PUBLICATION I

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Biomass Bioenergy 46, 60–69.

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Ethanol and biogas production from waste fibre and fibre sludge – The FibreEtOH concept

Katariina Kemppainen [a,], Liisa Ranta [b], Esa Sipilä [c], Anders Östman [d], Jari Vehmaanperä [e], Terhi Puranen [e], Kim Langfelder [f], Janne Hannula [g], Anne Kallioinen [a], Matti Siika-aho [a], Kai Sipilä [a], Niklas von Weymarn [a]*

[a] *VTT Technical Research Centre of Finland, P.O. Box 1000, 02044 VTT, Espoo, Finland*
[b] *UPM-Kymmene Oyj, P.O. Box 380, 00101 Helsinki, Finland*
[c] *Pöyry Management Consulting Oy, Jaakonkatu 3, 01620 Vantaa, Finland*
[d] *Skandinavisk Kemiinformation AB, Birkagatan 35, 11339 Stockholm, Sweden*
[e] *Roal Oy, Tykkimäentie 15, 05200 Rajamäki, Finland*
[f] *AB Enzymes GmbH, Feldbergstraße 78, 64293 Darmstadt, Germany*
[g] *Lassila & Tikanoja Oyj, Sääksvuorentie 4, 40530 Jyväskylä, Finland*

ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form
6 October 2011
Accepted 29 March 2012
Available online 24 April 2012

Keywords:
Lignocellulosic ethanol
High consistency hydrolysis
SSF
Waste fibre
Fibre sludge

ABSTRACT

The FibreEtOH concept was developed to tackle major challenges in the production of ethanol from lignocellulosics. The two feedstocks, waste fibre fractionated from solid recovered fuel, and pulp and paper mill fibre sludge, provide all-year-round supply of biomass with high hexose content (44–56%) and acceptable ash content (13–14%). They can be liquefied and hydrolysed by enzymes rapidly without a thermal or acidic pre-treatment, although they contain some recalcitrant mannose- and galactose-containing polysaccharides that require additional helper enzymes for complete hydrolysis to monosaccharides. Fractionation of solid recovered fuel, continuous liquefaction, and simultaneous saccharification and fermentation to ethanol, as well as biogas production from the fermented residue were demonstrated in pilot-scale with good results. Total yield consisting of C6 sugar hydrolysis yield (57%) and fermentation yield (84%) was 48% after only 6 h continuous liquefaction and 21 h fermentation. Average biogas production rate was 655 $dm^3\ kg^{-1}$ for fermentation residue from waste fibre and 400 $dm^3\ kg^{-1}$ from fibre sludge with methane content of 69–75%. Based on other results a hydrolysis yield of 75% is reachable within the process concept if the residence time in fermentation is extended. In this scenario 1000 kg of dry feedstock would produce 170 kg ethanol, 310 kg biogas, 360 kg waste sludge and 170 kg CO_2.

1. Introduction

The demand for road transport biofuels is growing rapidly [1]. The current global market is dominated by ethanol, which is predominantly produced from either sucrose or starch – both key raw materials in several food industry value chains. The R&D community, on the other hand, is vigorously trying to develop improved biofuels or more sustainable ways to produce the biofuels already on the market. In respect to ethanol, various non-edible, cellulose-rich raw materials,

* *Corresponding author*. Tel.: +358 20 722 7559; fax: +358 20 7071.
E-mail address: katariina.kemppainen@vtt.fi (K. Kemppainen).
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doi:10.1016/j.biombioe.2012.03.027

so-called lignocellulosics, have been the main focus of several new concepts. The lignocellulosic raw materials most interesting in terms of economic and environmental sustainability include residues of sugar cane, sugar beet, corn, wood and grain processing, or alternatively energy crops as well as industrial and municipal wastes. In Europe, lignocellulosic ethanol is already today produced commercially using the side-stream of sulphite pulp mills as the raw material (e.g. Domsjö Fabriker in Sweden and Borregaard in Norway).

In the current European biofuels policy framework, new biofuels products should meet the minimum target of greenhouse gas reduction in the production value chain. A threshold value of 35% is valid for current units and for new production plants the threshold is 60% reduction starting in 2017. The lignocellulosic materials from waste streams are attractive sources; for business opportunities the key question will remain on raw material availability and the price of the raw material.

Full commercial deployment of lignocellulosic ethanol is, however, still facing several major constraints [2]. The challenges relate both to the raw material logistics in general as to the actual conversion process. The FibreEtOH concept was developed to tackle most of these challenges (Fig. 1). The concept is based on the utilisation of a side-stream of a pulp and paper integrate (fibre sludge) and waste fibre separated from solid recovered fuel (SRF) and thus, all-year-round supply of inexpensive raw material would be ensured. Moreover, all technologies involved in the production chain except enzymatic hydrolysis, are available on commercial scale. On the other hand, process microbiology, in general, is an additional challenge when processing waste materials.

FibreEtOH is one of the four consortium projects that received funding under the 7th Framework Programme of the European Commission, when the large demonstration projects were selected in 2009. The FibreEtOH project is coordinated by UPM-Kymmene Oyj, Finland, a major global player in the pulp and paper industry. The other partners include VTT Technical Research Centre of Finland (R&D), Pöyry Management Consulting Oy, Finland (process design), Skandinavisk Kemiinformation AB, Sweden (process design), Roal Oy, Finland and AB Enzymes GmbH, Germany (industrial enzyme production and sales), Lassila & Tikanoja Oyj, Finland (supplier of SRF), and St1 Oy, Finland (distributor of fuel ethanol).

