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Hydrolysis and composition of recovered fibres fractionated from solid recovered fuel

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HIGHLIGHTS

- Recovered fibres were fractionated from solid recovered fuel and characterised.
- Recovered fibres have a high content of carbohydrates and ash.
- The carbohydrates in recovered fibres can be hydrolysed to sugars with enzymes.
- The effect of solids loading and surfactants on enzymatic hydrolysis was studied.

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ABSTRACT

Fibres fractionated from solid recovered fuel (SRF), a standardised market combustion fuel produced from sorted waste, were considered as a source of lignocellulosic fermentable sugars. The fibre yield from four samples of SRF was 25–45%, and the separated material consisted of 52–54% carbohydrates, mainly glucan, with a high content of ash (12–17%). The enzymatic digestibility of recovered fibres was studied at low and high solids loading and compared with model substrates containing only chemical and mechanical pulps. Above 80% hydrolysis yield was reached at 20% solids loading in 48 h, but variation was observed between different samples of recovered fibres. Surfactants were found to improve the hydrolysis yield of recovered fibres especially in tumbling-type of mixing at low solids loading, where hydrolysis was found to stagnate without surfactants. The results suggest that SRF is a potential source of easily digestible lignocellulosic carbohydrates for use in biorefineries.

1. Introduction

Sugars are raw material for microbial and chemical processes producing biofuels such as ethanol and butanol, polymer precursors like lactic and succinic acid, and other chemicals including xylitol and furfural (Menon and Rao, 2012). In addition a vast fermentation industry producing e.g. amino acids, antibiotics and industrial enzymes is using starch- or sucrose based glucose as a raw material. As the sustainability of using sugars from the food chain for the production of non-food products has been disputed, there is a need to find alternative, non-food sources of sugar for the non-food industry. Various lignocellulosic feedstocks could, in this respect, serve as an interesting alternative. The first commercial-scale lignocellulosic ethanol plants are currently under construction or have recently started operations (Balan et al., 2013). Corn stover, sugar cane bagasse, wheat straw and the energy crop *Arundo donax* are favoured as feedstocks in these first industrial plants. Other potential biomass sources besides agricultural residues are forest industry related biomass streams. Native woody streams like logs, harvest residues, saw dust and bark, and some more processed streams like pulp and paper mill sludges are rich in carbohydrates, but may be expensive or challenging to process. Recovered pulp and paper industry products are an alternative non-food source of sugars, which could be used for

Abbreviations: CBH, cellobiohydrolase; CHP, combined heat and power; EG, endoglucanase; GMO, genetically modified organism; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; MUL, 4-methyl-umbelliferyl-β-D-lactoside; PEG, polyethylene glycol; SRF, solid recovered fuel; TMP, thermomechanical pulp.

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the production of transport fuels and chemicals instead of being directly combusted to energy.

Solid recovered fuel (SRF) is defined as a solid fuel prepared from non-hazardous waste meeting the classification and the specification requirements laid down in the standard EN15359 (ERFO, 2013). SRF is typically municipal solid, industrial, or commercial waste, which is homogenised and upgraded to a quality that can be traded amongst producers and users. SRF is a heterogeneous fuel with a net calorific value from 3 to above 25 MJ/kg depending on the source. The current production of SRF is circa 12 Mt/a in the EU, however, the potential is much higher, estimated at circa 70 Mt/a (Straetmans, 2010). The production of refuse-derived fuel (RDF), which is shredded and sorted but not standardised, is even larger (Rotter et al., 2011). SRF is utilised for energy production in cement kilns, coal-fired power plants, lime kilns, industrial boilers and combined heat and power (CHP) plants reducing the amount of waste going to landfill. The largest European CHP and power plant capacities for SRF are currently in Germany, Finland and Sweden (ERFO, 2013).

The lignocellulose content of SRF is a key attribute as it is the climate neutral part of the fuel (Flamme and Geiping, 2012). Astrup et al. (2009) estimated the paper content of SRF to be 54% of dry content, and reviewed the biogenic carbon content to be from 45% to 85% of the total carbon, which covers 45% of SRF dry weight. This biogenic carbon present mostly in the form of cellulosic and non-cellulosic carbohydrates offers a waste-based low-cost source of fermentable sugars alternative to agricultural residues, which are seasonal and more recalcitrant. The fractionation of carbohydrates from SRF in the form of recovered fibres, and their recalcitrance towards enzymatic hydrolysis are the key factors in producing fermentable sugars from SRF. We have recently published pilot-scale trial data on an interesting biorefinery concept consisting of the fractionation of SRF, the hydrolysis of recovered fibres, ethanol fermentation and biogas production (Kemppainen et al., 2012). What is yet to be reported are the fractionation yields of recovered fibres from SRF, their composition, and the detailed behaviour of the material in enzymatic hydrolysis compared to its pure main constituents, chemical and thermomechanical pulp. The present literature on similar concepts is typically based on experiments at low processing consistency although a relatively high processing consistency appears to be necessary for feasible conversion processes of lignocellulosics (Modenbach and Nokes, 2013). It is thus important to understand the effect of solids loading on the hydrolysis yield of a particular feedstock. Surfactants are widely used chemicals that have been shown to improve the enzymatic hydrolysis of several different types of biomass (Eriksson et al., 2002). Assessing the effect of surfactants on a new type of biomass can bring down enzyme costs in an envisioned industrial plant. This paper analyses the suitability of recovered fibres fractionated from solid recovered fuel as a source of sustainable fermentable sugars.

