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BIOENERGY AND BIOFUELS

Wet fractionation of the succulent halophyte *Salicornia sinus-persica*, with the aim of low input (water saving) biorefining into bioethanol

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Abstract In this study *Salicornia sinus-persica,* a succulent halophyte was assessed for its potential to be used as a feedstock for bioethanol production. For such succulent, salty, green biomasses, direct fractionation and fermentation allow for water preservation in the process. Fresh biomass of *S. sinus-persica* was collected and split into two fractions by wet fractionation; liquid (juice) and solid (pulp). Sugar contents were found to be 1.0–1.5% for the juice fraction and 50% (*w*/w) for the fresh pulp. Direct fermentation of the juice using *Saccharomyces cerevisiae* showed no salt inhibition of the yeast and ethanol yields of ~70% were achieved. A pretreatment study was carried out for the pulp fraction applying mild hydrothermal pretreatment. Cellulose convertibility was found to be significantly higher for severity factors above 2.00, and the highest ethanol yield (76.91 ± 3.03%) was found at process severity of 3.06 (170 °C, 10 min).

Keywords *Salicornia sinus-persica* · Halophyte · Biomass · Green biorefinery · Bioethanol · Hydrothermal pretreatment

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Introduction

Biomass covering sugar and starch-rich crops, lignocellulosic woody and herbaceous material, and vegetable oils can provide a wide range of fuels and chemicals, which are currently made from petroleum (Wyman et al. 2005). The United Arab Emirates (UAE), like many other coastal countries, is not suitable for plant cultivation, due to scarceness in fertile soil and freshwater reserves. Yet, some native plants have shown the ability to grow in arid lands under harsh conditions; such as high temperatures and seawater irrigation (Cybulska et al. 2014b; Galvani 2007). Hence, vegetation existing in salty environments (such as algae, sea weeds, mangroves, and halophytes) should be studied in terms of chemical composition and ability to produce biofuels to substitute depleting fossil fuels, in addition to the economic feasibility for production and large-scale utilization of such vegetation.

Salicornia is an annual, small, vascular, salt-tolerant plant (halophyte), which is ordinarily found in coastal estuaries and salt swamps (Bassam 2010). It shows ability to yield as much biomass and seed as conventional crops even with soil salinity exceeding 70 ppt (about twice the salinity of seawater) (Glenn et al. 1991). *Salicornia sinus-persica* was found in Iran, specifically in the central provinces (Akhani 2003) and also in areas surrounding the Arabian Gulf.

Salicornia sinus-persica has been described by Akhani (2003) growing in Iran as an annual plant, smooth, dark green in color, and becoming orange-reddish on the lower part of the stems. Its height ranges between 25 and 60 cm, with a canopy diameter reaching 80 cm. Stems ascend in dense form, getting salt accumulations on the lower older shoots forming tubercles. With increasing salinity, water potential in *S. persica* became more negative; indicating that *S. persica* osmotically adjusts in response to salinity intensification (Ahmad et al. 2012).

Biomass pretreatment is a key step for utilizing lignocellulosic biomasses in the biofuel's production process, since it improves the enzymes accessibility, which is hindered by lignin and hemicelluloses by solubilizing the amorphous hemicellulose. Hydrothermal pretreatment is a promising pretreatment method that has successfully been applied to many herbaceous biomasses (Chandra et al. 2012; Cybulska et al. 2014b; Petersen et al. 2009; Thomsen et al. 2008). Optimal hydrothermal pretreatment is advantageous, since it positively impacts the production yields, product concentration, hydrolysis, and fermentation extents while decreasing the needed enzyme loading (Kristensen et al. 2008; Wyman et al. 2005).

A study on dry lignocellulosic biomass of Salicornia showed that the dried biomass needs to be washed with fresh water in order to bring down the salt content prior to processing (Cybulska et al. 2014a). However, if the plant is harvested in its green succulent state, wet fractionation can allow for water preservation in the process.

