for example pH 5 and at 46 °C, the solubility of lignin in the hydrolysate is low. As a result of such optimal conditions during the hydrolysis stage, it can be expected that the decrease in the fiber size of the biomass is significant. Fig. 2A shows the glucose and xylose concentrations, measured by HPLC, from the samples taken during

the hydrolysis. The final glucose concentration was 39.5 g/L, which corresponds to a yield of 59 % (based on glucose only). The xylose concentration at various points during the hydrolysis was about 33 % of the glucose concentration.

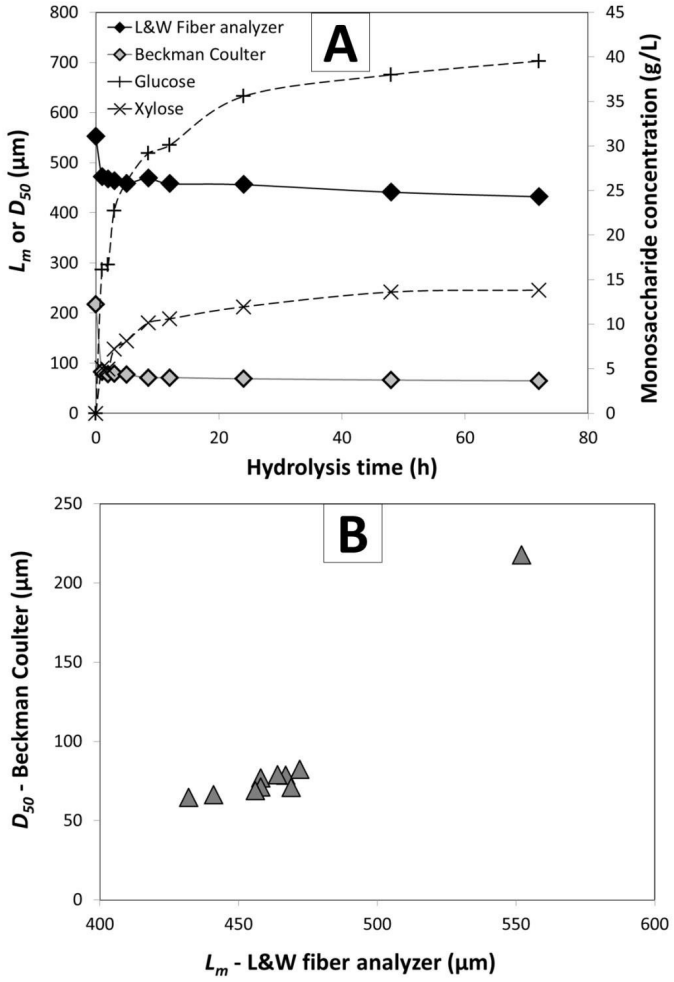

Figure 2: A) The mean fiber length (L_m) and the median particle size (D_{50}) of the solids, determined with an L&W fiber size analyzer and a Beckman Coulter particle size analyzer during the enzymatic hydrolysis. The corresponding concentrations of glucose and xylose in the liquid phase are also shown. B) Correlation between the mean length of fiber and the median particle size measured with the two analyzers

It is normal that the initial rate of enzymatic hydrolysis is rapid. The importance of the initial period is also observed in Fig. 2A which presents the mean fiber (L_m) and median particle (D_{50}) sizes. When determining fiber length, the arithmetic average fiber length was not the most suitable indicator, because of the high proportion of short fibers. Therefore, the length weighted average fiber length L_m was used. The median particle sizes (D_{50}) were determined from volumetric particle size distributions. An approximate correlation between the measured L_m and D_{50} sizes is presented in Fig. 2B. The most probable reason for the nonlinear shape of the 2nd order fit is that the proportion of fines increases immediately after the start of hydrolysis. The laser diffraction analyzer is able to include these fine particles in the PSD, because they are within its range of measurement. If the non-hydrolyzed sample was excluded, the correlation presented in Fig. 2B would be quite linear. The relative standard deviation for 6-9 parallel analyses with the Beckman Coulter analyzer ranged from 0.9 to 2.2 %. The fiber tester also produced very consistent data, but it is not possible to accurately evaluate the deviation based on two parallel measurements. After the first 2-3 hours of hydrolysis, the particle size does not appear to decrease significantly (Fig. 2A). The increase in glucose concentration is also rather moderate after the first 12 h. It was observed, using the laser diffraction analyzer, that the measurable size of the finest 10 % was reduced by 54 % during the first 12 hours, whereas the size limit for the largest 10 % fraction was reduced more, proportionally, by 73 %. There are a few factors that could explain why the conversion rate slows down rapidly after the enzyme addition: 1) the lack of suitable adsorption sites for the enzymes, 2) end product inhibition caused by the released sugars (not very significant in this case), 3) the presence and formation of other inhibitors, and simply 4) a lack of readily degradable cellulose. The slowdown in rate of hydrolysis has been more extensively studied[35,36] and also modeled[37-39] by other authors.

The length and width distributions of the fibers during the hydrolysis are shown in Fig. 3 A-D. During enzymatic hydrolysis, the most noticeable change in fiber length takes place during the first hour (Fig. 3 A,B). However, the fiber width distributions (Fig. 3 C,D) do not clearly correlate with the degree of enzymatic conversion.

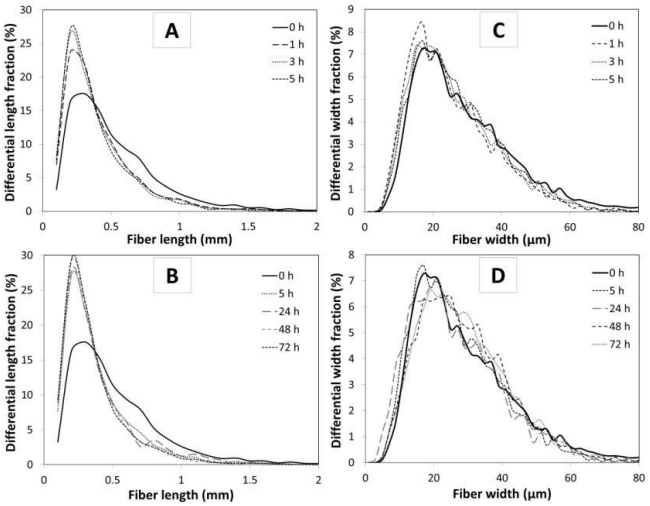

Figure 3: A) Differential fiber length distributions determined during the initial stage of hydrolysis, B) Differential fiber length distributions determined during the total course of hydrolysis, C) Differential fiber width distributions determined during the initial stage of hydrolysis, D) Differential fiber width distributions determined during the total course of hydrolysis. The distributions determined after 2, 8.5 and 12 h of hydrolysis are excluded for clarity.

The particle size distributions during the initial stages (0-5 h) and the total time of hydrolysis (72 h) are presented in Figs. 4 A-B. A comparison between Fig. 3 A-D and Fig. 4 A-B indicates that there are some similarities between the fiber and particle size distributions, even though the absolute values are not equal, as illustrated above in Fig. 2B. The particle size analyzer is capable of accurately measuring the particle size distribution of fines, such as inorganic fillers and fragments of fibers, while fibers are more reliably characterized using the fiber tester.

