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Chemical characterization and hydrothermal pretreatment of *Salicornia bigelovii* straw for enhanced enzymatic hydrolysis and bioethanol potential

Iwona Cybulska [a], Tanmay Chaturvedi [a], Grzegorz P. Brudecki [a], Zsófia Kádár [b], Anne S. Meyer [b], Robert M. Baldwin [a], Mette Hedegaard Thomsen [a,*]

[a] Masdar Institute of Science and Technology, P.O. Box 54224, Abu Dhabi, United Arab Emirates
[b] Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

HIGHLIGHTS

- First published work on chemical composition and biofuel potential of the halophyte *S. bigelovii*.
- *S. bigelovii* straw is a promising new lignocellulosic substrate.
- Halophyte biofuels production is a promising alternative for arid coastal areas.

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ABSTRACT

Salicornia bigelovii straw was characterized and evaluated as a potential lignocellulosic bioethanol feedstock. *S. bigelovii* used in the study was grown in the United Arab Emirates using saltwater (40 ppt) for irrigation. Salt removal was performed prior to pretreatment to protect the processing equipment and avoid inhibition of enzymes and yeast. Composition of the washed biomass was comparable to traditional lignocellulosic biomasses with relatively high glucan and xylan content (26 and 22 g/100 gDM, respectively) but with lower lignin content (7 g/100 gDM). The washed feedstock was subjected to hydrothermal pretreatment, producing highly digestible (up to 92% glucan-to-glucose conversion) and fermentable (up to 100% glucose-to-ethanol conversion) fiber fractions. Liquid fractions obtained in the pretreatment did not show inhibition towards *Saccharomyces cerevisiae*. No significant differences among the enzymatic convertibility and microbial fermentability of the fibers as well as low xylose recoveries suggest that lower severity pretreatment conditions could be exploited for *S. bigelovii*.

1. Introduction

Current energy needs and increasing crude oil prices are the driving force for exploring new energy resources. Lignocellulosic biomass is considered very attractive as a feedstock for generation of bioproducts (including bioethanol), due to its complex structure and natural abundance (Alvira et al., 2010). Traditional lignocellulosic feedstocks include mainly energy crops (e.g. prairie grasses) and agricultural residues (e.g. corn stover, wheat straw, sugar cane bagasse). However, even though being abundant and easily accessible in many regions of the world, these materials are not available in challenging climatic conditions, such as arid, salty soils of Middle East or Africa. An ongoing search for more attractive biofuel feedstocks for all climate regions has led researchers to examine new materials, such as halophytes. Halophytes are remarkable plant species that can survive high salinity environments (10–100 ppt); conditions which are not conducive to growth for 99% of other plants (Flowers and Colmer, 2008). *Salicornia bigolovii* is a halophyte that has attracted attention due to its oil seeds and it is a potential feedstock for biodiesel or bio-SPK (Synthetic Paraffinic Kerosene) production (Warshay et al., 2010). Lignocellulosic part of the plant (stems and seed spikes) is expected to have a similar composition to other halophytic shrubs (10–30% cellulose, 10–30% hemicellulose and 2–10% lignin) (Kraidees et al., 1998). This makes the biomass leftover after the seed separation a potentially attractive lignocellulosic feedstock for production of ethanol, biogas and other value-added by-products. *S. bigelovii* is a native plant for North America and the Caribbean (Zerai et al., 2010). The plant is currently grown in the Middle East for fodder for lamb, sheep and goats, which have the ability to tolerate high-sodium diet (Kraidees et al., 1998).

As 98% of water reserves are saline, and current fertile soils are getting salinized due to rising sea level, plants that can tolerate these conditions can become an attractive new feedstock for

* Corresponding author. Tel.: +971 2 810 9190.
E-mail address: mthomsen@masdar.ac.ae (M.H. Thomsen).

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biofuel production, not competing with food crops for the fertile soil (Rozema and Flowers, 2008).

Lignocellulosic materials require physical–chemical pretreatment prior to biological processing to decrease the recalcitrance of the biomass by breaking the lignin–carbohydrate bonds and decreasing the crystalinity of cellulose (Alvira et al., 2010; Galbe and Zacchi, 2007). Hydrothermal pretreatment has been found to be an effective and cost efficient method for a wide range of lignocellulosic materials including poplar, olive tree residues, corn stover, wheat straw, prairie cordgrass and many more, producing highly digestible fiber fractions (60–100% glucan-to-glucose convertibility) (Cybulska et al., 2009; Petersen et al., 2009; Thomsen et al., 2008).

This method utilizes high-temperature water (having lower pH) to initiate hydrolysis of the acetyl bonds in the lignocellulosic structure, thus removing hemicellulosic oligomers to the solution. Hydrolysis of the acetyl bonds drops the pH further, inducing more acetyl bond cleavage, thus the alternate name of the process is autohydrolysis (Wyman et al., 2005). The effect of elevated temperature can be partially replaced with a catalyst (e.g. a mineral acid), which allows for the processing temperature and time to be lower. The most commonly used catalysts include sulfuric acid, sulfur dioxide or carbon dioxide (Luterbacher et al., 2010; Taherzadeh and Karimi, 2008). Alkaline catalysts have also been widely used (Thomsen et al., 2006). In general, temperature has been found to have major effect on the pretreatment effectiveness towards producing highly digestible fibers. Residence time has a much lower influence on the pretreatment efficiency, and is often found as non-significant factor in statistical modeling (Yu et al., 2010). These observations have been summarized in the severity theory, which generated an experimental severity factor that shows a greater significance of temperature for the pretreatment efficiency (Galbe and Zacchi, 2007; Hendriks and Zeeman, 2009). The optimal severity factor suggested by Aita and Kim (2010) should be between 3.0 and 4.5 for maximum digestibility of the produced fibers. This corresponds to 160–210 °C at processing times between 10 and 30 min (Yu et al., 2010). As the severity of the process increases, sugar degradation reactions become favorable (>170 °C for pentoses and >210–220 °C for hexoses) (Garrote et al., 1999; Zhang et al., 2011) and undesirable by-products are formed. These by-products (including mainly acetic acid, furfural and 5-hydroxymethyl furfural) are known for their inhibitory effect on the microorganisms even in very low concentrations (<1 g/L), which can result in low ethanol yields (Klinke et al., 2004; Thomsen et al., 2009). Furthermore, high pretreatment severity results in alteration of the lignin structure via melting, coagulation, and repolymerization on the cellulose fibers. Sugars released during the autohydrolysis are incorporated in the lignin structure in the process of condensation, leading to losses of carbohydrates and an increase in the acid insoluble residue measurements, giving artificially high values for lignin recovery (Garrote et al., 1999; Young, 1998).