In green biorefineries, fresh biomass is split into a juice and a pulp fraction (wet fractionation). A fresh juice is obtained; containing active enzymes, vitamins, amino acids, and monomeric sugars (Thomsen 2005; Weimer and Digman 2013). Inhibitor formation is minimized, and cost of pretreatment decreased because only the green pulp needs pretreatment (Kamm and Kamm 2004; Kerfai et al. 2011). Some green biomass biorefinery projects are now wet separating the green biomass into green juice, press cake (pulp), and extracting proteins by applying mechanical force (Kromus et al. 2004; Thomsen et al. 2004). Pulp can be used either to produce forage for animal feed or for energy production, applying different methods, like combustion, fermentation to produce several liquid fuels, or digestion to produce gaseous fuels (Weimer and Digman 2013). The fresh green juice contains high value protein, vitamins, and phyto-chemicals, which could be extracted from the liquid blend before using the residual green juice as fermentation medium (Johnston et al. 2013; Thomsen et al. 2004; Thomsen et al. 2015). There have also been attempts for wet-fractionation to extract and concentrate soluble leaf proteins to be used in food products, especially in countries scarce of important nutrients like proteins (Lamsal 2004). Perie (1975) showed that pulping and pressing of different feed stocks results in 50–70% of crude protein yield of the extraction phase (depending on the feedstock).

In this study, fresh biomass of *Salicornia sinus-persica* was collected from the Umm al-Quwain shores in the United Arab Emirates (UAE), and studied for its potential to be used as a feedstock for bioethanol production using wet fractionation. The juice fraction was investigated for direct fermentation, and a hydrothermal pretreatment study was conducted on the fiber rich pulp fractions. Mild pretreatment was attempted, in order to minimize energy inputs. The impact of the applied pretreatment conditions was evaluated by studying the sugar recovery as well as the biomass fermentability.

Methodology

Feedstock collecting and handling

A native strain of Salicornia (i.e., *Salicornia sinus-persica*) was collected from Umm Al Quwain shores in United Arab Emirates. Total solids (TS) and total ash (TA) were measured in the original plant following the NREL protocol (Sluiter et al. 2008a; Sluiter et al. 2008b). The biomass sample was placed in the crucibles and dried at 105 °C overnight to determine the total solids content (also called dry matter—DM). In order to measure TA, the crucibles were ignited at 575 °C in the muffle furnace.

Fresh biomass fractionation and processing

Freshly collected green biomass of *Salicornia sinus-persica* was fractionated into a juice fraction and a pulp fraction using a SoloStarII, low rpm single auger juicer, model SS-9002. Both fractions were preserved at −18 °C until further processing. A portion of the pulp was dried at 105 °C before being used for carbohydrate composition analysis.

Total solids and total ash contents were evaluated for each fraction. Moreover, protein content was measured for the fractionated (pulp and juice) by measuring the total nitrogen content applying the Kjeldahl method (Janssen and Koopmann 2005).

Fresh juice analysis and processing

Juice sugar composition

Juice monomeric sugar content was determined before and after weak acid hydrolysis (WAH) (Sluiter et al. 2006). Monomeric sugars including glucose, xylose, fructose, and arabinose in the juice were analyzed by running the juice directly on HPLC as described below. WAH of the juice was done by taking a sample of 10 ml and digesting it with 10 ml of sulfuric acid (8% *w*/w). The solution was cooked at 121 °C for 10 min. After cooling the solution to room temperature, it was analyzed for carbohydrates and other by-products using HPLC (Agilent 1260 Infinity Bio-inert Binary LC). The Hi Plex-H column (Agilent) and RI detector were used to determine the concentrations of glucose, xylose, and arabinose at a temperature of 65 °C by means of 0.005 M H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min. The Hi Plex-Pb column (Agilent) and RI detector were used to determine the concentrations of glucose and fructose at a temperature of 80 °C with deionized water as the mobile phase at a flow rate of 0.6 ml/min.

Juice lipid composition

Lipid composition of the juice was gravimetrically determined for both, fresh and dried salicornia juice. First, total lipids extraction was done by soxhlet extraction, using a

chloroform—(methanol solvent mixture in a ratio of 2:1). Then the composition was gravimetrically evaluated.