The FibreEtOH process consists of raw material handling including the fractionation of SRF, liquefaction of the feedstock, simultaneous saccharification and fermentation (SSF), distillation and absolution of ethanol, and biogas production from the fermented residue. It can be highly integrated to other commercial processes resulting in sound end-uses of all main fractions of the raw material. The process takes advantage of the easy hydrolysability of waste fibres without a thermal or chemical pre-treatment, which is a major capital cost factor in most lignocellulosic ethanol production processes [3]. The advantage of using thermophilic enzymes is also employed. Prehydrolysis with thermostable enzymes can be carried out at higher temperatures, which can increase enzymatic activity, decrease the viscosity of the slurry enabling easier mixing, pumping and working in higher consistency, i.e. lower water to dry matter ratio [4]. Instead of having a residence time of four to six days in fermentation, the process takes a 'quick & dirty' approach hydrolysing and fermenting the easy part of the polysaccharides, approximately 75%, in only two to three days. Complete conversion is not a necessity because the rest of carbohydrates are utilized efficiently in biogas production, which is an important part of the concept from an economical point of view.

In the Helsinki metropolitan area there is a population of 1 million inhabitants. The amount of commercial and industrial waste after source separation to material recovery is about 450 000 t a^{-1}. In the project, the amount of SRF of 200–300 000 t a^{-1} has been estimated to be available for advanced energy applications. Additional raw fibre may be

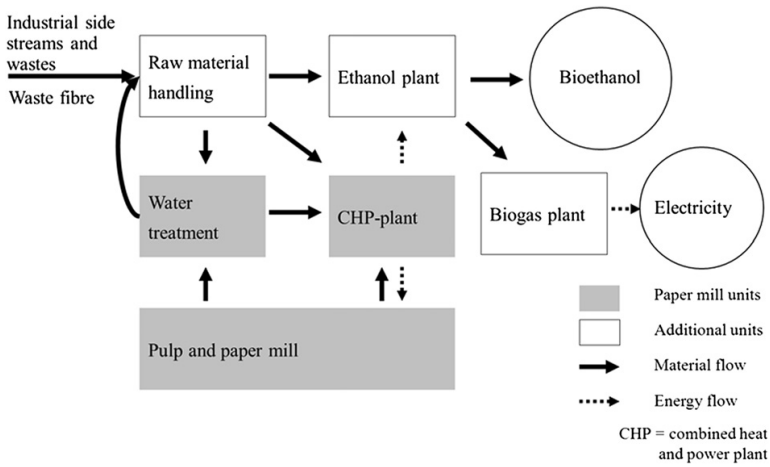

Fig. 1 – The FibreEtOH concept consists of raw material handling including the fractionation of SRF, liquefaction and fermentation of the feedstocks, distillation and absolution of ethanol, biogas and electricity production from the fermented residue, and a shared water treatment and a combined heat and power (CHP) plant with a pulp and paper mill.

available when the ethanol production facility is integrated to pulp and paper industry operations. In addition to fibre sludge, waste fibre fractions from pulp or paper recycling could also be utilized.

Extensive research has been carried out from laboratory scale to pilot-scale and from enzyme development to process design optimization during the past three years. In this paper, R&D results from the FibreEtOH project from all the main areas of the successful bioethanol production concept are presented. The composition of the feedstocks is described in detail and the lack of need for thermal or acidic pre-treatment is demonstrated. High consistency liquefaction is discussed separately before reporting results from a continuous pilot-scale bioethanol production campaign. Finally biogas production from the fermented residue is demonstrated.

2. Material and methods

2.1. Materials

2.1.1. Feedstocks

Two lignocellulosic feedstocks were used in the process: waste fibre and fibre sludge. Waste fibre was fractionated from solid recovered fuel (SRF) provided by Lassila & Tikanoja from their Turku and Kerava facilities. Most plastics, sand, and other contaminants were removed from SRF by screening after repulping the material with water. Material for the pre-treatment experiments was dried on a board machine to 95% mass fraction of dry matter (referred to as dry matter content in this paper). Material for high consistency liquefaction studies and pilot-scale continuous campaigns was dried with a filter press to ca. 50% dry matter content after a 60 min sanitation period at 95 °C. This dried product is called waste fibre. Fibre sludge was obtained from UPM Kaukas pulp and paper mill in 2–3% dry matter content and dried to 40–50% dry matter content using a filter press. Material for pilot campaigns was also produced by mixing 75% waste fibre and 25% fibre sludge (dry matter basis) before drying.

2.1.2. Enzymes

Enzymes used in the experiments have been provided by Roal Oy. The effect of pre-treatment to the hydrolysability of waste fibre was evaluated with commercial Econase CEP enzyme product (10 filter paper units (FPU) g^{-1} dry matter) and a pre-commercial β-glucosidase product from *Acremonium thermophilum* (1000 nkat g^{-1}). A pre-commercial thermostable enzyme mixture was used in the high consistency liquefaction study and pilot-scale bioethanol production with cellobiohydrolase I (CBH I) from *A. thermophilum*, cellobiohydrolase II (CBH II) from *A. thermophilum*, an endoglucanase component from *Thermoascus aurantiacus*, xylanase from *Nonomuraea flexuosa*, mannanase from *Trichoderma reesei* and β-glucosidase from *A. thermophilum*. Enzymes were dosed based on the FPU activity of the mixture. FPU activity was analysed according to Ghose [5] and β-glucosidase activity according to Bailey and Nevalainen [6].