This research study evaluates lignocellulosic biomass of *S. bigelovii*, a halophytic oil plant, as a potential lignocellulosic bioethanol feedstock. A hydrothermal process was chosen as a pretreatment method applied prior to enzymatic hydrolysis and fermentation in order to facilitate high efficiency of both. Optimization of the pretreatment temperature was performed as a screening test for future choice of the range of pretreatment conditions.

2. Methods

2.1. Raw material

S. bigelovii seeds were provided to Masdar Institute by International Center for Biosaline Agriculture (ICBA), Dubai as a part of the Masdar Institute Integrated Seawater Energy Agricultural System (ISEAS) project (ICBA, 2011). The plant was cultivated using 40 ppt NaCl water salinity and 1.0–2.0 gN/m^2 fertilization. Seeds were separated from the plant after harvesting and the resulting biomass (stems, seedless inflorescences, and branches) was dried and used in this study. The seedless and dried material was milled using a knife mill (IKA, 10 MF Basic) to pass through a 1 mm screen.

2.2. Biomass chemical composition characterization

Finely ground lignocellulosic *S. bigelovii* was subjected to a biomass compositional analysis before and after washing with fresh water at 25 °C (Sluiter et al., 2008a,b). The analysis consisted of two steps: (1) determination of extractives and; (2) determination of structural carbohydrates and lignin. Total solids (dry matter) and ash measurements were performed for raw and extractives-free material.

2.2.1. Determination of extractives

First steps of the characterization included sequential water and ethanol extraction using a Soxhlet apparatus to determine total weight of the extractives (Sluiter et al., 2008b). Dry biomass (5 g) was loaded into a cellulose thimble and the extraction was carried out with 200 g of the solvent for 7 h (for each solvent used). Number of siphon cycles per hour was set to 3 for water extraction and 6 for ethanol extraction. Upon completion the thimble contents were removed and dried. Water and ethanol extracts were analyzed for solids content by evaporating to dryness. Water- and ethanol-soluble extractives (total and non-volatile) content in the raw biomass was calculated using Eqs. (1) and (2).

$$\mathrm{NE}\left(\frac{\mathrm{g}}{100\ \mathrm{gDM}}\right) = \frac{\mathrm{W}_{dw/etex}}{\mathrm{DM}} * 100 \tag{1}$$

where NE = non-volatile extractives [g/100 gDM]; $W_{dw/etex}$ = weight of the dried water or ethanol extract (evaporated to dryness) [g]; DM = dry matter of the raw sample [g].

$$\mathrm{TE}\left(\frac{\mathrm{g}}{100\ \mathrm{gDM}}\right) = \frac{\mathrm{DM} - \mathrm{DM}_{\mathrm{ef}}}{\mathrm{DM}} * 100 \tag{2}$$

where TE = total extractives [g/100 gDM]; DM_{ef} = extractives-free dry matter [g].

2.2.2. Determination of carbohydrates and lignin

The extractives-free material was subjected to a strong acid hydrolysis according to (Sluiter et al., 2008a). Dried samples were treated with 72% (w/w) sulfuric acid at 30 °C for 1 h, and then the solutions were diluted with deionized water to achieve 4% (w/w) of sulfuric acid concentration. Diluted samples were autoclaved at 121 °C for 1 h. The hydrolyzates were filtered through fritted ceramic funnels, and the Klason lignin content was determined as the weight of the acid insoluble residue. The hydrolyzates were analyzed for sugars using High Performance Liquid Chromatography (Agilent 1260 Infinity Bio-inert Binary LC). The Hi Plex-H column (Agilent) and refractive index detector (RID) were used to determine the concentrations of glucose, xylose, and arabinose at 65 °C using 0.005 M H_2SO_4 as the mobile phase (eluent) with a flow rate of 0.6 mL/min. Eqs. (3)–(5) summarize the calculations made for the carbohydrates and Klason lignin content in the dry biomass.

$$\mathrm{Car}_{\mathrm{ef}}\left(\frac{\mathrm{g}}{100\ \mathrm{gDM}}\right) = \frac{C_{\mathrm{anhydro}} * V_{\mathrm{h}} * \frac{1\ \mathrm{g}}{1000\ \mathrm{mg}}}{\mathrm{DM}_{\mathrm{ef}}} * 100 \tag{3}$$

where Car_{ef} = carbohydrate content in the extractives-free biomass in the polymer form [g/100 gDM]; $C_{anhydro}$ = concentration of the sugars converted into their polymeric form (glucose in form of glucan, etc.) using an anhydro correction (0.88 for pentoses and 0.90

for hexoses) also corrected for any degradation that may have occurred during the dilute-acid step of the hydrolysis (using a recovery factor calculated from replicates spiked with known concentrations of the sugars analyzed) [g/L]; V_h = volume of the hydrolyzate [mL].

The percentage of each sugar on an "as received" basis (including extractives), was calculated to demonstrate the true content of carbohydrates in the raw samples (Eq. (4)).

$$\mathrm{Car_{ar}}\left(\frac{\mathrm{g}}{100\ \mathrm{gDM}}\right) = \mathrm{Car_{extractives\ free}} * \frac{(100 - \mathrm{Extractives})}{100} \quad (4)$$

where $\mathrm{Car_{ar}}$ = carbohydrate content in the sample containing the extractives ("as received") [g/100 gDM].

Acid insoluble lignin (Klason lignin) content in the extractives-free material was calculated using Eq. (5), while lignin content in the "as received" sample was calculated following the pattern of Eq. (4)

$$\mathrm{AIL_{ef}}\left(\frac{\mathrm{g}}{100\ \mathrm{gDM}}\right) = \frac{(W_{\mathrm{AIL}})}{\mathrm{DM_{extractives\ free}}} * 100\% \quad (5)$$

where AIL = acid insoluble lignin [g/100 gDM]; W_{AIL} = weight of AIL after drying at 105 °C [g].