2. Methods

2.1. Raw materials, enzymes and chemicals

SRF samples were received from three suppliers in the United Kingdom and one supplier in Finland. A sample of pulp and paper mill fibre sludge was received from an integrated pulp and paper mill in Finland. Never-dried birch and spruce kraft pulps, received as samples from Finnish pulp mills, were mixed in ratio of 51/49 w-% for use in hydrolysis experiments. A model substrate was composed by mixing birch kraft pulp, spruce kraft pulp and Finnish spruce thermomechanical pulp in ratio of 35/34/31 w-%.

Several different enzyme mixtures were constituted from nonpurified monocomponent enzymes provided by Roal Oy, Finland. The basic enzyme mixture contained Cel7A from *Acremonium thermophilum* (cellobiohydrolase I) and Cel5A from *Thermoascus aurantiacus* (endoglucanase), and was supported by the addition of Cel6A (cellobiohydrolase II) from *A. thermophilum* or *Chaetomium thermophilum,* Cel7B (endoglucanase) from *Trichoderma reesei*, xylanase from *Nonomuraea flexuosa* or *T. aurantiacus,* mannanase from *T. reesei* and β-glucosidase from *T. aurantiacus* or *A. thermophilum*. Protein content of the enzymes used in Figs. 1–3 was determined by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA), which is based on the Lowry assay using bovine gamma globulin as standard. Protein content of the enzymes used in Figs. 4 and 5 was measured using Bovine Serum Albumin as standard.

The nonionic surfactant products used in the experiments were PEG 4000 (Merck, Germany), Lutensol AT 50 Flakes (BASF, Germany), and Softanol 90 (Ineos, Switzerland). Other chemicals were analytical or technical grade depending on their purpose of use.

2.2. Fractionation of SRF

Fibres were fractionated from SRF in small (7.5 kg dry weight SRF treated per batch) and large pilot-scale (300 kg SRF treated per batch). Fibre batches A, B and C were produced by re-pulping the SRF samples from the United Kingdom in small pilot-scale for 20 min at 5% consistency (150 kg total mass) in a 200 L and a 11 kW batch pulper (Tampulping, Finland). The disintegrated fibre sludge was let out of the reactor through a sieve plate with 3 mm width holes. The reject was washed 3 times with a total of 130 L water. The sludges and washing waters from two pulping batches were combined, and pH was adjusted to 5 using strong sulphuric acid. The fibre sludge was dewatered in a decanter centrifuge producing a stream of recovered fibres at 34–38% dry matter content, and heat treated at 95 °C for 60 min to reduce microbial load.

Batch D fibres were fractionated from SRF from the same supplier as batch A fibres, but in large pilot-scale using a 8 m^3 pulping tank with a 110 kW motor. Sample E was fractionated similarly to sample D but before pH adjustment a volume of dilute pulp and paper mill fibre sludge was added to make up 25% of the dry matter of the total sludge. Heat treatment was conducted after adjustment of pH with strong phosphoric acid. The dewatering of the recovered fibres was carried out on a belt press to produce circa 320 kg (on dry) recovered fibres per batch at 43% average dry matter content.

2.3. Hydrolysis experiments

Hydrolysis experiments were carried out as triplicates in two experimental setups: in test tubes in a water bath under magnetic mixing, and in round plastic bottles in a rotating drum placed in a heat cabinet. Conditions for test tube hydrolysis were: 1% solids content, 3 ml total volume, 400 rpm magnetic mixing. Conditions for bottle hydrolysis were: 1–25% solids content, 50 ml total volume (40% bottle fill volume), 24 rpm of the rotating drum. The bottles were moving freely in the rotating drum. Temperature was controlled at 50 °C and sodium acetate buffer with 0.02% sodium azide was used in all experiments. Buffer strength of 50 mM was used in the test tube experiments, whereas in the bottle experiment the final buffer strength in the reaction was adjusted to 100 mM. Enzyme mixtures were dosed as mg protein per g of substrate using dosages in the range of 4–16 mg/g. Surfactants were dosed 1% w/w of the substrate dry weight and added to hydrolysis by dissolving them to the buffer. Hydrolysis was stopped by boiling

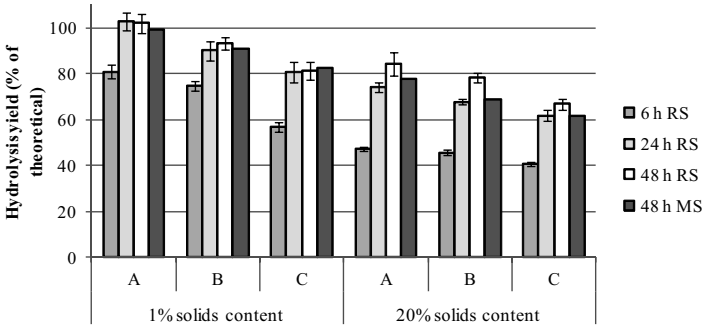

Fig. 1. Enzymatic hydrolysis of recovered fibres from SRF batches A–C at low (1%) and high (20%) initial solids content, 1% w/w Lutensol AT 50. RS = reducing sugars, MS = monosaccharides. The basic enzyme mixture was supplemented with *Acremonium thermophilum* Cel6A, *Trichoderma reesei* Cel7B, *Thermoascus aurantiacus* xylanase, *Thermoascus aurantiacus* β-glucosidase, and *Trichoderma reesei* mannanase (total enzyme dosage 8 mg/g).