2.1.3. Yeast

The yeast strain used in fermentation was commercial Red Star yeast (Le Saffre). Yeast inoculum was cultivated before every piloting campaign in the previous week in aerobic conditions to maximise the growth of yeast. The yeast was cultivated on a glucose-based medium (70 g dm^{-3}) with peptone (20 g dm^{-3}) and yeast extract (10 g dm^{-3}), from shake flask scale up to 1200 dm^3 via 28 dm^3 and if necessary 200 dm^3 seed fermenter stages. The yeast culture was separated by continuous centrifugation (BTPX-205 SGD, Alfa-Laval) and the concentrated yeast cream was stored in 4 °C until needed during process operation in the following week.

2.2. Feedstock composition analysis

Carbohydrate compositions of the dried feedstocks were analysed by high performance liquid chromatography (HPLC) after air drying, grinding and acid hydrolysis by 70% sulphuric acid to monosaccharides [7,8]. Ash content was analysed from wet samples in a muffle oven by heating samples stepwise first into temperature 103 °C for 7 h and then to 550 °C for 16 h to ash the samples. Ashed samples were cooled and the residues weighed. To analyse the content of extractives and lignin, air dried samples (1 g) were extracted with heptane in a soxhlet extraction system. The heptane extract was dried and the weight of the residue was measured as the gravimetric extractive content. The lignin content was analysed from extracted samples after acid hydrolysis with 70% sulphuric acid. Klason lignin was obtained by weighing the acid-insoluble residue after drying, and soluble lignin was obtained by analysing the UV absorbance of the liquid with wavelength of 203 nm and using absorptivity constant of 128 $dm^3\ g^{-1}\ cm^{-1}$ to estimate lignin concentration. It must be noted that other acid-insoluble substances in the materials such as plastics showed up as Klason lignin in the analysis.

2.3. Pretreatment experiments

The pilot-scale pre-treatment experiments were carried out for waste fibre in a 400 dm^3 multipurpose reactor (Jaro Oy, Finland) at VTT Rajamäki pilot plant. The pH of the pulped and dried waste fibre was adjusted to 5 or to 2 with H_2SO_4, after which the pulps were pretreated in conditions presented in Table 1 with saturated steam. After 5–10 min residence time the pressure was released by "exploding" the pulp to an unpressurized reactor. Batches 4 and 6 were continued from batches 3 and 5 by heating the material again after cooling and sampling.

Table 1 – Batch parameters in the pre-treatment experiments with waste fibre.

Batch	pH	T (°C)	Waste fibre concentration (g dm^{-3})
1	5	120	10–30
2	5	160	10–30
3	5	200	10–30
4	5	200 + 200	10–30
5	2	160	60
6	2	160 + 180	60

2.4. Laboratory scale hydrolysis experiments

Hydrolysis experiments were performed in Schott bottles in 50 ml volume in 100 g kg^{-1} substrate concentration with magnetic mixing. Temperature was adjusted to 45 °C with a water bath, and pH was adjusted to 5 with 100 mol m^{-3} sodium acetate buffer containing 0.02% sodium azide to prevent microbial activity during hydrolysis. Hydrolysis yield was analysed by stopping the reaction by boiling, removing solids by centrifuging, and analysing the reducing sugars in the supernatant by 3,5-dinitrosalicylic acid (DNS) assay [5].

A Hobart pulper was used for liquefaction studies performed in high consistency. It contains a metal vessel with a heat jacket and a lid, and a flat-beater type mixing element. Reaction volume was 1.2 dm^3 and substrate concentration at the start of the reaction was 300 g kg^{-1}. pH was adjusted to 5 with phosphoric acid and temperature was adjusted to 50 °C. Substrate concentration increased from 300 g kg^{-1} to 325 g kg^{-1} during the reaction due to evaporation. Thus dry matter content of the material was routinely measured and taken into account in yield calculations. Due to the high consistency used in the experiments, DNS analysis was performed from 10-fold diluted samples (triplicates) to diminish the solids effect caused by a large share of insoluble solids [9].

Prediluted hydrolysis samples were analysed by HPLC for their total neutral monosaccharide content according to Tenkanen and Siika-aho [8]. A secondary acid hydrolysis with 70% sulphuric acid (1 part acid, 20 parts sample) was carried out for supernatant samples in an autoclave in 120 °C for 1 h. After secondary acid hydrolysis dissolved oligosaccharides could be quantified as monosaccharides from supernatants with HPLC. An oligosaccharide analysis was performed with HPLC for some samples according to Tenkanen et al. [10].

2.5. Pilot-scale continuous experiments

2.5.1. Liquefaction and SSF

Continuous liquefaction and SSF were carried out for 86 h in a pilot-scale system presented in Fig. 2. The dried waste fibre in ca. 50% dry matter content was fed continuously into the process through a screw lift. The material had a high bridging tendency and regular attention was needed in order to ensure continuous feed to the system. In addition to the sanitation conducted before drying, a short steam sanitation was demonstrated by leading steam to the screw lift from a connection above the feed opening. The temperature of the material in the end of the screw was 70–80 °C and the residence time in the screw was approximately 10 min.