2.3. Raw feedstock wash

Washing experiment was carried out using 10% (w/v) of biomass loading in wash water at varying temperature (25, 50 and 95 °C) with 10 min residence time. Wash water contained 0.00, 0.03, 5.00, 10.00, and 30.00 ppt of sea salt content. The reason for using salt water was to investigate the feasibility of minimizing fresh water use due to the regional fresh water deficiency. Total solids and ash content were measured in the washed samples to evaluate the washing effectiveness.

2.4. Hydrothermal pretreatment

Hydrothermal pretreatment experiments were carried out at Technical University of Denmark in Roskilde (Risø Campus), Denmark. The process was performed at 6% w/w dry matter loading, at three temperature levels (190, 200 and 210 °C) without a catalyst and at one temperature level (200 °C) with a catalyst (0.5% w/w sulfuric acid). Processing time was maintained at 10 min. Severity factors (calculated using Eq. (6) (Overend et al., 1987) and Eq. (7) (Galbe and Zacchi, 2007)) corresponding to each condition tested are presented in Table 1.

$$\log(R_o) = \log\left(t \exp\left(\frac{T - 100}{14.75}\right)\right) \quad (6)$$

where T = pretreatment temperature [°C]; t = pretreatment time [min]; R_o = severity factor.

$$\mathrm{CS\ (combined\ severity)} = \log(\mathrm{R_o}) - \mathrm{pH} \quad (7)$$

where pH = pH of the slurry after the pretreatment.

In all the experiments, the raw material used for the pretreatment was washed with fresh water (as explained in the "raw feedstock wash" section) to bring down the salt content of the plant to avoid corrosion of the equipment and to remove part of the extractives that may trigger undesired reactions and interfere with the pretreatment conditions. The pretreatment was conducted in a loop-reactor which has been designed and constructed at Risø National Laboratory. The total volume of the loop-reactor is 26 L, while the working volume in these experiments was 1 L. Material of construction for the reactor was Sandvik Sanicro 28 (27% Cr, 31% Ni, 3.5% Mo, 1% Cu). The reactor was rapidly heated to a desired temperature using a salt mixture melt (composed of a 1:1 mixture of sodium nitrite and potassium nitrate with a melting point of 150 °C) and then rapidly cooled in a water bath (to 35 °C) after the treatment (using a moving rack). Due to efficient heat transfer conditions, the heating and cooling times were short (<10 min). After the treatment, the reactor was cooled to 35 °C and the pretreated material was separated by filtration into solid (fibers) and liquid fraction (filtrate). Both fractions were kept at 4 °C until analysis and further processing. Further processing included: total solids/ash measurements of the fiber fractions, analysis of the sugar degradation by-products in the liquid fractions, strong acid hydrolysis (with the measurement of the acid insoluble residue containing Klason lignin), enzymatic hydrolysis and fermentation by *Saccharomyces cerevisiae* of both fractions. Mass balance of all the important components (glucose, xylose, arabinose and ash) was performed using Eqs. (8)–(11). Content of these components in the solid fractions was measured using strong acid hydrolysis, while their amount in the liquid fractions (filtrates) was measured using dilute-acid hydrolysis described later in this section.

Table 1
Severity factors of all the pretreatment conditions tested.

Pretreatment conditions	Severity factor (log R_o)	Combined severity factor (CS)
190 °C, 10 min	3.65	−0.94
200 °C, 10 min	3.94	−0.20
210 °C, 10 min	4.24	0.32
200 °C with catalyst, 10 min	3.94	1.99

$$\mathrm{DM\ in\ (g)} = \frac{\mathrm{DM_{raw}} * W_{\mathrm{ib}} * C_{\mathrm{raw}}}{100\%} \quad (8)$$

where W_{ib} = weight of the initial biomass fed into the pretreatment [g]; $\mathrm{DM_{raw}}$ = total solids in the raw *S. bigelovii* [%]; C_{raw} = content of the specific component in the raw *S. bigelovii* measured by strong acid hydrolysis [g/gDM].

$$\mathrm{DM\ out\ (g)} = \frac{\mathrm{DM_p} * W_f * C_f}{100\%} \quad (9)$$

where W_f = weight of the fiber fraction after the pretreatment [g]; $\mathrm{DM_p}$ = total solids of the pretreated *S. bigelovii* [%]; C_f = content of the specific component in the fiber fraction after the pretreatment measured by strong acid hydrolysis [g/gDM].

$$\mathrm{Fiber\ fraction\ recovery\ \%} = \frac{\mathrm{Dry\ mass\ out\ (g)}}{\mathrm{Dry\ mass\ in\ (g)}} * 100\% \quad (10)$$

$$\mathrm{Liquid\ fraction\ recovery\ \%} = \frac{\mathrm{Amount\ of\ the\ component\ in\ the\ filtrate\ (g)}}{\mathrm{Dry\ mass\ in\ (g)}} * 100\% \quad (11)$$

2.5. Fiber fraction analysis and further processing

2.5.1. Strong acid hydrolysis

The pretreated fibers were subjected to strong acid hydrolysis to determine sugar and lignin recoveries. The process was carried out following the same protocol as in the case of acid hydrolysis of the extractives-free raw and washed *S. bigelovii* described above (Sluiter et al., 2008a).

2.5.2. Enzymatic hydrolysis

Enzymatic convertibility assay based on commercial Cellic CTec2 and Cellic HTec2 (Novozymes A/S, Denmark) enzymes was used to determine the efficiency of the pretreatment. The hydrolysis was performed according to National Renewable Energy

Laboratory protocol (Selig et al., 2008), using 50 g/L dry biomass loading, 15 FPU cellulase enzyme loading (with cellulase-to-hemicellulase ratio of 1:9), in total volume of 25 mL using 50 mL centrifuge tubes. The process was performed in the presence of 50 mM citrate buffer (pH 5) at 50 °C and samples were shaken at 150 rpm for 72 h. Glucose released during the enzymatic hydrolysis was quantified using HPLC at the same operating conditions as applied in the acid hydrolysis samples (described above).