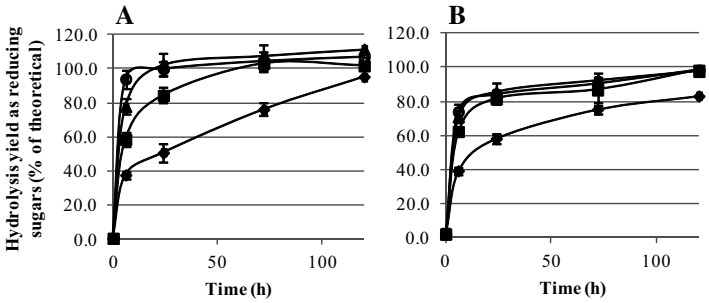

Fig. 2. Dosage response curves for chemical pulp (A) and recovered fibres from batch E (B) at low (1%) initial solids content. Enzyme dosages: Open circles 16 mg/g, triangles 12 mg/g, squares 8 mg/g, diamonds 4 mg/g. The basic enzyme mixture was supplemented with *Acremonium thermophilum* Cel6A, *Trichoderma reesei* Cel7B, *Nonomuraea flexuosa* xylanase, *Acremonium thermophilum* β-glucosidase, and *Trichoderma reesei* mannanase.

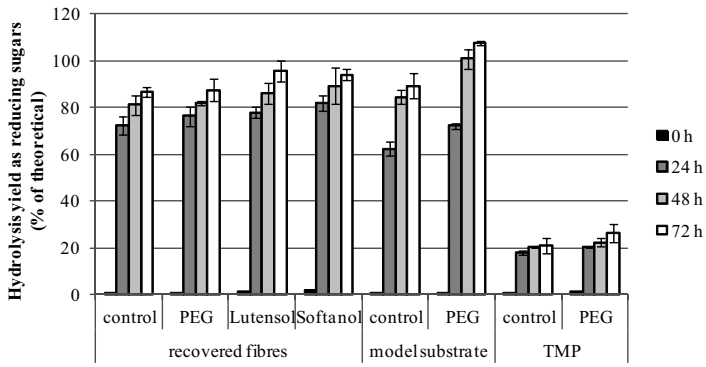

Fig. 3. Hydrolysis of recovered fibres (batch E), model substrates, and TMP in low initial solids content (1%) with different surfactants (1% w/w). The basic enzyme mixture was supplemented with *Acremonium thermophilum* Cel6A, *Trichoderma reesei* Cel7B, *Nonomuraea flexuosa* xylanase, *Acremonium thermophilum* β-glucosidase, and *Trichoderma reesei* mannanase (total enzyme dosage 4 mg/g).

a sample for 15 min, and insoluble solids were removed by centrifuging the sample. All samples taken from the bottle set-up were pre-diluted by a factor of ten before solids removal to eliminate the effect of high content of water insoluble solids on yield calculations (Kristensen et al., 2009). Hydrolysis yields were expressed as percentage of the theoretical maximum hydrolysis yield.

2.4. Analysis of chemical composition and hydrolysis products

The composition analysis of the fibre samples and the analysis of hydrolysis products were carried out as described by Kemppainen et al. (2012) except the acid soluble lignin content of fibre samples A–D was analysed according to Goldschmid (1971). As the acid soluble lignin content was very small, the

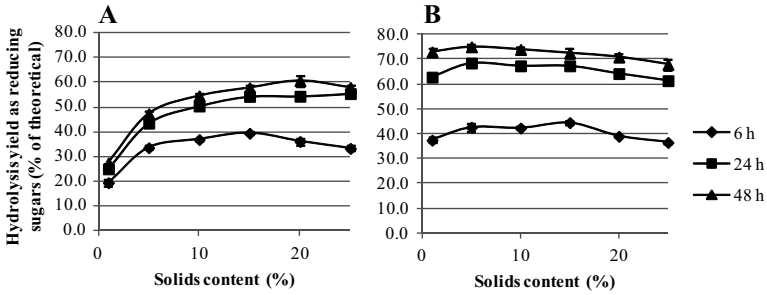

Fig. 4. Enzymatic hydrolysis of recovered fibres (batch E) under gravitational mixing at varying solids loading without (A) or with (B) surfactant (1% w/w Lutensol AT 50). The basic enzyme mixture was supplemented with *Chaetomium thermophilum* Cel6A, *Thermoascus aurantiacus* xylanase and *Thermoascus aurantiacus* β-glucosidase (total enzyme dosage 6 mg/g).