Juice fermentability

The extent of glucan-to-glucose convertibility of the juice was studied in fermentations with *Saccharomyces cerevisiae* in 1.0 l bioreactors (without juice pre-processing). pH was adjusted and maintained at 4.8 and the temperature was maintained at 32 °C. Commercial *Saccharomyces cerevisiae* (dry yeast, Malteserkors tørgær, De Danske Spritfabrikker A/S, Denmark) was inoculated in a concentration of 2.0 g/L, where continuous shaking at 200 rpm was applied.

Fresh pulp processing

The wet pulp was split into two fractions, one was dried at 105 °C overnight to be analyzed for its sugar composition and the other was kept (wet) in the freezer (at −18 °C), for further processing (discussed in the fermentability section).

Chemical characterization by strong acid hydrolysis of the pulp fraction

Carbohydrate composition was analyzed for the fresh pulp (without pretreatment) after being dried. The analysis followed standard analytical protocols developed by the National Renewable Energy Laboratory (NREL) and was achieved by determining the lignocellulosic components, carbohydrates and lignin. The dry pulp was hydrolyzed by strong acid (Sluiter et al. 2008c). Each sample was analyzed in duplicate. Dried samples (0.3 g) were treated with 72% (*w*/w) H_2SO_4 (3.0 ml) at 30 °C for 1 h with constant centrifugation (every 10 min). The solutions were diluted with 84 ml of water (acid diluted to 4%) and cooked at 121 °C for 1 h. Spiked samples were prepared (also in duplicate) in which 82 ml of water and 2 ml of spike solution were added in dilutions after concentrated acid hydrolysis. The spike solution was composed of a mixture of the three sugars (glucose, xylose, and arabinose) at concentrations of 30 g/L of each.

The hydrolysate generated after strong acid hydrolysis was filtered, and the Klason lignin content was determined as the difference between the weight of the insoluble residue and the ash content. The samples were analyzed for carbohydrates and other by-products (organic acids) using HPLC (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 a temperature of 65 °C by means of 0.005 M H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min. Accordingly, the content of sugars and organic acids was calculated (Cybulska et al. 2014a).

Pulp pretreatments

To study the effect of the pretreatment temperature and residence time, the pulp fraction was mildly pretreated at process severity ranging from 1.62 to 3.06. The used severities were achieved as follows: 1.62: 121 °C, for 10 min, 2.10: 121 °C, for 30 min, 2.40: 121 °C, for 60 min, 2.77: 150 °C, for 10 min, and 3.06: 170 °C, for 10 min, thereby examining both short and long residence times at moderate temperatures. Pretreatments carried out at 121 °C were conducted in a 500 ml total volume, containing 6% dry matter (DM) in an autoclave. The temperature-changing pretreatment was conducted in a 1.0 L reactor, with a vertical setup. After cooling the pretreated biomass (slurry form), the fiber fraction was separated from the pretreatment liquid by filtration through a cloth. The pretreated biomass (both fractions) was preserved in a freezer at −18 °C until further processing. Both fractions underwent sugar- and inhibitor-composition analysis as described below.

Chemical characterization of the solid fraction of the pretreated pulp

A portion of the solid fraction of the pretreated pulp was dried overnight at 105 °C, after which dry matter (Sluiter et al. 2008a), ash content (Sluiter et al. 2008b), and strong acid hydrolysis (Sluiter et al. 2008c) (described earlier) were conducted. The analysis of the sugars and organic acid contents was done by HPLC (Agilent) as already explained.

Chemical characterization of the pretreatment liquid produced from pulp pretreatment

The sugar composition of the pretreatment liquid was assessed by applying WAH. Carbohydrates and other by-products analysis was done by HPLC. The Hi Plex-H column (Agilent) and RI detector were used to determine the concentrations of glucose, xylose, and arabinose at the same conditions mentioned for strong acid hydrolysis. Accordingly, the content of sugars and organic acids was calculated per total liquid of the original sample.