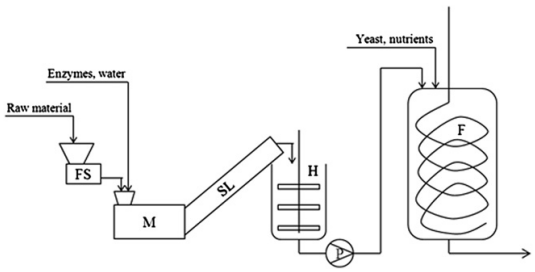

Fig. 2 – The layout of the liquefaction and SSF pilot process. SL = screw lift, H = liquefaction reactor, P = hose pump, F = fermenter.

The liquefaction of the material was carried out in a 100 dm^3 stirred-tank reactor with residence time of 6 h. Counter-current 4-impeller mixing elements were used with a 1.5 kW motor. Enzymes and water were pumped in continuously and biocide sodium bisulfite was dosed to the water feed to give 0.3 g dm^{-3} concentration in the process. Liquefaction pH was adjusted to 5 by adding 80% phosphoric acid to the vessel when needed and temperature was adjusted to 50 °C. Total flow through the system was 14 kg h^{-1} and total solids concentration at start was 300 g kg^{-1}. The liquefied material was pumped to the fermenter with Bredel SPX32 hose pump, which was able to pump very thick paste-like materials without much trouble. Because of the large size of the pumps, the pumping was pulsed with and electric timer (for example 10 s ON, 3 min OFF) to achieve correct average flow.

The fermenter used was a 300 dm^3 vessel with a top-down spiral-type of mixing element with online pH and temperature measurements. Residence time in the fermenter was 21 h, pH was adjusted to 4.5, and temperature to 35 °C. The process was started with 70 dm^3 water and 3 g dm^{-3} yeast in the fermenter. Yeast slurry was pumped in continuously with the feed to obtain 3 g dm^{-3} yeast concentration. Yeast extract (0.5 g dm^{-3}) was added to the fermenter continuously to ensure sufficient trace elements for the yeast. No other additional nitrogen source was used. Antifoam agent (Struktol J633, Schill & Seilacher, Germany) was used when needed. Nitrogen gas was fed to the fermenter headspace to ensure anaerobic conditions.

Samples were taken every 4 h and pre-diluted before analysis because of the high solids content. Metabolite concentrations were determined by HPLC using Aminex HPX-87H column (BioRad Laboratories, USA) with 2.5 mol m^{-3} H_2SO_4 as eluent and flow rate 0.3 ml min^{-1}. The column was maintained at 55 °C. Peaks were detected using a Waters 410 differential refractometer and a Waters 2487 dual wavelength UV (210 nm) detector.

2.5.2. Biogas

Continuous biogas pilot-scale experiments were conducted in a 300 dm^3 reactor at 35 °C. Mixing propeller was timed for sequential low speed mixing. Feeding of the evaporated residue from a waste fibre and fibre sludge pilot-scale fermentations was done manually once or twice a day depending on the amount of the feed. Disposal of the material was done from the bottom of the reactor through overflow principle. Biogas was collected from the upper part of the reactor and routed via gas volume meter.

3. Results & discussion

3.1. Composition of the feedstocks

Feedstock carbohydrate composition sets the limits for the obtainable yield as litres of ethanol per kg of dry feedstock. In the case of feedstocks originating from pulp and paper industry, the ash content and composition is also a significant

factor affecting the hydrolysability and processability of the material as well as acid consumption in the process. Several batches of both feedstocks were prepared over the period of 2 years and their carbohydrate composition was analysed to determine theoretical ethanol yields in the process. The SRF bales arriving from Lassila & Tikanoja were visibly different from each others in terms of their content, and it was clear that the heterogeneity of the materials would have to be accounted for in process design and yield calculations. Table 2 presents the range in the monosaccharide composition analysis results from several measurements. Waste fibre had a higher glucan and xylan content than fibre sludge, while the latter contained more galactomannan type of polysaccharides. The mixture of 25% fibre sludge and 75% waste fibre was evaluated to correspond to a mixture of 35% birch kraft pulp, 34% spruce kraft pulp and 30% spruce thermomechanical pulp based on its monosaccharide profile.

Both feedstocks contained ash originating mostly from paper filling and coating materials as well as extractives and lignin (Table 3). Main components of the ash were determined as $CaCO_3$, kaolin and talc. Ash content in UPM Kaukas mill sludge was lower than in many other reported paper mill sludges [11]. Acid-insoluble material, i.e. Klason lignin, showed all acid-insoluble components in the feedstocks including plastics, which are left as impurities in waste fibre after pulping. Fibre sludge does not contain plastics and so the acid-insoluble material presumably represents Klason lignin.

The glucan content of waste fibre was higher than for example that of corn stover, sugar cane bagasse, wheat straw and switchgrass [12] making it a very promising feedstock for bioethanol production. Because of the high C6 sugar content, the fermentation of C5 sugars is not needed to achieve high ethanol yield per ton of dry matter (theoretical maximum is 224–286 kg t^{-1}). The high ash content has been found problematic in bioethanol production from paper sludge because $CaCO_3$ contained in the ash interferes with pH control demanding high amounts of acids to achieve pH suitable to enzymes [13]. However in these feedstocks, the ash level was rather low compared to waste celluloses in general and although buffering capacity of the material was notable, the acid consumption did not become excessive in the process.