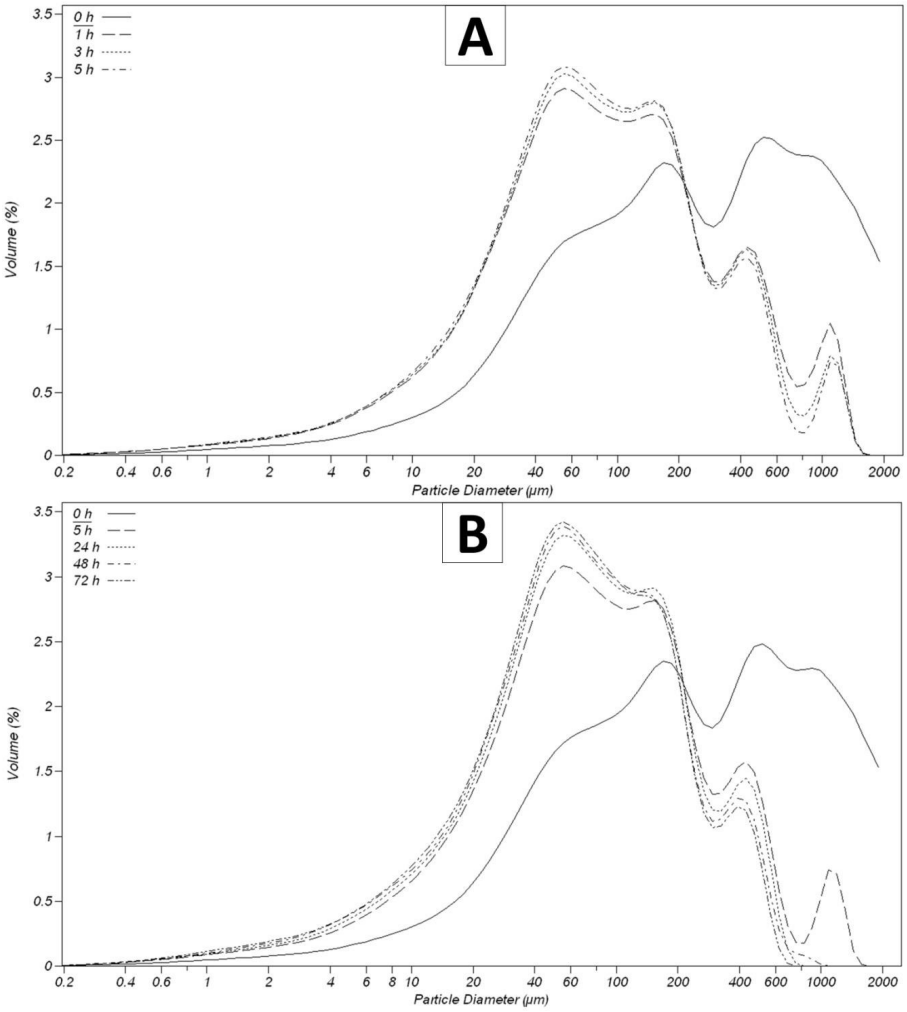

Figure 4: A) Differential particle size distributions determined during the initial stages of hydrolysis (0-5 h), B) Differential particle size distributions determined during the course of hydrolysis (72 h)

Clarke et al.[40] obtained a 92 % reduction in the fiber length of bleached Kraft pulp in 9 h, while a reduction of only 15 % (in 8.5 h) was obtained in this study. The negligible lignin content and the low solid loading (2 %) were probably the main reasons for such significant fiber length reduction. Mooney et al.[19] concluded that the role of particle size of the raw material was of the highest importance during the initial period of enzymatic hydrolysis. In their study, small fibers and fines in heterogeneous

lignocellulosic substrates were cleaved to sugars rapidly, resulting in a high hydrolysis rate at the beginning of hydrolysis. According to Zhu et al.[41], the fines are hydrolyzed first and the large particles can therefore retain their original size for many hours of hydrolysis. The experimental results presented in Figs. 2-4 are unable to confirm whether similar effects occurred in this study, although it is likely that fine fibers were hydrolyzed more rapidly, because of their large specific surface area.

Fig. 5 illustrates how the structure of the fiber network changes during the hydrolysis. Because the images have been taken after the fibers have been dried, evaluation of the exact dimensions of single fibers in their suspended state is not possible. However, it is possible to evaluate approximately the size and outer structure of the fibers. This type of general evaluation supports the results that were obtained from the fiber and particle size analyses.

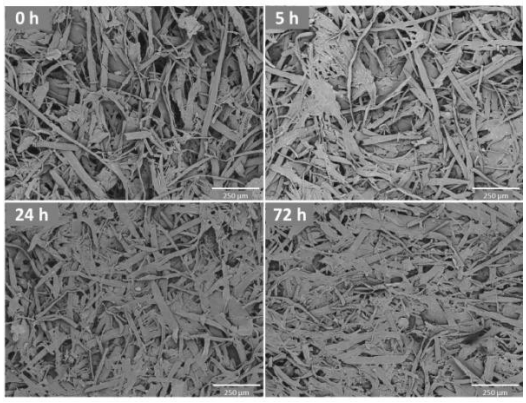

Figure 5: Scanning electron microscope (SEM) images of the fiber residue taken during the hydrolysis

Little dramatic breakage of fibers was seen, unlike that observed in the study of Clarke et al.[40]. Although, in each individual fiber, both the porosity and the specific surface area are likely to increase during the course of hydrolysis, the overall porosity of a dried sample seems to decrease as the saccharification proceeds. In other words, the fiber network seems to become more densely packed as a result of degradation of cellulose and hemicellulose that support the fibers. This phenomenon may also have an influence on the subsequent solid-liquid separation.

Particle size during mixing

During mixing of the hydrolyzed suspensions at rotation speeds of 100 and 500 rpm (1.67 and 8.33 1/s), the particle sizes of the solids in the suspensions did not change dramatically. The fiber size distributions for these agitated samples were not measured, because the initial assumption was that measurement of fine particles and cut fibers would be more important. It was surprising how small the effect on particle size was, even though high-intensity mixing with the Rushton turbine continued for 4 h (Fig. 6A). In comparison with the propeller (Fig. 6B), the Rushton turbine had a stronger influence on the measured particle size. The data in Figs. 6A and 6B are presented showing the particle sizes (D10 – D90) of the undersize distribution: 10 % - 90 % of particles, on a volumetric basis, were smaller than the particle size in question. Generally, the data collected during the hydrolysis stage (Fig. 2A-B) and the results presented in Fig. 6A-B imply that omitting the fiber size analyses at this stage was reasonable.

The differences between the effect of high (500 rpm) and low (100 rpm) rotational speeds were clear only in the case of the Rushton turbine (Fig. 6A). In the case of high-speed mixing (500 rpm) with the Rushton turbine, the D_{10} and D_{90} particle sizes were decreased by 21 and 16 % during the mixing time of 4 hours. Generally, the size limit of the smallest (D_{10}) fraction was reduced relatively more than that of the largest (D_{90}) fraction.

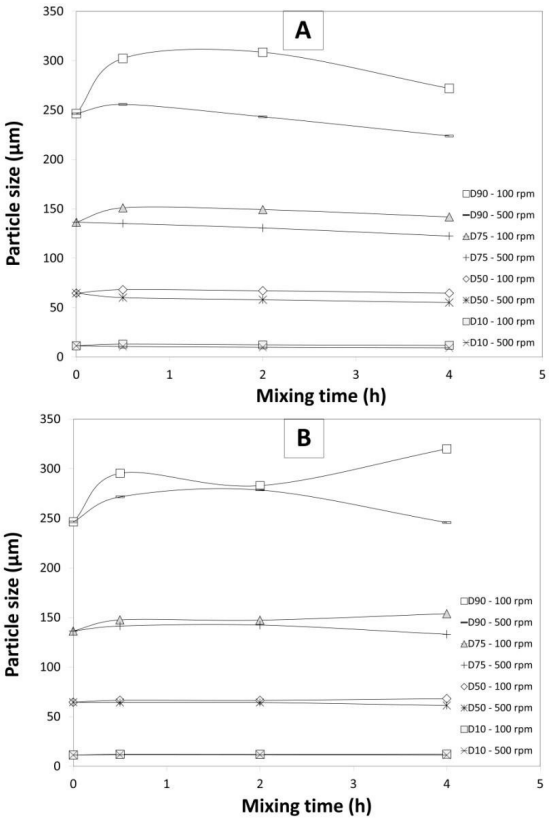

Figure 6: Particle size of hydrolyzed solids before and during agitation (0.5, 2, 4 h), at different agitation rates (100 and 500 rpm), obtained from the particle undersize distributions. A) Rushton turbine and B) Propeller

However, the use of the propeller did not lead to such notable differences in the measured particle size. It is probable that there is a relatively high degree of uncertainty in the D90 curves, because of the low number of particles in the largest decile. This is the most likely explanation for why, in Fig. 6B, the D90 particle size seems to get larger when the mixing is continued after sampling at 2 h. Other reasons for the increasing particle sizes could be presence of air bubbles in the analyzer and inaccuracy resulting from sampling and sample preparation. This could be explained by an increase in the fiber width (and volume) as a result of low-intensity mixing that is, however, unable to reduce the fiber length.