Enzymatic convertibilities of cellulose to glucose and xylan to xylose follow the relationship presented by Eq. (12).

$$\text{Convertibility } (\%) = \frac{C_{\text{glu/xyl}}}{L * \frac{C_f}{100 \text{ gDM}}} * 100\% \quad (12)$$

where $c_{\text{glu/xyl}}$ = concentration of glucose/xylose measured in the enzymatic hydrolyzate [g/L]; L = fibers loading [g/L]; C_f = content of the specific component (glucose or xylose) in the fiber fraction after the pretreatment [g/100 gDM].

2.5.3. Simultaneous saccharification and fermentation (SSF)

In order to evaluate the ability of *S. cerevisiae* yeast to ferment the cellulose-rich fiber fraction obtained after the pretreatment, simultaneous saccharification and fermentation (SSF) was performed. Fermentation mixture was prepared in 250 mL flasks, at 100 mL total volume. The flasks were secured with glycerol-filled yeast locks, to ensure anaerobic conditions, while enabling carbon dioxide release. The mixture was composed of enzymes (types and doses same as for enzymatic hydrolysis), pH buffer (to maintain the pH at 4.8), and 10% DM biomass loading. The mixture was pre-hydrolyzed for 24 h at 50 °C and 150 rpm in a shaking bed incubator. After the pre-hydrolysis, 0.2 g of dry Baker's yeast was added along with urea as nutrients source (0.2 mL of a 24% urea solution). The fermentation process was performed at 30 °C, with constant shaking (90 rpm) for 72 h. Weight measurements of the flasks were recorded throughout the duration of the process. The weight loss (representing release of carbon dioxide) was translated to ethanol yield using Eq. (13). Final ethanol concentration was determined by the HPLC analysis.

$$W_{\text{et}} = \frac{M_{\text{et}}}{M_{\text{CO}_2}} * W_{\text{CO}_2} \quad (13)$$

where W_{et} = weight of ethanol (EtOH) produced [g]; M_{et} = molar mass of ethanol [46 g EtOH/mol EtOH]; M_{CO_2} = molar mass of CO_2 [44 g CO_2/mol CO_2]; W_{CO_2} = weight of CO_2 produced = weight loss of the fermentation flask [g].

Theoretical ethanol yield was determined based on the available glucose released during enzymatic hydrolysis, following Eq. (14). Ethanol yield (%) was calculated as a percent ratio of the actual ethanol amount produced to the theoretical ethanol yield (Eq. (15)).

$$\text{TY}_{\text{et}} = 0.51 * m_{\text{glu}} \quad (14)$$

where TY_{et} = theoretical ethanol yield [g]; m_{glu} = amount of glucose released during enzymatic hydrolysis [g].

$$Y_{\text{et}} = \frac{\text{Ethanol produced (g)}}{\text{TY}_{\text{et}} \text{ (g)}} * 100\% \quad (15)$$

where Y_{et} = ethanol yield [%].

2.6. Liquid fractions (filtrates) analyses

2.6.1. Analysis of free sugars and pretreatment by-products

Liquid fractions were analyzed for the free released sugars including glucose, xylose and arabinose as well as for the pretreatment by-products (mostly sugar degradation products) including acetic acid, furfural and HMF. The analysis was performed using HPLC with the same operating conditions as described above.

2.6.2. Dilute-acid hydrolysis

Oligomeric carbohydrates released to liquid fractions have to be hydrolyzed to monomeric sugars prior to the HPLC measurement. To determine the quantity of the oligomers of xylose, arabinose and glucose in the pretreated liquid fraction (filtrate), dilute-acid hydrolysis was carried out with 8% w/w H_2SO_4 solution. The ratio of the liquid fraction sample to acid solution (8% w/w H_2SO_4) was 1:1, producing final acid concentration of 4% w/w. The solution was autoclaved at 121 °C for 10 min. Sugar recovery measurement was performed and the recovery factor was included in the calculations to account for sugar losses to degradation products. The dilute acid hydrolyzates were analyzed using the HPLC system to measure the concentrations of the simple sugars released. The results were then used in the mass balance recovery calculations for the sugars, as described above.

2.6.3. Fermentability study

Fermentability study was carried out on the liquid fractions obtained from the pretreatment process in order to test the possibility of using the filtrates as fermentation medium. Depending on the type of the lignocellulosic material and pretreatment conditions, the concentration of inhibitors in the filtrate will differ and their influence on the microorganisms and the fermentation performance will consequently vary (Olsson and Hahn-Hägerdal, 1996). Microbial constraints on parameters such as pH, temperature, and nutrient supplementation were controlled in this fermentation study. Since the liquid fraction contains fewer hexoses (as compared to solid fraction) and more inhibitors, glucose had to be added to the fermentation solution as well as additional nutrient mixture to facilitate the yeast with the optimal growth conditions. Fiber fractions are more abundant in nutrients therefore only nitrogen source (urea) was added as an external nutrient.

A 100 mL of the aqueous stock solution contained 6.25 g of ammonium sulfate, 2.50 g of mono potassium phosphate (KH_2PO_4), 5.00 g of yeast extract and 0.75 g of hydrous magnesium sulfate ($MgSO_4 \cdot 7H_2O$). *S. cerevisiae* pre-cultures were centrifuged and washed by water twice and then re-suspended in water. The dry matter loading of *S. cerevisiae* was 6.72% (2 g/L). This stock solution was used for preparation of the fermentation solution. The fermentation solution contained 35 g/L of glucose and 4% of the stock solution. Sodium hydroxide was used to adjust the pH of the filtrates to 4.8. Flasks containing the fermentation solution were incubated at 35 °C and 100 rpm. Weight loss measurements were taken over the following 45 h, and the weight loss was converted to ethanol yield using Eq. (13). The results of ethanol production were plotted to show the kinetics of the fermentation process in the pretreatment filtrates and thus any possible lag phase resulting from the inhibitory effect of the pretreatment by-products.

3. Results and discussion

3.1. Raw feedstock characterization

Characterization of the seedless *S. bigelovii* revealed extremely high ash content (43.08 g/100 gDM). Other than that, the material shows typical lignocellulosic crop characteristics, containing cellulose, lignin and hemicellulose as dominant components. Results of the compositional analysis are as follows: 9.1 ± 1.5 g/100 gDM glucan, 7.7 ± 0.4 g/100 gDM xylan, 5.5 ± 2.1 g/100 gDM arabinan, 6.8 ± 1.4 g/100 gDM Klason lignin, 6.8 ± 0.1 g/100 gDM structural ash, and 53.7 ± 3.6 g/100 gDM total extractives (including extractable ash).