3.2. *The effect of a thermal and acidic pre-treatment to hydrolysis yield*

The target for a pre-treatment of biomass is to improve the rate of hydrolysis and the yield of fermentable sugars by altering or removing structural and compositional impediments to hydrolysis. Liquid hot water treatment with or without pH control is a major class among the pre-treatment technologies reported in literature, often combined with steam explosion [14]. The effect of a thermal and acidic pretreatment to the hydrolysability of waste fibre was studied in pilot-scale. Experiments were run with temperatures from 120 to 200 °C in pH 5 or in pH 2, and the obtained materials were subjected to enzymatic hydrolysis with 10 FPU g^{-1} Econase CEP and 1000 nkat g^{-1} β-glucosidase. Treatments with temperature of 160° and higher visually changed the material making it significantly darker and the rate of sedimentation of the material appeared to increase. According to the results presented in Fig. 3 the thermal and acidic pretreatments did not improve hydrolysis yield but rather decreased the hydrolysis rate especially in the beginning of the hydrolysis. The results were confirmed by HPLC analysis of the released monosaccharides and in another experiment in lower substrate concentration (10 g kg^{-1}, data not shown). According to the results the material appeared to contain a major part of the carbohydrates in easily hydrolysable form (e.g. fibre derived from chemical pulping methods), and a minor part in resistant form (e.g. mechanical pulp, wood residues). The results also suggest that the feedstocks contain components such as lignin that re-distribute within the material as a result of high-temperature pre-treatment and consequently may cover and mask partially the cellulose, make the access of cellulase enzymes to cellulose difficult, and slow down hydrolysis.

Laboratory scale hydrolysis of fibre sludge with commercial cellulases (10 FPU g^{-1}) produced hydrolysis yield over 80% in 48 h without any pre-treatment to the material (data not shown). Thus it can be concluded that both feedstocks are readily available to enzymes as such. This result is in line with results reported in the literature. For example Lin et al. [15], and Lynd et al. [11] have obtained high hydrolysis yields with waste fibre and fibre sludge type of biomasses without any pre-treatment. On the other hand Yamashita et al. [16] found that phosphoric acid treatment alone and combined with ball milling improved the hydrolysability of a batch of paper sludge, which hydrolysed very poorly without any pre-treatment. They concluded that chemical swelling through acidic treatment enhanced the enzymatic saccharification, which supports the conclusion that the high temperature was the detrimental factor reducing the hydrolysability of waste fibre in these experiments.

3.3. *Liquefaction in high consistency*

In order to produce a high ethanol concentration in the broth and thus minimise tank size and the energy consumption in

Table 2 – Composition of carbohydrates from waste fibre and fibre sludge after acid hydrolysis and their combination containing 75% waste fibre and 25% fibre sludge. The values represent the fraction (%) of individual monosaccharides per dry matter released by analytical acid hydrolysis.

Raw material	Glc	Xyl	Man	Gal	Ara	Rha	Monosaccharides	Hexoses
Waste fibre	43.7–50.5	9.6–11.2	2.7–4.3	0.2–0.6	0.2–0.4	0	57.5–65.3	46.1–55.4
Fibre sludge	38.3–40.8	7.1–9.7	4.4–4.7	1.2	0.7	0.2	51.9–57.0	44.1–46.6
Combination	43.5–51.2	7.9–10.1	3.5–4.9	0.5–0.9	0.4–0.5	0	57.8–64.6	47.5–56.1

Table 3 – Ash, extractives, and lignin content of waste fibre and fibre sludge, and their combination containing 75% waste fibre and 25% fibre sludge used in piloting as a fraction (%) of dry matter.

Sample	Ash (%)	Extractives (%)	Acid insoluble material (%)	Acid soluble lignin (%)
Waste fibre	13.3	6.8	19.4	0.5
Fibre sludge	13.7	3.1	30.4	1.4
Combination	13.8	5.7	23.7	0.4

distillation, a high consistency process (>150 g kg^{-1} substrate concentration) is required in the production of ethanol from lignocellulose [17]. Prehydrolysis is an effective way to decrease the viscosity of high consistency biomass to enable easier mixing in further process stages [4].

Liquefaction of waste fibre in high consistency was studied with a table-top Hobart pulper, which produces very efficient mixing already from the beginning of a batch hydrolysis. A mixture of pre-commercial thermostable enzymes was used with 8 FPU g^{-1} dosing and temperature was adjusted to 50 °C. Relatively high hydrolysis yield of 40% from theoretical analysed as reducing sugars was obtained after 8–10 h (Fig. 4). Dissolved monosaccharides were analysed from hydrolysates before and after secondary acid hydrolysis to assess the hydrolysis yield as mono- and dissolved oligosaccharides.

The results show that hydrolysis yield to monosaccharides was lower than hydrolysis yield to mono- and oligosaccharides throughout the reaction time. This indicates that high end-product concentration in the reactor during the reaction (15–50 g dm^{-3} glucose, 17–55 g dm^{-3} total monosaccharides) inhibited the enzymes catalysing the last steps in the chain of reactions needed to hydrolyse polysaccharides to monosaccharides. Literature supports this conclusion as β-glucosidases from *Aspergillus niger* and *T. reesei* have been reported to lose 85% of their activity in 30 g dm^{-3} glucose [18]. The results also suggest that not only does DNS analysis respond to the reducing ends of oligosaccharides in the sample, but it also appears to degrade them to monosaccharides during the analysis. According to this, the analysis of reducing sugars in high consistency hydrolysis is a measure of the conversion of the substrate rather than a measure of the yield of the monosaccharide end-products.