Filtration characteristics after mixing

Average specific cake resistance

In traditional applications, where the properties of wood fibers are tailored, for instance, in the pulp and paper industry, mechanical treatment by pulping, classification, pumping, etc. may change the properties of the fibers.[42,9] The main changes include the dimensions, as well as physical properties. These changes can greatly affect the filtration properties of the fibers. The ability of the fibers to retain water, to swell and to be compressed under pressure is among the most important properties influenced by mixing. The average specific cake resistance is a scalable measure of the ability of a filter cake to drag the flow of liquid that passes through its pores. In practice, high average specific cake resistance signifies difficult filtration that is observed as a low filtration flow rate per unit area. Prediction of the average specific cake resistance of heterogeneous and compressible fiber suspensions based on the fiber size is problematic, because many other factors should also be taken into account. The difficulties are related to the non-uniform particle size, shape, flexibility, presence of fines, and many other factors, which affect the pore structure of the cake.[43,44] It is, therefore, not reasonable to predict the filtration properties based on particle size data.

In this study, the aim was to investigate how significant the effect of mixing is on the pressure filtration of enzymatically hydrolyzed suspensions. An increase in the mixing rate, i.e. the local shear rate, resulted in considerably elevated average specific cake resistances (Fig. 7).

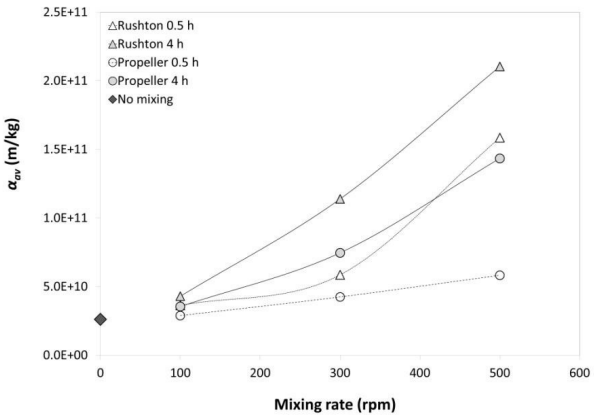

Figure 7: Average specific cake resistances obtained by pressure filtration experiments after 0.5 and 4 hours of mixing with the Rushton turbine and the traditional propeller at three different mixing rates. The zero mixing value is shown for comparison

This was especially clear in the case of the Rushton turbine. However, the particle size data do not support the assumption that this was the result of a significant decrease in the fiber size. The most important conclusion from this is that the fiber properties, which could not be directly measured, such as an increase in the number of microfibrils and other structural changes in the fiber, increase α_{av}. Partial fibrillation of the fibers is likely to be one main reason for the increased resistance to filtration. This explanation is analogous to drainage of non-hydrolyzed wood fibers on a paper machine, where excessive fibrillation of fibers should be avoided, in order to obtain a good drainage rate. However, fibrillation cannot be observed in the SEM images prepared using dry fibers. Another interesting point is the behavior of lignin in the process. Lignin was almost exclusively in the solid state at the filtration conditions (pH 5, 23 °C) and may have also been affected by agitation. In comparison with inorganic fines, small lignin particles are typically more difficult to be separated by cake filtration. At the process pH, most of the lignin was in the solid state, attached to the carbohydrate polymers in the fibers. Quantification of the amount of free lignin-rich particles in the suspension would have been interesting but technically challenging.

The average specific cake resistances shown in Fig. 7 are presented as a function of the mixer head in Fig. 8A (Rushton turbine) and 8B (propeller). The mixer head, calculated from Eq. (5), corresponds to shear pressure (Pa) produced by the impeller.

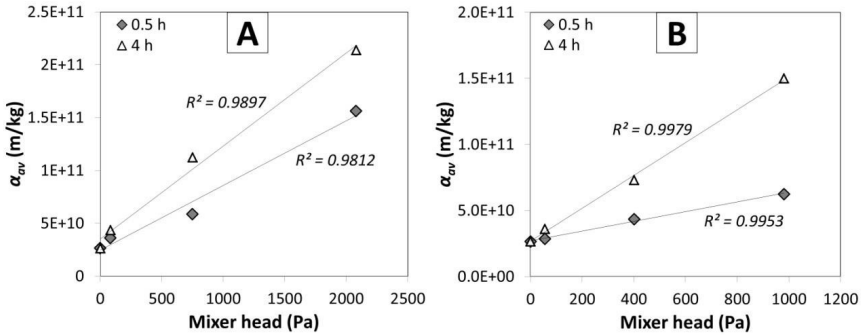

Figure 8: Average specific cake resistances obtained by pressure filtration experiments after 0.5 and 4 hours of mixing at different shear pressures (the zero mixing value is also shown). A) Rushton turbine and B) Propeller

It was observed that the filtration performance depends on the mixing time and shear pressure. The correlation between shear pressure and filtration performance is linear. The linear fit for the data was good (R^2 = 0.981 – 0.998). The results presented in Figs. 7 and 8 illustrate the importance of the mixer geometry with respect to solid-liquid separation. It is apparent that the results obtained using the Rushton turbine and the propeller are more comparable when the influence of mixing geometry is taken into account, in addition to the mixing rate.

Cake compressibility and porosity

Determination of the cake compressibility index *n* for the non-mixed and the strongly mixed (Rushton turbine, 4 h, 500 rpm) hydrolyzed suspensions was performed by plotting the average specific cake resistance, α_{av}, against the filtration pressure (Fig. 9A). The exponent in the power function fitted to the data represents the compressibility index. The more sharply α_{av} increases with pressure the more compressible is the filtered material. In spite of the high average specific cake resistance in the

separation of the mixed suspension and even though, in this case, the cake resistance was strongly affected by the filtration pressure, the compressibility index did not increase greatly as a result of mixing.

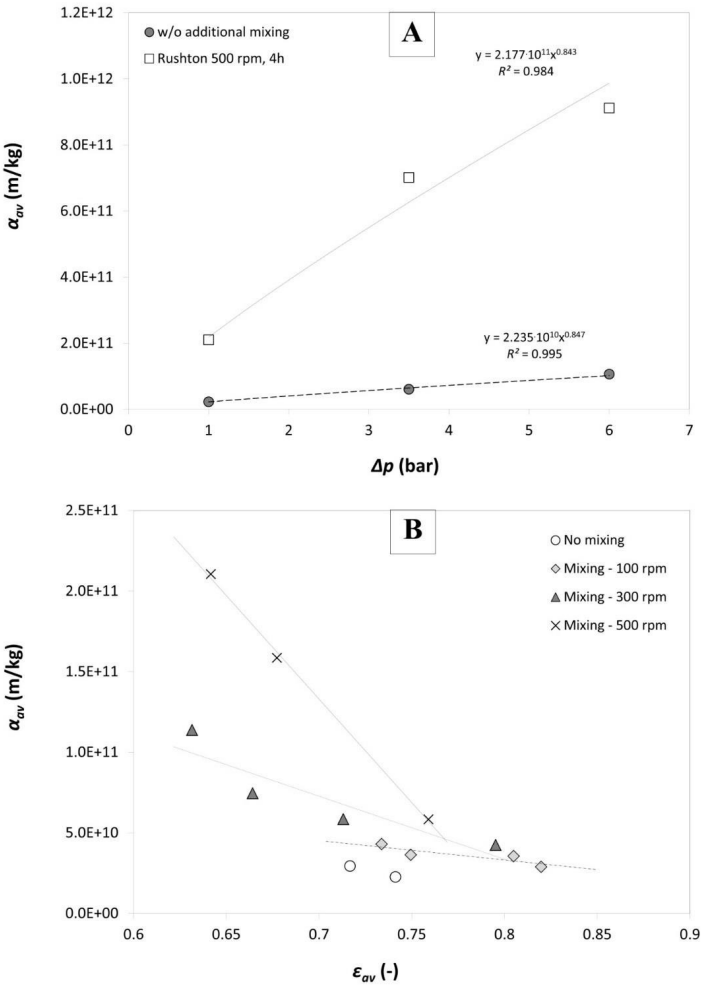

Figure 9: A) Dependence of the average specific cake resistance on the filtration pressure (Δp). The pressure filtration experiments were performed immediately after hydrolysis and after 4 hours of mixing with the Rushton turbine at 500 rpm. B) The average cake porosity and its relationship with the average specific cake resistance, obtained at different mixing rates