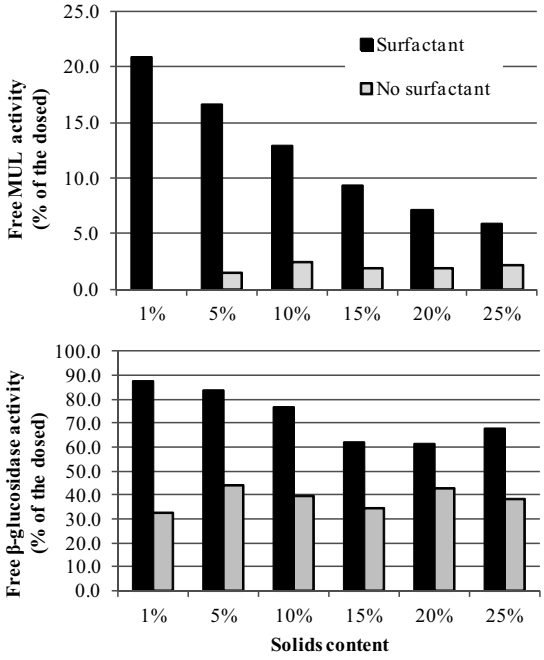

Fig. 5. Free MUL- and β-glucosidase activity in the solution after 48 h hydrolysis with or without surfactant at 1–25% solids loading. The basic enzyme mixture was the same as in Fig. 4.

change of method is not expected to have a significant effect on the reliability of the results. The elemental composition of ash was analysed by inductively coupled plasma mass spectrometry after microwave assisted hydrofluoric acid–nitric acid–hydrochloric acid dissolution.

2.5. Enzyme activity assays

The cellulase activity present in the hydrolysis supernatants was assayed using 4-methyl-umbelliferyl-β-D-lactoside (MUL) according to Bailey and Tähtiharju (2003) except that the only inhibitor compound used in the experiment was glucose. β-glucosidase activity was assayed using 4-nitrophenyl-β-D-glucopyranoside as described by Bailey and Nevalainen (1981).

3. Results and discussion

3.1. Separation of recovered fibres from SRF

Prior to using the fibre components from SRF as a source of lignocellulosic sugars, they have to be separated from the plastics and other main components in the feedstock, and chemically characterised. This paper, to the authors' knowledge is the first description of separation yields and the chemical composition of the fractions. The first target was to study the yield and composition of recovered fibres separated from SRF obtained from three different suppliers. Visually the fuel samples looked very different from each other. The SRF used to produce the fibre sample A had the smallest particle size (circa <5 cm) and most homogenous appearance being composed only of soft plastics, paper, and board. Sample C SRF had the largest particle size and contained hard objects like metal cans, pieces of wood, hard plastics, and rocks, that were removed manually prior to pulping. The particle size of the SRF for sample B was between the previous two, and the material contained long flexible pieces of hard plastic that were removed prior to pulping. The separation of recovered fibre batches A–C was carried out by high force repulping in small pilot scale to disintegrate the paper and board components. The fibre sludge was let out of the reactor through a sieve plate and the fractionation yield was increased by washing the reject 3 times with water. The dry matter content of the combined sludge and washing water was between 0.8% and 1.1%. Depending on the sample 35–44% of the dry mass of the SRF was recovered in the combined sludge and washing waters forming the accept (Table 1). The accept contained some small pieces of plastics but the majority of plastics was retained in the reject. The differences in yields may reflect the fibre content

Table 1
Separation yields and chemical composition of recovered fibres, batches A–E.

Batch	A	B	C	D	E
Fractionation yield, %	44.1	41.1	34.7	51.2	n.a.
Dewatering yield, %	75.7	64.4	72.3	87.1	n.a.
Total yield, %	33.4	26.5	25.1	44.7	n.a.
Carbohydrates, % of dry matter	53.6	53.8	52.4	52.4	53.8
Glucan	43.1	43.4	42.8	42.2	40.9
Xylan	6.7	6.4	5.5	6.6	7.8
Mannan	2.9	3.1	3.2	2.9	3.9
Arabinan	0.4	0.5	0.4	0.3	0.4
Galactan	0.4	0.5	0.5	0.4	0.7
Lipophilic extractives, % of dry matter	8.2	3.6	5.3	6.3	6.4
Acid insoluble material, % of dry matter	19.7	22.8	24.2	24.0	23.4
Acid soluble lignin, % of dry matter	0.6	0.6	0.8	0.6	0.5
Ash, % of dry matter	11.9	17.0	15.0	12.5	12.7

of the SRF sample but also the type and origin of the fibre components. Products prepared using wet-strength chemicals like liquid packaging boards, paper bags and some tissues disintegrate less efficiently and may remain in the reject. In efficient industrial pulping equipment a slightly increased particle size may be beneficial for the process limiting the size reduction of the plastics during pulping and thus reducing the amount of impurities in the fibre fraction. In this study the SRF sample with the smallest particle size and most homogenous appearance produced the highest fractionation yield, but as observed later, also had a high content of carbohydrates.

The separation efficiency of dewatering carried out using a decanter centrifuge is affected by the pumping rate and the particle size of the material. The dry matter content of the filtrate was 0.3–0.4%, whereas the dry matter content of the dewatered fibre fraction was 34–38%. The dewatering yields achieved ranging from 64% to 76% probably reflect the content of fines in the fibre sludge as some insoluble material was lost during dewatering despite of the slow pumping rate of the slurry to the decanter centrifuge. Ash and other material solubilised during pH adjustment and very fine insoluble ash may also constitute a part of the dry matter lost during dewatering.