Water extractives (Table 2) represent a substantial part of the plant, containing "extractable" ash which is mostly represented by salty deposit on the plant tissues. This suggests that most of the ash measured in the original material can be removed by water wash. In order to improve the efficiency of the ethanol production per unit mass of *S. bigelovii* as well as to protect the pretreatment equipment, a wash of the original samples was investigated.

3.2. Raw feedstock wash

Results of the wash experiment (Table 3) revealed that fresh water is needed in order to remove salt deposits from the biomass. Even when using low-salinity salt water (5 ppt) a relatively low amount of salt was removed from the biomass. The results also showed that when using fresh water, a large amount of the salt can be extracted at low temperature in a short time (10 min). Samples washed at 25 °C for 10 min were used as the pretreatment feedstock with composition as follows: 25.8 ± 2.0 g/100 gDM glucan, 21.6 ± 1.5 g/100 gDM xylan, 5.7 ± 1.8 g/100 gDM arabinan, 7.7 ± 0.1 g/100 gDM Klason lignin, 5.5 ± 0.5 g/100 gDM structural ash, and 18.3 ± 1.2 g/100 gDM total extractives (including extractable ash).

The washed feedstock was found to contain far less ash (9.97 g/100 gDM including extractable and structural ash) and thus more cellulose per unit mass than the original material. No losses of the other components were observed after the wash. This fact is a strong argument for the washing step to be applied prior to any processing of the biomass.

3.3. Hydrothermal pretreatment of the washed biomass

S. bigelovii was found to follow the trend of a typical lignocellulosic biomass when treated with high temperature and pressure in aqueous environment. Major part of cellulose (up to 85%) was retained in the solid, as was lignin and part of hemicellulose (Fig. 1a–d). Xylan was mostly dissolved into the filtrate, and partly degraded to furfural due to high severities used. Xylan removal has been proven to be associated with higher enzymatic digestibility of the pretreated fibers (Holopainen-Mantila et al., 2013). As can be observed from Fig. 1b, a temperature higher than 200 °C results in increased production of furfural, which can be inhibitory for fermentative microorganisms. However, furfural is a valuable compound which can be utilized as a value-added product to generate platform chemicals (Bozell et al., 2000). Lignin recovery in the pretreated fibers was found to be higher than lignin content in the feedstock (over 100% of the total input), which is suspected to be the result of a high extent of condensation and reprecipitation reactions resulting in high acid insoluble residue content (AIR) also referred to as pseudolignin and low recoveries of hemicellulose carbohydrates (Fig. 1b and c). Extractable (non-structural) ash was partly removed to the filtrate to an extent dependent on the temperature used (Fig. 1d). The results suggest that temperature increase has a positive influence on the removal of salts from the biomass.

Mean comparisons (using Tukey's Honestly Significant Difference, HSD) revealed that temperature does not have influence on the glucose distribution between the solid and the liquid, however adding a catalyst results in a significantly ($p < 0.05$) higher glucose extraction to the filtrate. Xylose recovery in the filtrate was significantly ($p < 0.05$) lower in the samples treated at 210 °C. Xylose recovery response is closely correlated (R^2 of 0.86) to the degradation of xylose to furfural (Fig. 2).

No significant differences were found among different treatments for the acid insoluble residue measurements. Significantly lower ($p < 0.05$) amount of ash was dissolved in the filtrate in the samples treated at 190 °C, suggesting that higher temperatures are more favorable to produce low-ash fibers for enzymatic hydrolysis and fermentation.

Table 2
Water and ethanol extractives analysis.

Extractives (non-volatile)			
	Content (g/100 gTS)	Ash content (%)	Ash-free content (g/100 gDM)
Water extractives	49.1 ± 0.2	73.9 ± 0.1	12.8 ± 0.1
Ethanol extractives	2.1 ± 0.1	20.5 ± 1.8	1.7 ± 0.1
Total extractives	51.2 ± 0.1		14.5 ± 0.1

Table 3
Washing experiment results.

Salinity of water [ppt]	Temperature of washing [°C]	Ash in the solids [% DM]
0.00	25.00	16.50
0.00	50.00	16.21
0.00	95.00	20.43
0.03	25.00	21.35
0.03	50.00	20.42
0.03	95.00	21.27
5.00	25.00	33.70
5.00	50.00	37.50
5.00	95.00	33.11
10.00	25.00	17.95
10.00	50.00	32.50
10.00	95.00	37.72
30.00	25.00	38.10
30.00	50.00	38.50
30.00	95.00	39.56

3.4. Enzymatic hydrolysis

All pretreatment conditions resulted in a significant ($p < 0.05$) enhancement of the initial glucan-to-glucose enzymatic convertibility of *S. bigelovii*. Enhancement in the enzymatic hydrolysis is typical when a pretreatment is applied to a lignocellulosic biomass, achieving high glucan-to-glucose convertibility for this feedstock (87–92%), when compared to the non-pretreated samples (26%). Similar trend has been found in previous studies using hydrothermal pretreatment applied to lignocellulosic feedstocks, including wheat straw (up to 93% enzymatic hydrolysis cellulose convertibility) (Petersen et al., 2009), wheat bran (up to 90% enzymatic hydrolysis cellulose convertibility) (Reisinger et al., 2013) prairie cordgrass (up to 95% enzymatic hydrolysis cellulose convertibility) (Cybulska et al., 2009). No significant difference among glucan-to-glucose conversions was found among the treatments. Similar observations were made for the xylan-to-xylose convertibility, achieving 62–100% (at the highest pretreatment severity), compared to 6% convertibility for the non-pretreated samples. The lack of significant differences among the various pretreatments suggests that the severity does not have influence on the glucose and xylose enzymatic hydrolysis yields in the range tested. Since all of the pretreatment conditions produced highly digestible fibers, it can be argued that even lower pretreatment severities should be explored.