Analysis of the oligosaccharides by HPLC showed concentrations of 5–9 g kg^{-1} cellobiose, 7–11 g kg^{-1} xylobiose, and 1 g kg^{-1} mannobiose in the reactor during the liquefaction. Oligosaccharides corresponding to standards with 5 and 6 xylose units, and 3 and 5 mannose units were also detected by HPLC. Monosaccharide analysis before and after secondary acid hydrolysis showed that after 10 h hydrolysis especially galactose, xylose and mannose remained bound to solubilised oligosaccharides whereas arabinans and glucans were hydrolysed more efficiently to monosaccharides (Fig. 5). Xylan solubilised most easily: as much as 33% of xylan had solubilised already after 30 min hydrolysis (data not shown).

Although there are studies available on the hydrolysis of biomass in high consistency conditions [17,19], very few report anything about the hydrolysis yield during the first 10 h of the reaction. Nevertheless, one very promising process option in ethanol production from lignocellulose is to conduct a short liquefaction prior to SSF to reduce viscosity of the material and to take advantage of the higher enzymatic reaction rate in elevated temperatures. This makes the phenomena during this period industrially relevant. Based on these results it can be concluded that waste fibre liquefies and hydrolyses very quickly in high consistency conditions, but end-product inhibition appears to be a significant factor affecting the hydrolysis rate to monosaccharides. Thus a short liquefaction stage followed by SSF is the preferred process choice as the hydrolysis rate reduces significantly when monosaccharide concentration increases.

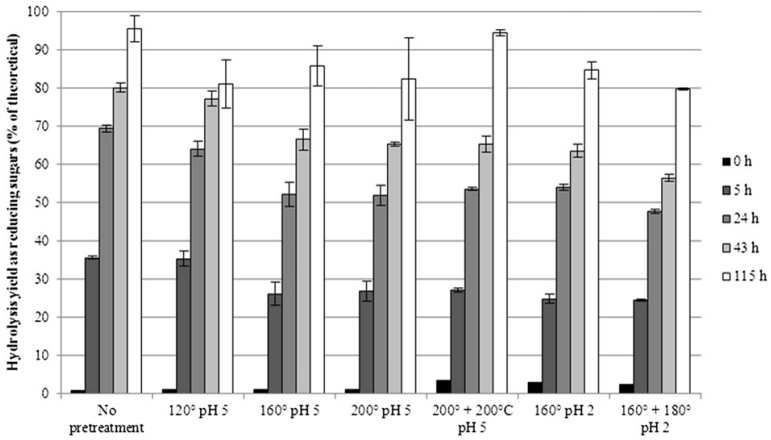

Fig. 3 – The effect of thermal and acidic pre-treatment to hydrolysis yield of waste fibre with 10 FPU g^{-1} cellulase and 1000 nkat g^{-1} β-glucosidase in 100 g kg^{-1} substrate concentration, 100 mol m^{-3} sodium acetate buffer pH 5 at 45 °C. Error bars show the standard deviation of triplicate samples.

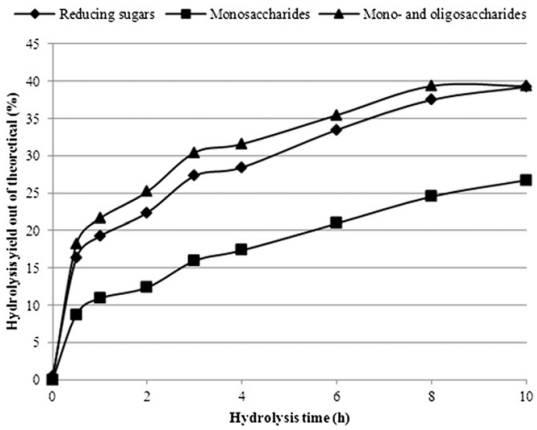

Fig. 4 – Hydrolysis yield as reducing sugars, monosaccharides (HPLC analysis), and mono- and oligosaccharides (HPLC analysis after secondary acid hydrolysis of the supernatant) in liquefaction of waste fibre with 8 FPU g^{-1} in 300 g kg^{-1} substrate concentration, 50 °C and pH 5.

According to the results obtained here, waste fibre contains mannans and galactans, which solubilise with the enzyme mixture containing cellulases, xylanases, and mannanases forming recalcitrant oligosaccharides, which require additional helper enzymes for hydrolysis to fermentable monomers. Rättö et al. [20] hydrolysed isolated galactoglucomannan from pine kraft pulp and found that whereas the hydrolysis yield was high (85%), only 3% of the hydrolysis products were mannose and the rest were mainly mannobiose (35%), mannotriose and larger unidentified oligosaccharides. Adding α-galactose and β-glucosidase only slightly improved hydrolysis to mannose (6%). β-mannosidase could prove beneficial and should be studied to obtain full hydrolysis of these recalcitrant polysaccharides from waste fibre.