Fractionation of SRF for batch D fibres in large pilot scale reached higher fractionation and dewatering yields compared to small pilot-scale. It is suspected that the disintegration was more efficient in the large pilot scale leaving fewer fibres in the reject. In the belt press the fibres may function as a filter aid improving the retention of fines. Overall the reported total yields in this study are expected to present the minimum, which can be reached, when fractionating fibres from SRF in a suboptimal pilot scale equipment. A process designed especially for these materials should be able to increase the total yield up to 50–55% and produce a relatively clean reject stream that could be either combusted or even recycled for plastics. In this case, assuming 54% paper-based content in SRF (Astrup et al., 2009) and 90% yield in fractionation, the plastic and other impurities would account for 3–12% of the recovered fibre fraction. However, it must be noted that also the paper-based fraction contains ash, lignin and other non-carbohydrate components.

3.2. Composition of recovered fibres

The composition of the recovered fibres was analysed (Table 1). The results indicated large variations between different suppliers and fractionation scales. Batches A–D comprised only fibres from SRF, but the batch E also contained 25% pulp and paper mill fibre sludge. The largest variation between the samples was observed in the content of ash and lipophilic extractives. Lipophilic extractives extracted with heptane may contain native wood extractives but also chemicals used in inks, glues and coatings. Ash in the recovered fibres is composed of mainly inorganic coating and filler materials, such as kaolin, calcium carbonate, and talc. Main inorganic elements found from the batch E materials were Si (2.0% of dry weight), Ca (1.7%), Fe (1.2%), Al (0.6%), Mg (0.3%), P (0.3%), S (0.2%) and K (0.1%). Si, Ca, Al and Mg originate from the abovementioned filler materials, but the source of iron can only be speculated. For comparison, Kang et al. (2010) concluded that the ash in a sample of kraft paper mill primary sludge (36%) was composed mainly of calcium carbonate, clay and titanium oxide. Both the ash and the extractives content of the recovered material is greatly affected by the type of the fibre product since the majority of these compounds do not originate from wood. The same applies at least partially to the acid insoluble material termed as Klason lignin for wood, because it covers also acid insoluble plastic impurities in the material. In addition it contains the acid insoluble portion of the ash. Chemical pulp contains 3–5% lignin, and the lignin content of mechanical pulp is close to that of native softwood, 25–35% (Biermann, 1996). Chemical pulp is used in sack papers, the lining of particle boards, some wrapping papers, liner boards, and on the top ply of packaging boards as well as in printing papers. Mechanical pulp often forms the centre ply of packaging board and is used in newspapers and other print products. Recycled fibre pulp is commonly used in making packaging materials and it may be composed of varying combinations of chemical and mechanical pulp. Wang et al. (2013) report 17.1% acid insoluble lignin in newspaper, 4.7% in office paper, 13.9% in magazines and 14.2% in cardboard. The determination of the actual lignin content of our materials would require additional separation methods, but it can be estimated that Klason lignin comprised less than 70–80% of the acid insoluble material.

There was little variation in the carbohydrate content or the monosaccharide profile of the recovered fibres. Having a high content of C6 sugars (50.6–52.2% as monosaccharides) the material is very suitable for concepts carrying out only C6 fermentation with common non-GMO yeasts. The content of xylan and mannan reflects the fact that the material is a combination of hardwood and softwood pulps. For example the xylan contents of bleached kraft hardwood and softwood pulps are 25% and 7.4% respectively, whereas their mannan contents are 0.5% and 6.6% (Sjöholm et al., 2000). High content of xylan could suggest a higher content of chemical pulp since mechanical pulp is made solely from softwood. A material similar to these fibres, paper pulp derived from MSW, was found to contain 55% glucan, 12% xylan and 6% arabinan/galactan/mannan (Puri et al., 2013). The higher total carbohydrate content (73%) was probably reached by removing ash by washing during the production of the paper pulp, as the ash content of the material was only 3%. Negative side to ash removal by washing is the loss of fines, which decreases the yield of easily hydrolysable material. For comparison, Kinnarinen et al. (2012) report 78% carbohydrate content, 11.5% lignin content and 9% ash content in cardboard waste.

The variation between the batches in the most important component regarding the production of sugars, the carbohydrates, was small. However, a similar carbohydrate content does not guarantee that the hydrolysability of these carbohydrates is also similar, as they may originate from many paper and board grades. The non-native extractives, ash and acid insoluble materials may likewise have a large impact on the behaviour of the material in enzymatic hydrolysis. In this work the scale-up of fractionation (comparison between batches A and D) produced a higher fractionation yield and increased the amount of acid insoluble material in the fibre fraction. It is possible that the fibre products containing more mechanical pulp, and thus lignin, have been more resistant to disintegration. In a more efficient pulping also they disintegrate increasing the fractionation yield and the relative amount of acid insoluble material in the fibres. Fibre sludge from a mill producing chemical and mechanical pulp and paper contains fillers and coating chemicals as well, which may contribute to the fraction of acid insoluble material in addition to lignin. However, the plastic residues present in fibres recovered from SRF are lacking from pulp and paper mill fibre sludge. Thus, although the content of acid insoluble material is similar in batches D and E, it is possible that the batch E contains more lignin, because it contains 25% fibre sludge. Based on the results, the composition of the recovered fibres is influenced by the source material and the fractionation efficiency.

3.3. Enzymatic hydrolysis of recovered fibres

The recovered fibres from batches A–C fractionated in the same set-up were hydrolysed with low and high solids loading with an enzyme mixture containing cellulases and hemicellulases (Fig. 1).