The compressibility indices *n* for the cakes obtained for the mixed and non-mixed suspensions were 0.84 and 0.85, respectively. In the case of dewatering of wastewater sludge, the separation is even more difficult: the compressibility index is typically 1-2, sometimes higher,[45] and the compressive pressure does not always affect the filtration rate.[46]

Mixing not only increased the cake resistance but also resulted in a simultaneous reduction in the cake porosity (Fig. 9B). It is clear that mixing speed had a major effect on both the porosity and the average specific cake resistance. The pretreatment and the enzymatic hydrolysis are generally thought to increase the porosity[8] of fibers and vice versa.[47] However, such well-hydrolyzed fibers are more susceptible to mechanical compression in a pressure filter, which leads to a decrease in the cake porosity and an increase in the average specific cake resistance.[48] This is also somewhat analogous to sludge dewatering, where the organic matter has been partly decomposed and the resulting cakes are viscoelastic, highly compressible and cause a high cake resistance.[46] The partial breakdown of the fiber network in the filter cake, as well as the apparent increase in the fibrillation of fibers, which take place while the suspension is mixed intensively, have a similar effect on the cake properties. The validity of the above speculations could be evaluated using more representative sample imaging techniques, preferably for wet fibers. Additionally, it would probably be useful to analyze the solids further in order to evaluate changes in the specific surface area and shape of the fibers and other particles.

Regression model with combined effect of mixing rate and time

Both mixing rate and time had an influence on the average specific cake resistance and the average porosity. The process was investigated employing a linear regression model supplemented with the combined effect of the variables (Eq. (6)):

$$y = \beta_0 + \beta_1 X1 + \beta_2 X2 + \beta_3 X1X2 \qquad (6)$$

where *X1* is the coded value of the rotation speed of the impeller (rpm) and *X2* is the coded value of the mixing time (h). The coded values of variables ranged from -1, which corresponded to the minimum value of the variable, to 1 that represented the maximum. The dimensionless coefficients $\beta_0 - \beta_3$ were determined (Table 2).

Table 2
Dimensionless coefficients $\beta_0 - \beta_3$ for each regression model

Mixer type	*Characteristic*	R^2	β_1	β_2	β_3	β_0
Rushton	α_{av}	0.955	$7.238 \cdot 10^{10}$	$1.899 \cdot 10^{10}$	$1.133 \cdot 10^{10}$	$1.035 \cdot 10^{11}$
	ε_{av}	0.982	$-9.205 \cdot 10^{-2}$	$-5.616 \cdot 10^{-2}$	$-5.600 \cdot 10^{-2}$	$6.571 \cdot 10^{-1}$
Propeller	α_{av}	0.983	$3.432 \cdot 10^{10}$	$2.064 \cdot 10^{10}$	$1.964 \cdot 10^{10}$	$6.391 \cdot 10^{10}$
	ε_{av}	0.965	$-6.393 \cdot 10^{-2}$	$-4.915 \cdot 10^{-2}$	$-3.357 \cdot 10^{-2}$	$7.422 \cdot 10^{-1}$

The coefficients of determination R^2 for all models were good. It is possible to obtain estimates for α_{av} and ε_{av} using Eq. (6), coefficients $\beta_0 - \beta_3$ and the coded values of the variables. In this case, the inclusion of the combined effect of mixing rate and time improved the correlation: for instance, the R^2 values for α_{av} in the case of Rushton turbine and propeller were improved from 0.93 to 0.96 and from 0.81 to 0.98, respectively.

The modeled and measured values for α_{av} and ε_{av}, obtained using both types of mixer, are shown in Fig. 10A-B. The agreement between the modeled values and those measured is good. However, the use of Rushton turbine seems to have resulted in and increased divergence between the measured and modeled α_{av}, although it is difficult to identify a reason for this effect. More generally, the main reason for the good accuracy of the model is that the effect of chemical phenomena, such as those taking place during the pretreatment and hydrolysis stages, were excluded by using the same batch of slurry in the mixing experiments.

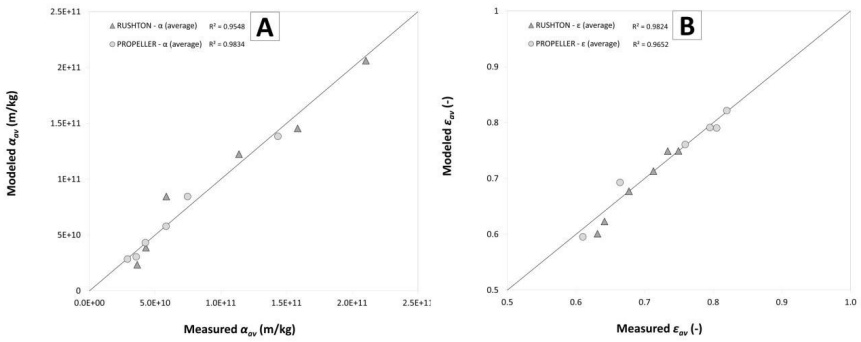

Figure 10: Measured and modeled values for A) the average specific cake resistances α_{av} and B) the average porosities of the filter cakes ε_{av}

CONCLUSION

In this study, the filtration properties of hydrolyzed and agitated biomass suspensions were determined using a pressure filter. It was shown that upstream process conditions have a pivotal role in the solid-liquid separation of a lignocellulosic hydrolysate. The average specific cake resistance increased linearly with the shear pressure caused by mixing. Intensive mixing and pumping of hydrolyzed suspensions should, therefore, be avoided. However, the success in the separation stage should not be predicted based solely on the fiber or particle size data. It is apparent that advanced analysis techniques are necessary in order to better understand the micro- and nano-scale changes that take place in the fibers and that consequently affect the cake filtration characteristics. These techniques include, in particular, 1) nano-scale imaging of the wet solids and 2) thorough investigation of the electrochemical phenomena and interactions.

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Influence of enzyme loading on enzymatic hydrolysis of cardboard waste and size distribution of the resulting fiber residue

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HIGHLIGHTS

- Fiber size of biomass during enzymatic hydrolysis has been investigated.
- Different cellulase and hemicellulase loadings were used.
- Cellulase was mainly responsible for the extent of saccharification.
- The size reduction of fibers occurred rapidly after the enzyme addition.
- The mean fiber length was reduced, at most, by 20%.

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ABSTRACT

Enzymatic hydrolysis of lignocellulosic biomass to sugars alters the properties of the cellulosic fibers. Several process variables, including enzyme loading, play an important role in these changes. Many physical properties of fibers are affected: their length and width, porosity, specific surface area, and degree of fibrillation, for instance, may undergo dramatic changes when subjected to enzymatic degradation. In this study, the influence of enzyme loading on the fiber size was investigated using milled cardboard waste as the raw material. The effect of cellulases and hemicellulases on the monosaccharide production and the resulting fiber size was studied using commercial enzyme products. It was shown that the cellulase loading largely determined the amount of sugars produced. The fiber length was reduced during the course of hydrolysis, although the size reduction was not especially dramatic. Based on the SEM images, no significant damage to the fiber surfaces occurred during the process.