3.5. Simultaneous saccharification and fermentation

All pretreated fiber fractions were fermented with high efficiency by *S. cerevisiae*, with no apparent lag phase. The maximum ethanol yield – between 1.72 and 1.99 g ethanol (17.2 and 19.9 g/L) corresponding to 77% and 100% of theoretical yield based on

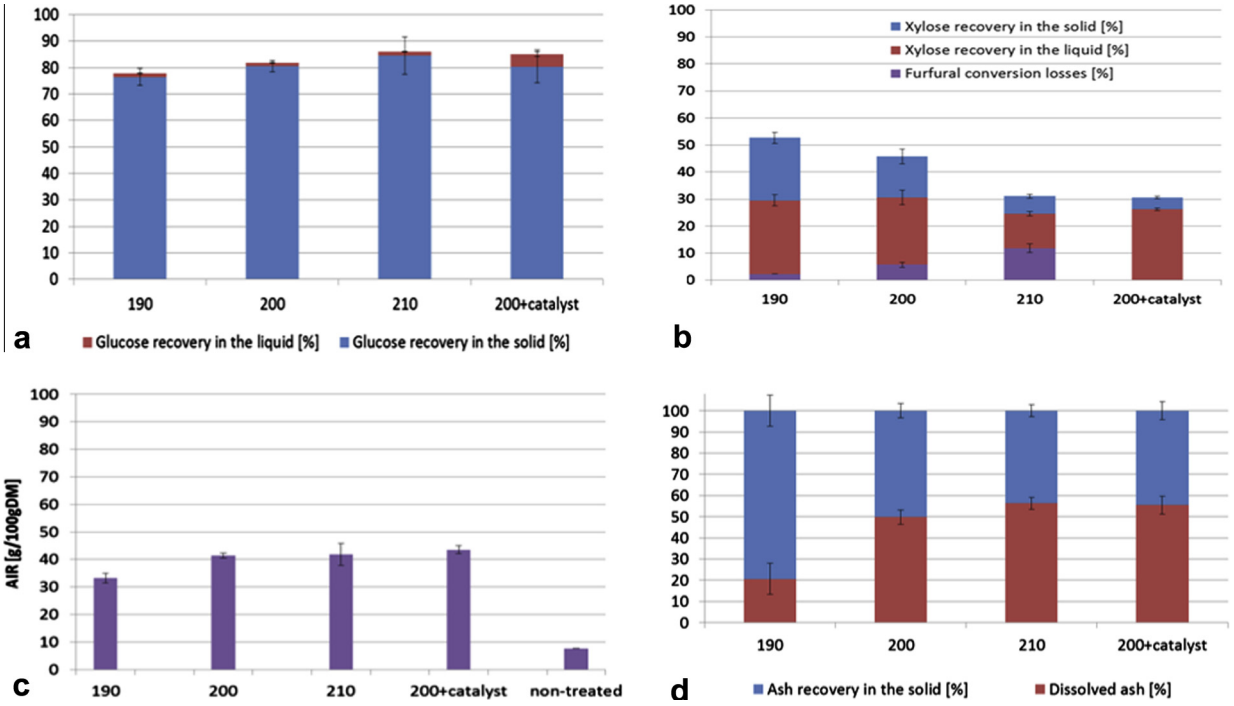

Fig. 1. Mass balance of the pretreatment process tracking the distribution of: a – glucose, b – xylose, c – lignin (contained in the acid insoluble residue, AIR) and d – ash.

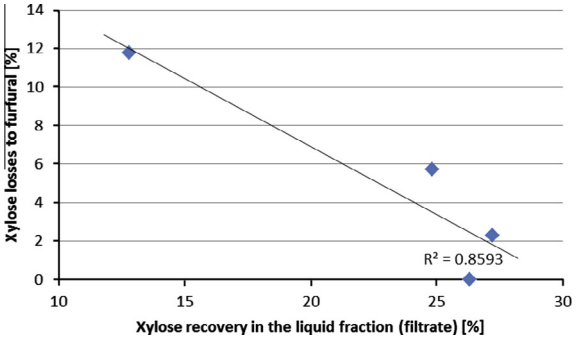

Fig. 2. Xylose recovery vs. xylose losses in degradation to furfural.

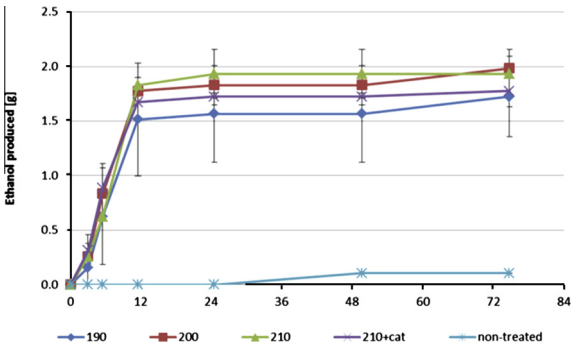

Fig. 3. Results of the simultaneous saccharification and fermentation (at-line monitored ethanol production).

available glucose only, respectively – was achieved after the first 12 h in all the samples tested (Fig. 3).

Tukey's HSD showed no significant difference among ethanol yields produced using samples pretreated at different severities. Significant differences in the ethanol yield were found among all of the pretreated samples and a non-treated sample. Similarly to the enzymatic hydrolysis results, this suggests that since severity did not have influence on the fermentability of the fibers in the range tested, lower severities of the pretreatment should be examined in the future studies. Ethanol yield measured using the gravimetric method was found not to be significantly different from the HPLC measurement, save one sample (pretreated at 200 °C without catalyst). This suggests that gravimetric measurement can be successfully used in the ethanol yield evaluation in most cases.

3.6. Liquid fractions

Liquid fractions obtained from the pretreatment (filtrates) have been analyzed in detail in order to evaluate their possible use as the fermentation medium. The severity factor (log R_o) had a high influence on the hemicellulose removal from the fibers and formation of pretreatment by-products, showing a typical trend for the lignocellulosic biomass (Galbe and Zacchi, 2007) (Fig. 4a). However, as the severity factor does not include the pH parameter, it does not reflect the true influence of the acid catalyst on all those responses. Fig. 4b presents the same response variables (hemicellulose removal from the fibers and concentration of pretreatment by-products formed) dependency on the combined severity factor, which includes the pH parameter, broadening the possibility of the result interpretation (Chum et al., 1990). It can be observed that the hemicellulose removal is even more dependent on the combined severity factor, but formation of all the by-products show little correlation (maximum R^2 of 0.54 for acetic acid). Based on these results, even though the hemicellulose removal increases with decreasing pH, adding a catalyst to the elevated temperature treatment of *S. bigelovii* results in slightly higher deacetylation and lower degradation of the sugars to the inhibitory by-products (Fig. 4b). A clear correlation between hemicellulose removal and severity factors is not always found in the lignocellulosic material pretreatment, as the equations used for the severity factor calculation are experimental and not always fit the conditions of the process, which has been observed for the catalyzed hydrothermal