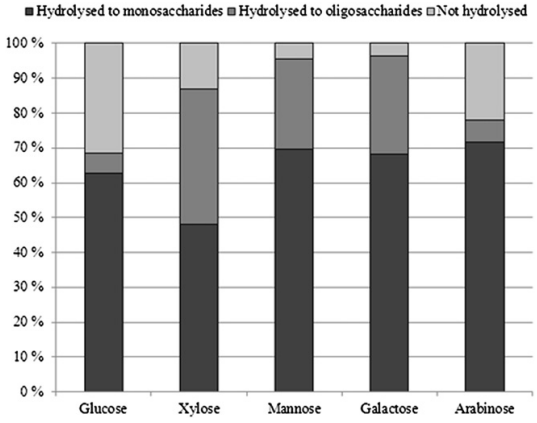

Fig. 5 – Hydrolysis yield of monosaccharides after 10 h liquefaction of waste fibre with 8 FPU g^{-1} in 300 g kg^{-1} substrate concentration, 50 °C and pH 5.

3.4. *Continuous pilot-scale liquefaction and SSF*

Although continuous processing could be an important factor in reducing the costs of producing ethanol from lignocellulose, the studies reporting results from continuous SSF runs are scarce. The advantages of continuous processing in ethanol production include higher volumetric productivity, reduced labour costs, reduced vessel down time for cleaning and filling, and the adaptation of the fermentative organism to inhibitors in the biomass [21]. Continuous processing differs from batch processing in many significant ways but especially high consistency continuous-type of experiments are difficult if not impossible to conduct in laboratory scale. The important hands-on experience on dealing with possible problems for example in raw material handling and microbial contamination can be achieved only by running continuous pilot campaigns. However, the size of the pilot-scale equipment at this stage of the process development does not have to be very large. Smaller volumes and flows ensure easy handling and give flexibility during the campaign but still give good indication of the obtainable yields and functional process parameters.

Several 1–2 week long continuous pilot-scale campaigns have been run over the past two years to verify laboratory results in larger scale experiments. Continuous campaigns were run at VTT Otaniemi bioprocess pilot plant consisting of liquefaction and SSF stages. Total flow through the system varied from 10 to 20 kg h^{-1} containing 250–300 g kg^{-1} solid substrate at the start. The object for the liquefaction stage (also called prehydrolysis) was to achieve rapid initial hydrolysis at a temperature optimal for the enzymes. However, because end-product inhibition reduced the hydrolysis rate significantly in high consistency conditions, the material was transferred to the fermenter almost as soon as it was transformed into pumpable form. In continuous processing mode this meant 6–8 h residence times in the liquefaction reactor. A regular stirred-tank reactor was used because of its simplicity and relatively low cost. Counter-current impellers ensured material flow in three dimensions. Hose pumps were chosen as the pump type because of the high viscosity of the material, and because they are little affected by sand, plastics, bark, or other impurities in the material. Enzyme dosage varied from 8 to 20 FPU g^{-1} in the campaigns.

The main results of one continuous campaign with waste fibre as feedstock are presented and discussed below. Fig. 6 presents the concentration of substrates and metabolites in the fermenter during the campaign. Concentration is presented as g kg^{-1} because of the weight-based predilution before analysis. The material fed to fermentation had a degree of hydrolysis of 43–49% as reducing sugars. Hydrolysis was slightly higher than what Larsen et al. [22] report as the hydrolysis yield of wheat straw in high consistency after 6 h liquefaction in a pilot-scale free-fall mixing system (30–40%). Hydrolysis yield to glucose was lower because of the high end-product inhibition, approximately 28–36%. The campaign was successfully run in continuous mode for 86 h except between 55 and 61 h when the mixer motor in the liquefaction reactor

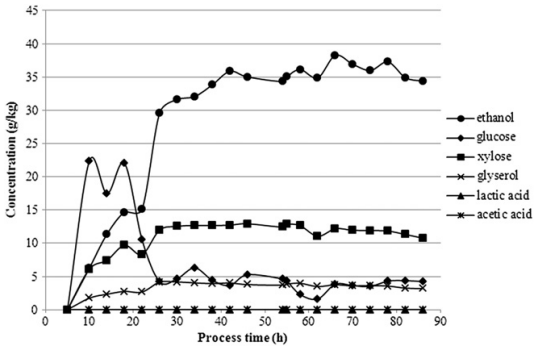

Fig. 6 – The main substrate and metabolite concentrations during a continuous SSF process with waste fibre after continuous 6 h liquefaction in 300 g kg^{-1} substrate concentration.

broke down and had to be changed. During that time no feed went in or came out of the fermenter, which can be seen as a reduction in the glucose concentration in the fermenter.

Fermentation residence time was kept at only 21 h to achieve reliable data from a continuous 5-day campaign and thus the total yield remained rather low. Because of the short residence time, some glucose passed through the reactor unused. This was prevented in later campaigns by dividing the residence time in SSF to two reactors. No lactic acid was produced during the campaign indicating good microbial control throughout the process. Glycerol production was 9–11% of ethanol production while no acetate was detected (detection limit 2 g kg^{-1}). Ethanol productivity was 1.8 g dm^{-3} h^{-1} at its highest. Hydrolysis yield of fermentable hexoses from polysaccharides during the stable face of the campaign was approximately 57% showing that hydrolysis continued further in SSF conditions. Ethanol yield from the hydrolysed hexoses was 84% and thus total yield was 48%. Maas et al. [23] obtained the same glucan-to-ethanol yield (48%) in a pilot-scale batch prehydrolysis and SSF of lime-treated wheat straw in over two times longer fermentation (48 h). This indicates that although final yield here was low, the ethanol productivity rate was rather high, which indicates that a generally shorter fermentation time of 50–70 h is required in this process concept compared to most other proposed process concepts. Good fermentation performance in high dry matter conditions also indicates the low toxicity of the raw materials to the yeast.