1. Introduction

Various lignocellulosic wastes, residues and crops are under consideration for industrial bioethanol production, in part due to their relatively high cellulose contents and excellent availability. Driven by rising oil prices and the increasing demand for sustainably-produced transportation fuels, the first commercial bioethanol plants for the demonstration of industrial production have recently been constructed (Larsen et al., 2012; Huang et al., 2009). Enzymatic hydrolysis, performed in order to cleave cellulosic polymers to monosaccharides, has been recognized as the key process stage to enable feasible bioethanol production. On the other hand, the main difficulties in the process are related to this challenging process stage which, together with required pretreatment and enzyme production, may contribute to over 40% of the total cost of bioethanol production (Banerjee et al., 2010). Several process configurations for bioethanol production have been proposed, but a number of technical difficulties remain (Cardona and Sanchez, 2007; Hamelinck et al., 2005; Huang et al., 2008).

Cellulases and hemicellulases are the enzymes most typically used to cleave cellulose and hemicellulose to monosaccharides. There are many characteristics that affect the enzyme choice and requirement, such as the substrate type, composition and lignin content (Van Dyk and Pletsche, 2012). The practicality of enzyme loading, in turn, is determined by cost factors, i.e. the cost of enzyme and the price of the end product, and can be optimized (Newman et al., 2013). In the case of industrial processes, in which a high sugar concentration must be obtained, the initial suspended solid concentration in the hydrolysis should be high, preferably over 200 g solids/kg suspension. However, high solid loadings are known to reduce the obtainable yield (Kristensen et al., 2009), which adversely affects the process economy.

Development of effective bioethanol production from lignocellulosic raw materials can be facilitated by an in-depth understanding

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of fiber properties during the enzymatic saccharification. During the degradation of solid biomass (cellulose and hemicelluloses) to their structural sugars, the suspended solids content in the solid–liquid system decreases while the concentration of dissolved solids correspondingly increases. The changes in the physical and chemical composition of the biomass, as well as rheological characteristics of the suspension (Nguyen et al., 2013), may be dramatic.

Previous studies have shown that the both the initial composition and upstream pretreatment of the feedstock have a large influence on the success of enzymatic hydrolysis. The selection of the pretreatment method is influenced, for instance, by the type of raw material and the costs of enzymes (Jorgensen et al., 2007). Pretreatment with steam and/or acids has been widely applied and recognized as effective, in spite of some drawbacks, such as formation of inhibitory compounds (Galbe and Zacchi, 2012). Reduction of the particle size and fiber dimensions of the biomass may improve enzymatic saccharification greatly in many cases (Hoeger et al., 2013; Yeh et al., 2010) but not without exceptions (Del Rio et al., 2012). The enzyme loading also has an important role in the process (Soares et al., 2011). Although high enzyme loading typically results in an enhanced degree of conversion, the relative improvement may be rather poor (Kinnarinen et al., 2012). The main focus of the intensive research on enzymatic hydrolysis has been on the pretreatment stage. However, there are some interesting industrial waste fractions, such as cardboard waste, that can be hydrolyzed even without any other pretreatment than particle size reduction. Consequently, when there is no pretreatment stage, the hemicellulosic sugars can be potentially recovered simultaneously with the main hydrolysis product, glucose. In this process, not only the saccharification itself is interesting, but the properties of non-degraded fiber residue largely determine its potential for utilization. The fiber residue can be pumped into the fermentation stage, recycled back to hydrolysis, perhaps after enzyme recovery by desorption (Moniruzzaman et al., 1997), deliquored (Kinnarinen et al., 2012) and dried for combustion, or utilized in some other way. Selection of the utilization method depends on the fiber properties, from which the size of the fibers is particularly important. Changes in fiber size during the enzymatic hydrolysis of lignocellulosic biomass have, up to date, only been evaluated in a few studies. Most of the previous attempts to study the subject have been made using strongly diluted fiber suspensions, which may have led to excessively high sugar yields and overestimation of the fiber length reduction, as compared to hydrolysis at more realistic solid loadings. The mechanism of fiber length reduction, caused by enzymatic attack, has been recently investigated by Clarke et al. (2011), who observed substantial fiber cutting already during the initial period of hydrolysis.

In this study, the influences of cellulase and hemicellulase loading on the degree of saccharification and fiber size were investigated. Commercially available cellulase and hemicellulase preparations (Cellic CTec2 and HTec, Novozymes, Denmark) were used. The fiber size distributions during the 24 h of hydrolysis were determined using a standard fiber testing instrument, in which the fibers were measured with an image analysis technique. In order to determine the respective sugar concentrations, the liquid phases of the same samples were analyzed using high-performance liquid chromatography (HPLC). Additionally, visual characterization of the fibers was performed using scanning electron microscopy (SEM).

2. Methods

2.1. Composition of cardboard waste

In the experiments, air-dry cellulosic waste was used as the raw material. The raw material consisted mainly of shredded corrugated cardboard, collected from Finland. In addition to cellulose, hemicelluloses and lignin, several impurities were present: pieces of plastic, metals and inorganic minerals were observed in the raw material. Prior to the analyses and experiments, the sample was milled, using a hammer mill, in order to reduce its particle size. The initial particle size is presented in the Results and Discussion Section. An approximate chemical composition of the raw material is presented in Table 1.

The composition was comparable to that of old corrugated cardboard, reported by Yáñez et al. (2004). The cellulose content of the raw material was determined according to the method of Black (1951), utilizing the anthrone reagent in strong sulfuric acid. The proportion of lignin was measured using a liquid chromatographic method (Phenomenex Luna 3u C18(2) column, 20 mM ammonium hydroxide/methanol, 50/50 vol-%, as eluent), described in more detail by Kinnarinen et al. (2012). The hemicellulose content was calculated, not measured, assuming that no extractives were present. Due to the presence of inorganic matter, such as calcium carbonate, used as a filler in paper and cardboard products, the ash content was relatively high. The ash content was determined according to the ISO 1762:2001 standard.

2.2. Preparation of fiber suspensions

The fiber suspensions of 10 wt.% were prepared in sealed plastic bottles ($V = 50\ cm^3$). In each bottle, the same weight of the milled raw material was added and extremely pure RO water (Millipore) was added to form the suspension. After thorough mixing, in a shaker, for one hour, sulfuric acid ($2.0\ mol\ dm^{-3}$) was added to the bottles to gradually adjust the pH to 5.0. In order to obtain an optimum temperature for the hydrolysis experiments, a water bath with bottle holders was prepared and set to a constant temperature of 46.0 °C, which was at an optimum level for the process, according to the enzyme manufacturer.

The enzymes used were commercial preparations (Novozymes, Denmark), consisting of different types of enzymes designed to degrade cellulose and hemicellulose. The cellulase was of the CTec2 type, while the product type of the hemicellulase was HTec. The activity of the cellulase product was not measured, but it was approximately $150\ FPU\ cm^{-3}$, according to Zhou et al. (2013). Based on literature (Eckard et al., 2012), the xylanase activity of Cellic HTec was $1090\ FXU\ cm^{-3}$. Both cellulase and hemicellulase were added simultaneously into the bottles, after which the bottles were closed, shaken manually for 30 s and placed in the temperature-controlled water bath. The experimental plan with the applied enzyme loadings is shown in Table 2.

The enzyme loadings were selected based on prior experience to obtain effective saccharification and enable easy sampling already during the initial period of hydrolysis.

2.3. Experimental procedure

In typical saccharification and fermentation processes for lignocellulosic materials, either separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) is applied for 48–72 h. This study aimed at investigating specifically

Table 1
Approximate composition of the cardboard waste used as the raw material for the experiments.

Component	Concentration (wt.%)	Method or reference
Cellulose	63 ± 1.6	Black (1951)
Hemicellulose	14	Calculated
Lignin	12 ± 0.4	Kinnarinen et al. (2012)
Ash	11 ± 0.2	ISO 1762:2001

Table 2
Experimental plan. The enzyme concentrations are given in relation to the mass of dry raw material.