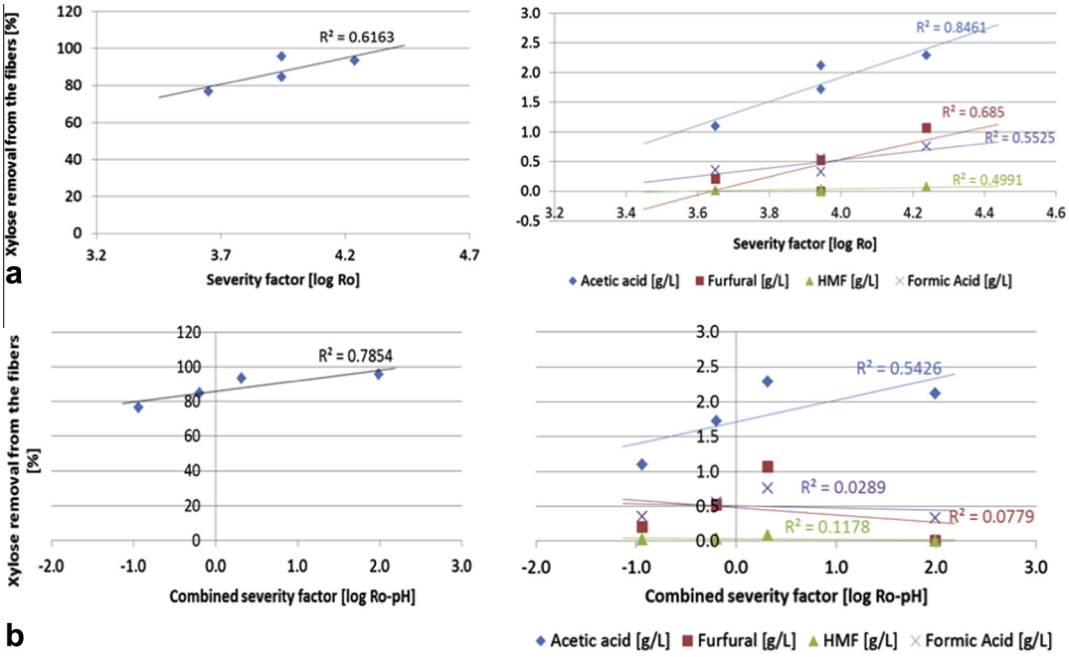

Fig. 4. Influence of: (a) severity factor (log R_o) and; (b) combined severity factor (log R_o-pH) on the xylose removal from the fibers and on the formation of the pretreatment by-products.

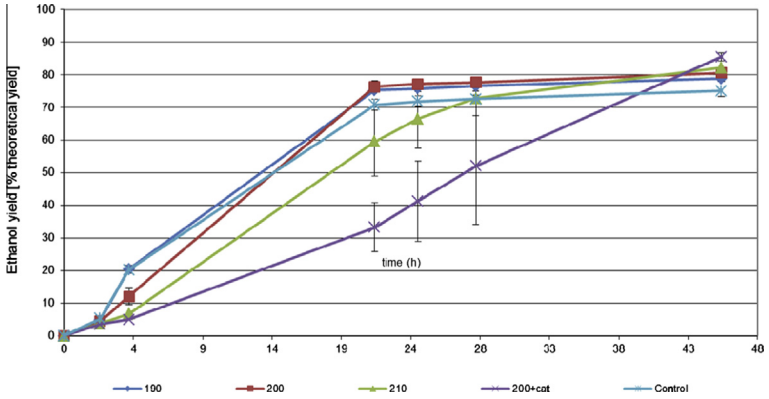

Fig. 5. Fermentability of the pretreatment liquid fractions illustrated by the ethanol yield produced using *S. cerevisiae*.

pretreatment of wheat straw (Pedersen and Meyer, 2010). Clear correlation occurring in the pretreatment of the *S. bigelovii* samples shows that the plant follows an expected outcome and these equations can be used to describe process conditions.

The fermentability study is an important tool for testing any possible inhibitory influence the liquid fractions may have on the fermentative microorganisms and their efficiency in ethanol production. It was observed that liquid fractions obtained at 190–200 °C were non-inhibitory for *S. cerevisiae* when compared to a control, achieving slightly better ethanol yields than the control. Liquid fractions obtained at 210 °C and at 200 °C with a catalyst resulted in a longer lag phase, suggesting initial inhibition of *S. cerevisiae* performance in ethanol production (Fig. 5). This result can be explained by a high content of acetic acid in samples obtained at both conditions, which has been considered inhibitory for *S. cerevisiae* in concentrations over 0.5 g/L (Jacques et al., 2003). Furthermore, relatively high furfural concentrations were detected in samples treated at 210 °C, which has also been found to cause microbial stress, even at low concentrations (1 g/L) (Modig et al., 2002). Lag phase observed in the samples treated with the sulfuric acid catalyst can be also caused by added content of sulfite (not removed from the pretreatment liquid), which has a known inhibitory effect on the metabolism of yeast (Schimz, 1980).

4. Conclusions

Seedless *S. bigelovii* examined as a bioethanol feedstock follows a typical trend for a lignocellulosic biomass. However, fresh water wash is required prior to any processing to remove the salt deposits. No significant difference was found among the enzymatic digestibility and fiber fermentability (both found to be high) in the severity range tested, suggesting that lower severities could be examined in the future, also since the content of lignin was found low in the feedstock. High hemicellulose losses were observed during the pretreatment, showing a correlation with increasing temperature, giving more reasons to lower processing temperature in future trials.

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References

Aita, G.M., Kim, M., 2010. Pretreatment technologies for the conversion of lignocellulosic materials to bioethanol. In: Sustainability of the Sugar and Sugar Ethanol Industries. American Chemical Society, pp. 117–145.

Alvira, P., Tomas-Pejo, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour. Technol. 101 (13), 4851–4861.