According to reducing sugar analysis the hydrolysis yields and rates were affected by both the source of the raw material and the solids loading. Batch A fibres hydrolysed the fastest and reached the highest hydrolysis yield resulting in practically a complete hydrolysis of the carbohydrates in 24 h. Batch C fibres hydrolysed slower and reached a lower yield at low solids loading (82%) even after 48 h. The lower hydrolysis yield obtained with batch C fibres could be caused by limited accessibility of the matrix, increased non-productive binding of the enzymes, or inhibition of the enzymes by some solubilised compounds. Batch C contained more acid insoluble material, which could correlate with a higher lignin content and explain the reduced hydrolysis yield due to limited accessibility and non-productive binding. Based on the results a high content of lipophilic extractives does not affect negatively the hydrolysis yield of the material.

Hydrolysis with high solids loading was in general slower and the final yield was decreased. However, relatively good hydrolysis yield was achieved already in 6 h showing promise for commercial concepts employing high consistency conditions in the process. HPAEC-PAD analysis of the released monosaccharides supported the conclusions. The monosaccharide concentration was close to the amount of reducing sugars at 1% solids content, but 8–12% less at 20% solids content. One explanation for this finding could be the higher relative share of oligosaccharides present in the high consistency hydrolysate due to stronger end-product inhibition. These oligosaccharides give response in the reducing sugar assay in addition to monosaccharides, and they may also be further hydrolysed in the assay conditions. For all materials glucan and xylan hydrolysed to monosaccharides most efficiently ($\geqslant$89% hydrolysis yield) at 1% solids content compared to mannan ($\leqslant$10%), arabinan ($\leqslant$46%), and galactan ($\leqslant$7%). Glucan and xylan hydrolysed more efficiently compared to other carbohydrates at 20% solids loading as well, but their hydrolysis rate was clearly reduced in 20% solids concentration as compared to 1% solids concentration. The poor hydrolysability of galactoglucomannans in recovered fibres was previously reported by our group (Kemppainen et al., 2012). Additional accessory enzymes such as mannosidases and galactosidases could help to increase to overall hydrolysis yield.

According to the results the hydrolysability of the carbohydrate fraction of SRF varies from supplier to supplier and could also vary from batch to batch. This fact needs to be taken into account when designing process concepts. The hydrolysis is at least partially limited by the recalcitrance of the material, especially the mechanical pulp portion. Thus adaptation is needed to accommodate the process for changes in the feedstock.

3.4. Comparison of recovered fibres to chemical and thermomechanical pulp

The hydrolysis of recovered fibres and chemical pulp was compared to evaluate the effect of mechanical pulp and impurities in the material compared to almost pure cellulose and hemicellulose (Fig. 2). Chemical pulp used in the experiment was a mixture of spruce and birch kraft pulps with 70.3% glucan content, 15.2% xylan and 3.2% mannan content. Its behaviour in the hydrolysis suggested that the hydrolysis rate was limited only by the enzyme dosage. On the other hand, the hydrolysis rate and yield of recovered fibres appears to be limited by the structure and accessibility of the material and possibly also inhibition of enzymes. Hydrolysis rate was on the same level with dosages from 8 to 16 mg/g indicating that the hydrolysis was mainly limited by accessibility.

To better determine the effect of thermomechanical pulp (TMP) in the material, a model substrate was created comprising of birch kraft pulp, spruce kraft pulp and spruce thermomechanical pulp in ratio of 35/34/31. The ratio of the components was chosen so that the monosaccharide profile of the model substrate was as close as possible to the profile of batch E fibres. Thermomechanical pulping of wood retains the chemical structure of wood fibres close to their native form, as only small amounts of water soluble polysaccharides are removed during pulping. TMP is resistant to enzymatic hydrolysis because its high content of softwood lignin is masking the carbohydrates and preventing the swelling of the fibre (Mooney et al., 1998) and causing non-productive adsorption and possible denaturation of the enzymes (Eriksson et al., 2002). Fig. 3 shows the results from the hydrolysis of recovered fibres, model substrate and TMP. Recovered fibres hydrolysed the fastest suggesting that they in fact contain perhaps less TMP than the model substrate. As the enzyme mixture was dosed as protein per gram of dry matter, it must be noted also that the enzyme dosage per gram of carbohydrate was higher for recovered fibres. Comparison to the model substrate also suggests that the impurities in the recovered fibres do not cause enzyme inhibition at least in low consistency conditions. The hydrolysis yield of the model substrate shows that TMP fibres can be hydrolysed to some extent, since the material contained 31% TMP but reached a hydrolysis yield higher than 69%. The hydrolysis yield of TMP alone was very low compared to the other substrates. It appears that the enzymes became soon non-productively bound and possibly inactivated halting the hydrolysis reaction before it was limited by the recalcitrance of the material. Probably the addition of fresh enzyme to TMP hydrolysis would have increased the hydrolysis yield further. The results are supported by Mooney et al. (1998) who observed a large difference in the hydrolysability of kraft pulp and mechanical pulp, as well as Li et al. (2012) who reached only 11% hydrolysis yield with TMP. However, Mooney et al. (1998) do not speculate on the possibility of non-productive binding of enzymes on lignin which was later discovered as one of the major factors limiting the hydrolysis.