Test	Enzyme concentration	
	CTec2[a] ($cm^3\ kg^{-1}$)	HTec ($cm^3\ kg^{-1}$)
1	192	5
2	192	10
3	192	20
4	192	40
5	24	40
6	48	40
7	96	40
8	192	40

[a] Estimated activity approximately 150 FPU cm^{-3} (Zhou et al., 2013).

the initial 24 h period of enzymatic hydrolysis, during which time, based on prior experiments, most changes in the fibers typically take place. The procedure for sample preparation and analytical operations is illustrated in Fig. 1.

Six samples were taken from each suspension: one before the enzyme addition and five during the hydrolysis period of 24 h. Prior to sampling, the bottles were shaken to homogenize the suspension. The sampling was performed using conventional Pasteur pipettes (3 cm^3), cut accordingly in order to obtain a significantly enlarged inlet/outlet diameter, which also enabled sampling from the thick, less hydrolyzed suspensions. The total volume of each sample, transferred into plastic test tubes for centrifugation, was intended to be 3–5 cm^3. A Jouan GT 422 bucket centrifuge, equipped with the appropriate holders for the test tubes, was used for separating the suspension into liquid supernatant and a settled fiber bed. The centrifugation was continued at 67 Hz for 120 s.

The supernatant was filtered through a syringe filter with a nominal pore size of 0.2 μm and analyzed for monosaccharides with high-performance liquid chromatography (HPLC). The HPLC procedure is described in Section 2.5.

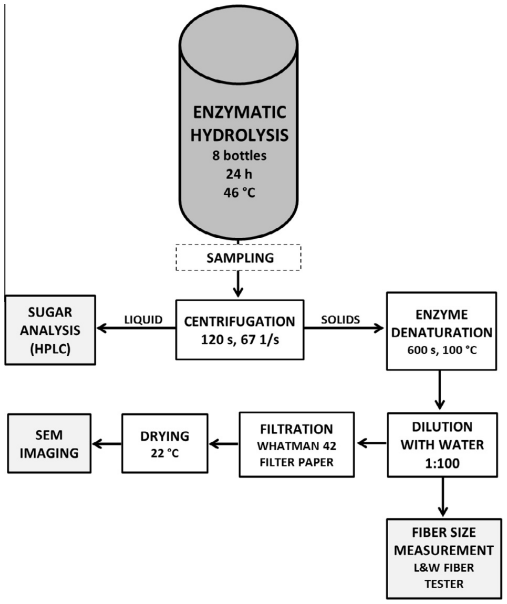

Fig. 1. The experimental procedure.

In order to avoid further degradation of fibers in the test tubes, the enzymes were denatured at 100 °C. The denaturation was performed by keeping the test tubes in a bath of boiling water for 600 s. The fiber samples were then dispersed in water, first in the test tubes with 5 cm^3 of Millipore water prior to placing the samples in a freezer. Prior to fiber size measurement, the samples were again diluted in water in order to separate the fibers from each other. After the fiber size measurements, these dilute suspensions were filtered through Whatman 42 filter paper and dried at room temperature to obtain a solid and relatively flat bed of fibers for scanning electron microscopy (SEM) imaging.

2.4. Fiber characterization

Size distributions of the fiber suspensions at different times during the hydrolysis were determined using a Lorentzen & Wettre fiber tester (Kista, Sweden). The main advantage of this instrument is that both the length and width distribution of the fibers is measured using a two-dimensional image analysis technique. The unit incorporates a very narrow gap between two plates in which the fibers are aligned so that the real dimensions of whole fibers can be measured from the images taken. As a result of milling the raw material prior to the experiments, the fiber length was reduced. This is the main reason why there are large amounts of fibers shorter than those obtainable from fresh wood. The maximum possible measurement range, according to the manufacturer, for the fiber length was 0.2 to 7.5 mm (±0.5%) and the range for the fiber width was 10 to 100 μm (±0.5%). All fibers shorter than 0.2 mm were classified as fines. In order to avoid emphasizing the small fibers, due to their high population, weighted distributions for the fiber length and width were calculated as described in previously by other authors, such as Robertson et al. (1999).

The mean length (L_m) and width of the fibers were determined from length-weighted distributions, which are commonly used in fiber analysis, because the relative number of fine fibers is too high for creating representative number-based population distributions. The calculations of fiber mean size and size distributions were therefore performed according to the principle presented in Eqs. (1) and (2), showing the calculations related to the fiber length:

$$L_m = \frac{\sum_{i=1}^{N} n_i l_i^2}{\sum_{i=1}^{N} n_i l_i}, \tag{1}$$

$$P_{l,i}(\%) = \frac{n_i l_i}{\sum_{j=1}^{N} n_j l_j}, \tag{2}$$

where n_i is the number of measured fibers in each length fraction and l_i is the average length of fibers in a fraction. $P_{l,i}$ is the percentage of length in category i in which the number of fibers is n_i and the average length of fibers is l_i.

A JEOL JSM-5800 scanning electron microscope with a voltage of 15 kV and a magnification of 200× was used for the evaluation of the appearance of the fibers before, during and after the hydrolysis.

2.5. Determination of sugar concentrations

Samples taken from the supernatant after the centrifugation of the primary sample (Fig. 1) were analyzed for monosaccharides. The concentrations of glucose and xylose in the produced hydrolysates were determined by high performance liquid chromatography (HPLC, HP Agilent 1100). A dilute (0.005 mol dm^{-3}) aqueous solution of sulfuric acid was used as the eluent while the column (Varian Metacarb 87H) was kept at 60 °C. The eluent flow rate was 0.01 $cm^3\ s^{-1}$. The injection volume was 10^{-6} dm^3 and a

precolumn was connected to the feed line to protect the column from solid particles.

3. Results and discussion

The effect of cellulase and hemicellulase loading on the degradation of cardboard waste to glucose and xylose, was studied using a wide range of enzyme loadings. In Fig. 2(a), the hemicellulase loading (volume of enzyme/weight of biomass) was constant, 40 $cm^3\ kg^{-1}$, and the effect of cellulase loading on the glucose production was investigated. Based on Fig. 2(a), it is clear that the rate of enzymatic hydrolysis slows rapidly after the first hours of hydrolysis. Another apparent conclusion from Fig. 2(a) is that the enzyme consumption increases relatively more sharply than the obtained glucose concentration: doubling the enzyme loading does not result in a doubling of the glucose concentration of the hydrolysate. The final yields after 24 h of hydrolysis ranged from 0.36 to 0.63 $kg_{glucose}\ kg^{-1}_{cellulose}$. As can be seen in the experimental plan, Tests 4 and 8 were identical. This test was performed twice in order to evaluate the repeatability of the experiments. The final yields obtained in Tests 4 and 8 were 626 and 629 $g_{glucose}\ kg^{-1}_{cellulose}$, respectively, which indicates good repeatability.

The influence of hemicellulase loading on glucose production is shown in Fig. 2(b). It is probable that in this case hemicellulose is not a major obstacle for the conversion of cellulose to glucose. The high cellulase loading may be the most important reason for the weak improvement in the hydrolysis of cellulose that was obtained as a result of increased hemicellulase loading.

In Fig. 2(c) and (d), the impacts of cellulase and hemicellulase loading on the xylose concentration of the hydrolysate are summarized. It is clear from Fig. 2(c) that degradation of cellulose was mainly responsible for increasing the availability of hemicelluloses for enzymatic degradation. Therefore, the hemicellulase loading (Fig. 2(d)) was of minor importance for hemicellulose conversion. These results imply that the cellulase preparation CTec2 itself, taking into consideration its high dosage, was so effective in hydrolyzing both cellulose and hemicellulose that the effect of hemicellulase addition becomes negligible. It is difficult to find the measured xylanase activity of Cellic CTec2 in the existing literature. However, results of previous studies (Zhou et al., 2013; Chen et al., 2013) indicate that this enzyme product has significant xylanase activity.