Another continuous campaign was carried out using raw material combined from waste fibre (75%) and fibre sludge (25%) (detailed results not shown). The hydrolysis and ethanol yields were on a similar level as with 100% waste fibre. Small pieces of bark and wood sticks originating from the fibre sludge were prone to get stuck in the narrow valves and hoses of the pilot-scale equipment, but otherwise the material was as easily processable as 100% waste fibre.

Schell et al. [24] reported results from the initial runs at the National Renewable Energy Laboratory bioethanol plant with corn fibre feedstock. They encountered significant problems with bacterial contamination in the process during the 10 and 15 day runs and later in longer campaigns [25]. Also Isci et al. [26] report problems with bacterial contamination and lactic acid production in pilot-scale fermentation of pretreated switchgrass. In this campaign reported here, no lactic acid or acetic acid production was observed and the lactic acid bacteria concentration was below 10^4 cfu ml^{-1} in the fermenter throughout the week (data not shown) indicating that sanitation by heat, the use of sodium bisulphite and the short residence time in the fermenter were effective measures for successful bacterial control. In longer campaigns we have also encountered problems with lactic acid bacteria and noted that having high yeast concentration in the fermenter helps to limit their growth. Lactic acid production appeared to start only after the contamination reaches a certain level and thus it is important to take defencive measures (pH shock, cleaning etc.) early on in the process.

3.5. *Biogas production from fermented residue*

Biogas production is an important factor in the FibreEtOH process because it allows the more complete utilization of the feedstock reducing waste and helping to overcome financial challenges related to ethanol production from lignocellulose. It opens up an opportunity to digest additional starch containing materials to biogas when attractive market prices are boosting the economy. The combined ethanol and biogas production will also increase the carbon conversion yield significantly compared to stand-alone ethanol production. Biogas can also be upgraded to biomethane which can also be used in transport applications.

Material from two continuous bioethanol pilot campaigns was used in a biogas production experiment that lasted little over 3 months. First fermentation residue came from a bioethanol pilot campaign using waste fibre and the second from a campaign using fibre sludge as the raw material. The produced ethanol was removed from both residues by evaporation.

During the first 2.5 months when waste fibre based residue was used, total of 655 dm^3 biogas per kg dry matter feed was produced. Loading of material was increased during the experiment from 0.1 to 3.0 kg m^{-3} d^{-1} and best result (990 dm^3 kg^{-1}) was obtained with the lowest loading whereas biogas production with the highest loading was 350 dm^3 kg^{-1}. For fibre sludge residue the total production of biogas during the experiment was 400 dm^3 kg^{-1}. Loadings were varied from 5.9 to 10 kg m^{-3} d^{-1} and the best result (580 dm^3 kg^{-1}) was obtained with the lowest loading. Methane content of the produced biogas was 69–75%. The treated waste fibre based stillage contained 3.6 g dm^{-3} BOD (91% reduction), 48 g dm^{-3} COD (66% reduction) and 51 g dm^{-3} suspended solids. The respective analysis results for fibre sludge based stillage were 12 g dm^{-3} (83% reduction), 104 g dm^{-3} (54% reduction), and 71 g dm^{-3}. Further experiments confirmed that additional nutrients (urea and phosphoric acid) were not needed for effective biogas production.

3.6. *Material balance in the process*

A material balance sheet has been produced based on the results from the pilot campaigns. According to the results

presented in this paper 1000 kg dry combined waste fibre and fibre sludge produces approximately 120 kg ethanol, 350 kg biogas (70% methane), 400 kg (d.m.) waste sludge, and 120 kg CO_2. In the calculations 4% of the feedstock glucan has been appointed for yeast propagation. Based on other pilot-scale experiments a glucan hydrolysis yield of 75% is reachable within the process concept if the residence time in fermentation is extended. For this case the corresponding numbers are 170 kg ethanol, 310 kg biogas, 360 kg waste sludge, and 170 kg CO_2.

4. Conclusions

Waste fibre fractionated from solid recovered fuel and pulp and paper mill fibre sludge are competitive bioethanol feedstocks because of their high hexose content and easy hydrolysability without thermal or chemical pre-treatment. High polysaccharide conversion could be obtained in high consistency liquefaction of the feedstocks in only 6–10 h although hydrolysis to monosaccharides was somewhat slower. Hydrolysis of waste fibre and fibre sludge released recalcitrant mannan- and galactan-containing polysaccharides that require additional helper enzymes to be further hydrolysed to monosaccharides. Continuous pilot-scale campaign consisting of liquefaction and simultaneous saccharification and fermentation was run stably for several days with efficient ethanol production and without problems from bacterial contamination. Biogas was produced with high yields from the evaporated residue of waste fibre and fibre sludge hydrolysis and fermentation. The results presented here demonstrate the feasibility of the FibreEtOH concept as a potential 2nd generation bioethanol process.

Acknowledgements

This project has been financed by the 7th Framework Program of the European Commission under grant agreement TREN/FP7EN/239341. In addition, we would like to thank the technicians, engineers and scientists at VTT, especially Juha Tähtiharju, Michael Bailey, Merja Aarnio, Arvi Wilpola, Toni Paasikallio, Pertti Tiitola, and Hanna-Mari Sinilehto for their excellent technical assistance and help in the pilot campaigns

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