3.5. High consistency hydrolysis of recovered fibres

To evaluate the suitability of a feedstock to industrial production of lignocellulosic sugars, the hydrolysis experiments have to be conducted at high solids concentration. Without high solids loading in the hydrolysis, the resulting low end product concentrations challenge the feasibility of the product separation and the overall concept. Increasing the solids concentration in enzymatic hydrolysis affects the system in many ways including effects on end-product and other inhibition mechanisms, mass transfer issues, and challenges with mixing (Modenbach and Nokes, 2013). Increasing solids loading has been reported to cause the so called 'solids effect', where at increasing substrate concentration the corresponding hydrolysis yield decreases (Kristensen et al., 2009).

The hydrolysis reaction for recovered fibres was carried out in round plastic bottles, which were freely tumbling inside a rotating drum. Very little yield decrease was observed in the high consistency hydrolysis of recovered fibres (Fig. 4). It appears that the aforementioned 'solids effect' is weak on recovered fibres making them a promising feedstock for biorefinery concepts where high end-product concentration is critical. However, an interesting difference was observed between this experimental set-up and the test-tube set-up, as hydrolysis yield in 1% solids content was dramatically reduced in the rotating drum. Only 27.4% hydrolysis yield was reached, which indicates that the enzymes were not working optimally in the reaction conditions. Hydrolysis yield increased as a function of the solids content up to 15% solids content, after which the yield at 6 h started to decrease even though the final yield remained at 58–61%. It appears that the enzymes suffered from the free-fall mixing at low solids loading resulting in most likely enzyme inactivation and thus stagnation of the hydrolysis. Magnetic mixing in test tubes is apparently gentler

for the enzymes in low substrate loading, as the hydrolysis yield of this material at 1% loading in test tubes resulted constantly in yields above 90% (data not shown). Le Costaouec et al. (2013) show similar results for pretreated spruce and wheat, where 2% consistency produced lower yields compared to 10% consistency under combined gravity and vortex mixing. However, the effect was less dramatic and was not discussed in detail in the paper. In our work the hydrolysis yields calculated from HPAEC-PAD analysis were constantly slightly lower but otherwise aligned with the reducing sugar yields. The yields based on HPAEC-PAD analysis of monosaccharides and post-hydrolysed oligosaccharides varied between 88% and 91% of the corresponding reducing sugar yield after 48 h hydrolysis (data not shown). Post-hydrolysis of the samples with mild acid hydrolyses oligosaccharides to monosaccharides. Therefore the difference between reducing sugar assay and HPAEC-PAD analysis results is caused by something else than different the reducing ends of oligosaccharides, for example the different response of the reducing sugar assay on different monosaccharides, or the presence of other reducing compounds in the substrate.

Cellulase and β-glucosidase activities were measured after the hydrolysis from the supernatants to see whether the amount of residual enzyme activity in the supernatant correlates with the hydrolysis yields or solids loadings. MUL is a substrate for certain classes of endoglucanases, cellobiohydrolases and β-glucosidases. MUL activity was analysed using glucose to inhibit β-glucosidase activity, and it thus shows the cellobiohydrolase and endoglucanase activity in the solution. As CBHII and EGII cannot hydrolyse MUL, the measured activity correlates with CBHI and EGI enzyme activities (Reinikainen, 1994). It needs to be noted that whereas the results show the residual activity in the solution after the hydrolysis, they do not show whether the rest of the enzyme is adsorbed on solids or inactivated in the solution. The analysis of MUL activity from the supernatants showed very low amounts of free activity in the liquid phase independent of solids loading. The activity loaded at 1% solids concentration was low and close to the detection limit of the analysis, thus no conclusions should be drawn on the zero activity measured at 1% solids loading. The free β-glucosidase activity was significantly higher compared to MUL activity and did not show correlation with the solids loading. Similarly Varnai et al. (2011) have shown that *Trichoderma reesei* Cel7A (CBHI) and Cel5A (EGII) remain highly adsorbed on steam pretreated spruce and Avicel at low substrate concentration, whereas β-glucosidase remains mostly free in the solution. This is natural as cellobiose is the substrate for β-glucosidase and it is soluble. In a later study it was found that the adsorption of intact Cel7A is even stronger at high solids loading compared to low solids loading (Varnai et al., 2013). According to these results, the free enzyme activity in solution did not correlate with solids loading in the hydrolysis of recovered fibres.

3.6. Effect of surfactants on enzymatic hydrolysis of recovered fibres

In an attempt to increase the hydrolysis yield of recovered fibres with low enzyme dosages the effect of surfactants was studied, as they have been shown to improve enzymatic hydrolysis (Ooshima et al., 1986; Helle et al., 1993; Eriksson et al., 2002). Two non-ionic surfactants Lutensol and Softanol, and polyethylene glycol (PEG) were dosed as 1% w/w of the substrate together with buffer to the reaction medium, and the effect of PEG was measured also on the model substrate and TMP (Fig. 3). Small positive effect could be detected with Lutensol and Softanol on recovered fibres. PEG notably increased the hydrolysis yield of the model substrate both in the beginning and the end of the hydrolysis. PEG addition did not significantly improve the hydrolysis yield of TMP.