Hu et al. (2011) obtained clear improvements in the glucose and xylose yield using a mixture of commercial cellulase (Celluclast 1.5L) and β-glucosidase (Novozym 188) with xylanase (Multifect Xylanase) addition in the hydrolysis of steam-pretreated corn stover. The xylanase activity of the Multifect xylanase was 2600 U cm^{-3} and the Celluclast product had also significant (440 U cm^{-3}) xylanase activity. The results of Hu et al. (2011) indicated that the positive effect of xylanase addition was more significant when the cellulase dosage was low. This observation supports the results of the present study, where the applied cellulase dosages were exceptionally high and hemicellulose addition did not improve the hydrolysis significantly. The results of Lin et al. (2010), obtained using artificial biomass (prepared from MCC from wood pulp, and xylan and lignin from birch wood) and

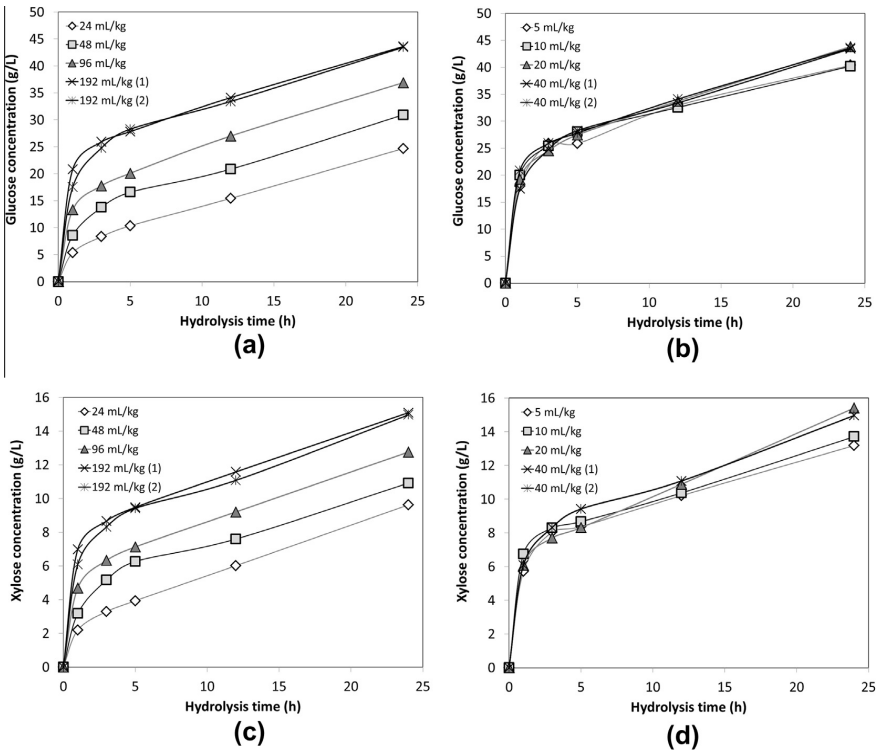

Fig. 2. Effect of cellulase (a) and hemicellulase (b) loading on conversion of cellulose to glucose. The hemicellulase loading in Fig. 2(a) was 40 $cm^3\ kg^{-1}$ and the cellulase loading in Fig. 2(b) was 192 $cm^3\ kg^{-1}$. Effect of cellulase (c) and hemicellulase (d) loading ($cm^3\ kg^{-1}$) on conversion of xylan to xylose. The hemicellulase loading in Fig. 2(c) was 40 $cm^3\ kg^{-1}$ and the cellulase loading in Fig. 2(d) was 192 $cm^3\ kg^{-1}$.

commercial cellulase (ACCELLERASE 1500) and xylanase (OPTIMASH™ BG) showed a relatively small improvement in the xylose yield. According to Lin et al. (2010), the former of these enzyme products contained also hemicellulases. Generally, the optimum cellulase/hemicellulase ratio seems to depend on the properties of the substrate, the amount of enzyme(s) used and, regarding commercial enzyme preparations, the composition of the cellulase or hemicellulase product.

There are three major phenomena that have been proposed to restrict the sugar release and to cause the typical shape of the sugar concentration curves, seen in Fig. 2: limited accessibility of cellulose, which is also related to the lignin content, deactivation of enzymes during hydrolysis, and product inhibition caused by sugars (MacLellan, 2010). In certain situations, for pretreated suspensions in particular, the negative effect of lignin on the hydrolysis has been shown to be smaller than the influence of cellulose accessibility (Wiman et al., 2012). In the case of the cardboard waste used in the present study, the cellulose content of the substrate is high and the lignin content is lower than that of fresh biomass substrates. The matrix of carbohydrate polymers and lignin has also been opened in the pulping process. However, enzymatic hydrolysis of cardboard waste to sugars is hampered by the fiber hornification, i.e. reduction of the water binding capacity of the fibers, resulting from fiber drying. This reduces the enzyme accessibility, which is one important reason for the relatively low yield obtained at reasonable enzyme loadings.

In most studies related to enzyme loading, or enzymatic hydrolysis in general, high amounts of enzymes have been used. The enzyme dosages used in the present study were also too high for a commercial process. In order to enable commercially viable saccharification, significantly lower enzyme loadings should be used. This can be achieved by proper pretreatment and by recycling the enzymes. Adsorption and desorption of enzymes for the purpose of enzyme recycling have been studied in some previous articles (Kristensen et al., 2007; Weiss et al., 2013).

The effect of cellulase loading on the mean length and width of fibers during enzymatic hydrolysis is illustrated in Fig. 3(a) and (b).

It can be observed in Fig. 3(a) that there is, at most, a reduction of approximately 20% in the fiber mean length. The lowest enzyme loading of 24 $cm^3\ kg^{-1}$ seems to result in smaller changes in the fiber length, which can be clearly observed after the initial 5 h of hydrolysis. On the other hand, it is difficult to say whether the slight increase in the measured mean length in the following two cases is only random variation resulting from sampling and measurement errors.

In Fig. 3(b), the shape of the graphs presenting the fiber mean widths during the hydrolysis is very interesting. As could be expected, the mean width of the fibers started to decrease rapidly after the enzyme addition. What happened after this very initial period is likely to result from swelling of fibers, increased fibrillation, which could increase the measurable fiber width, or simply size reduction of small fibers to such an extent that they were classified as fines, and therefore excluded from the width distribution.

Under those experimental conditions where the cellulase loading was exceptionally high, hemicellulase addition was not significantly responsible for a reduction in the fiber length (Fig. 3(c)). The use of high cellulase loading caused an initial reduction in the fiber width, after which the fiber width increased slowly by about 17% (Fig. 3(d)). The hemicellulase loading was not observed to be of any importance in this process.

Unlike most previous studies, the present study was performed in non-mixed conditions, in order to investigate the sole effect of enzymatic hydrolysis on the fiber dimensions. Factors other than the enzyme loading also appear to have an influence on the fiber size. Samaniuk et al. (2011) studied the effect of mixing on the average fiber length during the very initial stage of enzymatic saccharification. Their mixing experiments were performed using a modified torque rheometer equipped with motorized counter-rotating screw elements. They also made control experiments, without mixing, and observed that the average fiber length was not significantly affected by the enzymes when the reaction suspension was not mixed. Because the duration of hydrolysis in the study of Samaniuk et al. (2011) was only 40 min, while it was 24 h in the present study, the results are not well comparable. Chinga-Carrasco et al. (2010) studied the same topic from a microscopic point of view by monitoring submicron-scale changes in Kraft pulp fiber surface during enzymatic hydrolysis carried out in shaken flasks. It was noticed in their study that enzymatic hydrolysis using Novozym 188 resulted in changes in the fibrillar structure of the fibers, including changes in the morphology and reduction of anisotropy, i.e. a measure of degree of orientation of the surface texture of the fibers. These changes may affect the behavior (e.g. the rheology and the water retention capacity) of the fiber suspension in the subsequent process stages more than the macroscopic size reduction of the fibers. In an earlier study (Ramos et al., 1993), a rapid size reduction of eucalyptus fibers was obtained, which consequently increased the proportion of fines in the suspension. The rapid size reduction obtained in the beginning of hydrolysis is in line with the results of the present study.