Bozell, J.J., Moens, L., Elliott, D.C., Wang, Y., Neuenscwander, G.G., Fitzpatrick, S.W., Bilski, R.J., Jarnefeld, J.L., 2000. Production of levulinic acid and use as a platform chemical for derived products. Resour. Conserv. Recycl. 28 (3–4), 227–239.

Chum, L.H., Johnson, D.K., Black, S.K., 1990. Organosolv pretreatment for enzymatic hydrolysis of poplars. 2. Catalyst effects and the combined severity parameter. Ind. Eng. Chem. Res. 29, 156–162.

Cybulska, I., Lei, H., Julson, J., 2009. Hydrothermal pretreatment and enzymatic hydrolysis of prairie cord grass. Energ. Fuel. 24 (1), 718–727.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179 (4), 945–963.

Galbe, M., Zacchi, G., 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. In: Olsson, L. (Ed.), Biofuels, vol. 108. Springer Berlin, Heidelberg, pp. 41–65.

Garrote, G., Dominguez, H., Parajo, J.C., 1999. Hydrothermal processing of lignocellulosic materials. Holz Roh – Werks 57 (3), 191–202.

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100 (1), 10–18.

Holopainen-Mantila, U., Marjamaa, K., Merali, Z., Käsper, A., de Bot, P., Jääskeläinen, A.S., Waldron, K., Kruus, K., Tamminen, T., 2013. Impact of hydrothermal pretreatment to chemical composition, enzymatic digestibility and spatial distribution of cell wall polymers. Bioresour. Technol. 138, 156–162.

ICBA. 2011. Evaluation of *Salicornia bigelovii* under different salinity levels. Annual report. The International Center for Biosaline Agriculture.

Jacques, K.A., Lyons, T.P., Kelsall, T.P., 2003. The Alcohol Textbook, fourth ed. Nottingham University Press, Nottingham, UK.

Klinke, H.B., Thomsen, A.B., Ahring, B.K., 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl. Microbiol. Biotechnol. 66 (1), 10–26.

Kraidees, M.S., Abouheif, M.A., Al-Saiady, M.Y., Tag-Eldin, A., Metwally, H., 1998. The effect of dietary inclusion of halophyte *Salicornia bigelovii* Torr on growth performance and carcass characteristics of lambs. Animal Feed Sci. Technol. 76 (1–2), 149–159.

Luterbacher, J.S., Tester, J.W., Walker, L.P., 2010. High-solids biphasic CO_2–H_2O pretreatment of lignocellulosic biomass. Biotechnol. Bioeng. 107 (3), 451–460.

Modig, T., Liden, G., Taherzadeh, M.J., 2002. Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. Biochem. J. 363 (Pt. 3), 769–776.

Olsson, L., Hahn-Hägerdal, B., 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb. Technol. 18 (5), 312–331.

Overend, R., Chornet, E., Gascoigne, J., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments [and discussion]. Phil. Trans. R. Soc. Lond. Ser. A Math. Phys. Sci. 321 (1561), 523–536.

Pedersen, M., Meyer, A.S., 2010. Lignocellulose pretreatment severity – relating pH to biomatrix opening. New Biotechnol. 27 (6), 739–750.

Petersen, M.Ø., Larsen, J., Thomsen, M.H., 2009. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass Bioenerg. 33 (5), 834–840.

Reisinger, M., Tirpanalan, Ö., Prückler, M., Huber, F., Kneifel, W., Novalin, S., 2013. Wheat bran biorefinery – a detailed investigation on hydrothermal and enzymatic treatment. Bioresour. Technol. 144, 179–185.

Rozema, J., Flowers, T.J., 2008. Crops for a salinized world. Science 322, 1478–1480.

Schimz, K.-L., 1980. The effect of sulfite on the yeast *Saccharomyces cerevisiae*. Arch. Microbiol. 125 (1–2), 89–95.

Selig, M., Weiss, N., Ji, Y. 2008. Enzymatic saccharification of lignocellulosic biomass: laboratory analytical procedure. National Renewable Energy Laboratory. NREL/TP-510-42629.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2008a. Determination of structural carbohydrates and lignin in biomass. National Renewable Laboratory. NREL/TP-510-42618.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2008b. Determination of extractives in biomass. National Renewable Energy Laboratory. NREL/TP-510-42619.

Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int. J. Mol. Sci. 9 (9), 1621–1651.

Thomsen, M., Thygesen, A., Thomsen, A., 2009. Identification and characterization of fermentation inhibitors formed during hydrothermal treatment and following SSF of wheat straw. Appl. Microbiol. Biotechnol. 83 (3), 447–455.

Thomsen, M.H., Thygesen, A., Jørgensen, H., Larsen, J., Christensen, B.H., Thomsen, A.B., 2006. Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of bioethanol and electricity. In: McMillan, J.D., Adney, W.S., Mielenz, J.R., Klasson, K.T. (Eds.), Twenty-Seventh Symposium on Biotechnology for Fuels and Chemicals. Humana Press, pp. 448–460.

Thomsen, M.H., Thygesen, A., Thomsen, A.B., 2008. Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicellulose recovery, high cellulose digestibility and low lignin hydrolysis. Bioresour. Technol. 99 (10), 4221–4228.

Warshay, B., Pan, J., Sgouridis, S., 2010. Aviation industry's quest for a sustainable fuel: considerations of scale and modal opportunity carbon benefit. Biofuels 2 (1), 33–58.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y., 2005. Coordinated development of leading biomass pretreatment technologies. Bioresour. Technol. 96 (18), 1959–1966.

Young, R.A., 1998. Environmentally Friendly Technologies for the Pulp and Paper Industry. John Wiley & Sons, Inc.

Yu, G., Yano, S., Inoue, H., Inoue, S., Endo, T., Sawayama, S., 2010. Pretreatment of rice straw by a hot-compressed water process for enzymatic hydrolysis. Appl. Biochem. Biotechnol. 160 (2), 539–551.

Zerai, D.B., Glenn, E.P., Chatervedi, R., Lu, Z., Mamood, A.N., Nelson, S.G., Ray, D.T., 2010. Potential for the improvement of *Salicornia bigelovii* through selective breeding. Ecol. Eng. 36 (5), 730–739.

Zhang, B., Shahbazi, A., Wang, L., Diallo, O., Whitmore, A., 2011. Hot-water pretreatment of cattails for extraction of cellulose. J. Ind. Microbiol. Biotechnol. 38 (7), 819–824.