PEG is composed of a polymerised ethylene oxide which is a carbon chain where every third atom is oxygen. Thus the hydrophobic carbon–carbon areas and more hydrophilic surroundings of the oxygen atom alter rapidly. Lutensol and Softanol products are composed of a polymerised ethylene or propylene oxide chain bound to a strongly hydrophobic saturated fatty alcohol. Softanol has 9 and Lutensol has on average 50 ethyl oxide groups whereas PEG 4000 is composed of 91 ethyl oxide groups. A PEG product and a non-ionic surfactant having an ethylene oxide chain of similar length have been shown to produce a positive effect of similar magnitude on steam pretreated spruce (Börjesson et al., 2007a) suggesting that the poly(ethylene oxide) part of the non-ionic surfactant is more important than the alkyl chain. Most effective surfactants have been shown to contain more than 75 ethylene oxide units in their structure (Börjesson et al., 2007a). Nevertheless, the alkyl chain appears to have contributed to the positive effect of Softanol and Lutensol on recovered fibres because of the lack of effect of PEG on recovered fibres in this experiment.

PEG adsorbs on lignin through hydrophobic interaction and prevents the deactivation of enzymes by exclusion of enzymes from lignin surfaces (Börjesson et al., 2007b). Improvements in hydrolysis yield and rate have been shown on steam pretreated spruce but not on delignified spruce (Börjesson et al., 2007a). PEG does not adsorb on Avicel (microcrystalline cellulose), but nevertheless improves its hydrolysis rate slightly (Börjesson et al., 2007b). PEG appears to improve hydrolysis yield most efficiently on materials with good enzyme accessibility and a relatively high content of lignin, such as the model substrate in this experiment. Jensen et al. (2011) found no positive effect of PEG on thermally treated municipal solid waste, which contains same components as the materials in this paper. The positive effect of surfactants containing the fatty acid part could be related to some non-fibre constituents in the material and their interactions together. The lack of effect on TMP may be related to the dosage of the surfactant. Non-ionic surfactants may improve the hydrolysis of crystalline substrates by varying the adsorption balance of endo- and exoglucanases. Surfactants may prevent the non-productive attachment of endoglucanases to the cellulose surface after reaction, which prevents the access of the saccharifying exoglucanase enzymes to the newly formed cellulose chain ends (Ooshima et al., 1986).

The effect of surfactant Lutensol AT50 was studied at varying solids loading to evaluate its effect in conditions required in industrial processes (Fig. 4). Interestingly, applying 1% of non-ionic surfactant (0.1 g/kg concentration in the reaction medium at 1% solids content) restored the hydrolysis yield at low solids loading in the rotating drum. Surfactant addition improved hydrolysis yield 166% at 1% solids content, 58% at 5% solids content, and 17–35% at solids content of 10–25%. There was a slight reduction in the final hydrolysis yield towards increasing solids content when surfactant was used. For comparison, Ma et al. (2011) obtained 5–30% improvement with surfactant Tween 80 in the hydrolysis of pretreated cassava bagasse at 10–25% solids loading. Kim et al. (2007) report the increase of hydrolysis yield from 49.5% to 70.6% on newspapers when using surfactant Tween-80 in low consistency hydrolysis.

The effect of surfactants on the free MUL activity after hydrolysis was clear and now correlated negatively with the solids loading (Fig. 5). The most dramatic difference was observed at 1% solids loading where now a significant portion of the dosed MUL activity was free in the liquid phase. Surfactant also increased the free β-glucosidase activity in the liquid phase. Also Börjesson et al. (2007b) reported a decrease in enzyme adsorption when using surfactants on pretreated spruce. They found 10.0% of the original Cel7A (CBHI) and 13.0% of Cel7B (EGI) activity (measured by ρ-nitrophenyl-β-D-cellobioside) in the solution after 6 h adsorption

to steam pretreated spruce on a well plate. The addition of PEG increased the amount of free activity correspondingly to 13.0% and 21.1% showing a larger effect on Cel7B. The positive effect of PEG on free enzymatic activity on isolated pure lignin was even larger compared to pretreated spruce. Park et al. (1991) reported significant increases in hydrolysis yields when using non-ionic surfactants, and correlate the improvement to decreased enzyme adsorption, suggesting that the effect comes from the surfactant's ability to improve enzyme desorption.

Results reported here show that an increase in free MUL activity positively correlates with improvement on the hydrolysis yield on this substrate. It appears that the surfactant was able to prevent the enzyme inactivation that took place in the rotating drum at low solids loading. The positive effect of surfactants could be related to their suggested capability to shelter enzymes from inactivation by shear forces, thermal inactivation, and non-productive adsorption resulting in denaturation of the enzyme on the lignin surface. The positive effect at high solids loading could also originate from the viscosity reducing effect of the surfactants (Modenbach and Nokes, 2013). It appears more probable that the surfactant in these experiments prevented thermal and/or shear force induced inactivation of the desorbed enzymes at low solids loading. High substrate concentration increases the possibility of enzyme-substrate interaction (Varnai et al., 2013), which would suggest that if the main effect was the prevention of non-productive adsorption, the improvement would be the largest at high solids loading. As a conclusion it appears that surfactants could be used to protect enzymes from inactivation in the hydrolysis of recovered fibres in high consistency conditions relevant to industrial concepts.

4. Conclusions

Fibres fractionated from SRF are a distinct source of fermentable sugars, unique in their origin and high carbohydrate and ash content. Although variation was detected between fibres from different SRF suppliers, SRF-derived fibres can in general be hydrolysed rapidly to soluble sugars even at high solids loading. The TMP present in the material slows down the hydrolysis compared to chemical pulp, but does not fully stop it. Non-ionic surfactants can be used to improve hydrolysis yield and rate, and they appear to shield enzymes from inactivation caused by tumbling type of mixing at low solids loading.

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