In papermaking, enzymatic modification of fibers has been performed for decades. The aim in this case is to make the pulp properties more beneficial, while avoiding conversion of high amounts of cellulose to sugars. The cutting of fibers to shorter fragments cannot typically be completely avoided when cellulases are used. Pere et al. (1995) used two different cellobiohydrolases and endoglucanases produced by *Trichoderma reesei* for the modification of unbleached pine kraft pulp. They obtained neither a significant yield of hydrolysis nor reduction in the fiber length, but the viscosity was significantly reduced. The primary reason for the small change in the fiber length may have been the low enzyme dosage. However, dramatic changes in the pulp viscosity were observed, which was mainly due to the presence of endoglucanases. With respect to the viscosity, similar observations have been reported in previous articles (Samaniuk et al., 2011; Nguyen et al., 2013).

As regards the fiber size distributions, clear changes in the shape of the differential length-weighted distribution occurred during the hydrolysis. The reduction in the fiber length is readily observed (Fig. 4(a)). Most of the length reduction took place during the first three hours of hydrolysis and the shape of the distribution became narrower and more regular as the largest fibers were hydrolyzed. Consequently, the peak height of the length distribution was increased by 60% during the hydrolysis time of 24 h. The final fiber length distributions after 24 h, on the other hand, were not much influenced by the enzyme loading, apart from one exception (Fig. 4(b)).

It is apparent that the lowest cellulase loading used in Test 5 was insufficient to affect the fiber length as effectively as the higher loadings. It can thus be concluded that the general level of cellulase loadings in this study was too high to produce observable differences, i.e. the length reduction was at the maximum level obtainable using the enzyme products in question.

A theory on fiber cutting during enzymatic hydrolysis of lignin-free fibers has been proposed by Clarke et al. (2011). They recognized certain dislocation sites, where the fiber cutting took place, while the decrease in the fiber length was extremely significant. The results of Clarke et al. (2011) show the essential importance of the first hours of hydrolysis for fiber size reduction, which supports the findings of the present study. However, the magnitude of size reduction in their study was greater, which is likely to have resulted from the absence of lignin and the low substrate loading (20 g kg^{-1}).

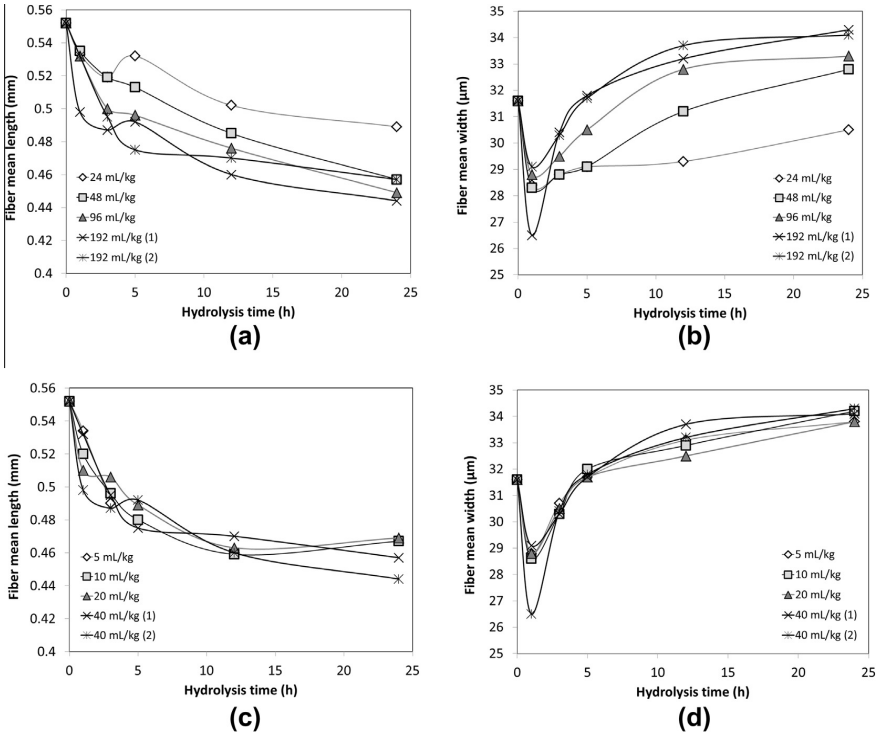

Fig. 3. Effect of cellulase loading ($cm^3\ kg^{-1}$) on the fiber mean length (a) and width (b) during the 24 h hydrolysis period when the hemicellulase loading was 40 $cm^3\ kg^{-1}$. Effect of hemicellulase loading ($cm^3\ kg^{-1}$) on the fiber mean length (c) and width (d) during the 24 h hydrolysis period. The cellulase loading was 192 $cm^3\ kg^{-1}$.

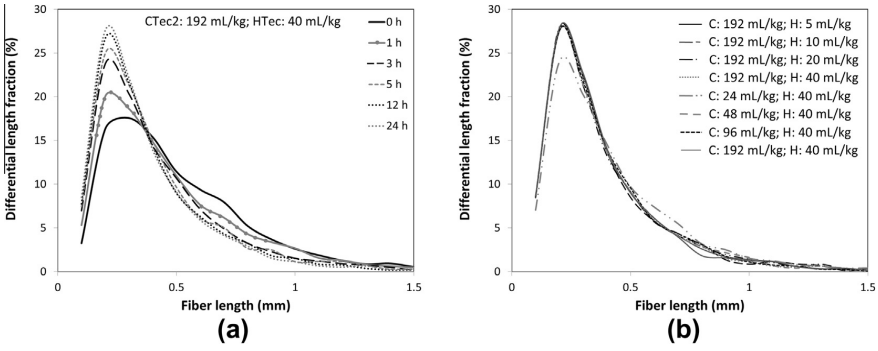

Fig. 4. Test 4: Changes in the fiber length distribution during the 24 h hydrolysis period at the highest enzyme loading, CTec2 192 $cm^3\ kg^{-1}$/HTec 40 $cm^3\ kg^{-1}$ (a). All experiments: influence of enzyme loading ($cm^3\ kg^{-1}$) on the final fiber size distribution after 24 h of hydrolysis (b).

Based on the results presented in Figs. 2 and 3, the repeatability of the experiments seems to be good. The largest uncertainty in the results is related to the early stage of hydrolysis, when the sampling error was probably larger than in the end of hydrolysis.

The fiber size analyzer was used to measure the fiber lengths within a range of 0.2–7.5 mm. All fibers shorter than 0.2 mm were classified as fines, the proportion of which increased during hydrolysis. The proportion of fines rose from the original 24% to 58% in the case of the highest cellulase loading and to 45% in the case of the lowest cellulase loading.

SEM images of the original fibers, prior to and during hydrolysis, are presented as electronic Supplementary material.

When it comes to the surface of the fibers, no clear changes can be observed. This can result from the drying procedure used for the sample preparation, which causes shrinkage of fibers and may, therefore, hide the most apparent changes. However, there may be some changes in the structure of the fiber network. It appears that the most hydrolyzed fibers form a denser and flatter network, compared to the other cases. This could result from the decrease of the fiber strength, which in turn is mostly caused by hydrolysis of

the main supporting component, cellulose. It is difficult to make more detailed conclusions from the SEM images, because in this case the images could only be visually evaluated. In order to analyze the SEM images, possibly to associate SEM imaging with the fiber size data, advanced software should be used.

4. Conclusion

In this study, the effects of enzyme loading on the saccharification of cardboard waste and characteristics of the residual fibers have been investigated. The results show that the reduction of fiber size is moderate and takes place very rapidly after the addition of enzymes. Visual observations from the SEM images supported these conclusions. Cellulase was observed to be responsible for most changes in the extent of saccharification and fiber length reduction. Future studies could focus on more detailed characterization of partially hydrolyzed fibers. The influence of impurities (minerals, ink, lignin, etc.) on the enzyme requirement could also be evaluated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.02.091.

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