Glucan-to-glucose convertibility and fermentability

For the mild pretreated samples, fermentation was conducted by preparing four batches; described in different pre-treatment residence times (i.e., 30 or 60 min) and different fermentation media (i.e., either deionized (DI) water or the liquid produced from pulp pretreatment). The aim was to assess any inhibitory effects which could be encountered by adding the pretreatment liquid as a fermentation medium. The inhibitory components are foreseen by-products of the pretreatment process. The inhibitory assessment is achieved by weighing the ethanol yields for both fermentation media. Fermentation (glucan-to-

glucose convertibility) was conducted for the fibers (solid fraction of the pretreated biomass), which was done using 10% dry content loading in 25 ml total volume for each of the described batches.

Simultaneous Saccharification and Fermentation (SSF) was applied. Enzymatic pre-hydrolysis was carried out at 50 °C for 24 h by adding Celluclast 1.5 and Novozyme 188 or Cellubrix, with enzymatic hydrolysis of 15 FPU/g DM and having Novozyme 188 to Celluclast ratio of approximately 1/9th. Intensive shaking was applied (110 rpm). After 24-h pre-hydrolysis, the samples were cooled to room temperature by the aid of a cold water bath. pH was measured and tuned (if needed) to a pH value of 4.8. After which another dose of enzyme was added together with 0.2 ml of sterile filtered urea and 2.0 g/L Baker's yeast (*Saccharomyces cerevisiae*, commercial dry yeast, Malteserkors tørgær, De Danske Spritfabrikker A/S, Denmark). Simultaneous saccharification and fermentation were carried out in an incubator 32 °C while shaking at 70 rpm for 7 days. Ethanol, residual sugars, and organic acids content analysis was achieved by HPLC using Hi Plex-H column (Agilent) and RI detector.

For the hydrothermal pretreated samples, the fermentability study was conducted on the pretreated pulp by preparing 5% of dry matter loading in a total volume of 100 ml. This experiment was done first by adding DI water and then by adding the pretreatment liquid as fermentation medium, both were done in duplicates.

Convertibility of the fibers was analyzed in SSF as described above. Yet, during the saccharification step, 150 rpm intensive shaking was applied. All other fermentation steps explained for mild pretreated pulp were applied.

Calculations

Total solids and ash content

Total solids (TS) and total ash (TA) were measured in the original plant following the NREL protocol (Sluiter et al. 2008a; Sluiter et al. 2008b). TS content (TS) was calculated using Eq. (1) and Eq. (2), while TA content was calculated using Eq. (3).

$$\text{Total Solids of Biomass (TS)}\% = \frac{W_d}{W_i} * 100 \quad (1)$$

$$\text{Total Moisture Content}\% = \left(1 - \frac{W_d}{W_i}\right) * 100 \quad (2)$$

where W_d is the weight of the biomass in grams after drying at 105 °C to constant weight and W_i is the weight of the initial biomass in grams.

$$\text{Total Ash}\% = \frac{W_r}{W_d} * 100 \quad (3)$$

where W_r is the weight of the residue in grams after drying at 575 °C to constant weight and W_d is the weight of the biomass in grams after drying at 105 °C to constant weight.

Protein content

The protein content was calculated by applying the factor 6.25 to the material's nitrogen content (Islam and Adams 2000) as shown in Eq. (4).

$$\text{Protein content}(\%) = \text{Nitrogen content}(\%) * 6.25 \quad (4)$$

Fresh juice sugar composition

Accordingly, the content of sugars and organic acids was calculated per total liquid of the original sample as explained in Eq. (5). The equation is multiplied by 2 to eliminate the effect of dilution, where the pretreatment liquid was diluted by a factor of 2.

$$\text{Sugar/acid}_{\text{extract}}\left(\frac{\text{g}}{\text{L liquid}}\right) = C_{\text{extract}} * \frac{1\text{g}}{1000\text{mg}} * \frac{1000\text{ml}}{1\text{ L}} * 2 \quad (5)$$

where C_{extract} is the concentration of a measured component (sugar or organic acid) analyzed by the HPLC [mg/ml].

Pulp's structural carbohydrates and lignin composition

The hydrolysate generated after strong acid hydrolysis was filtered and taken for analysis for carbohydrates and organic acids using HPLC. Accordingly, the content of sugars and organic acids in the pulp fraction was calculated per total liquid of the original sample as shown in Eq. (6) (Cybulska et al. 2014a).

$$\text{Sugar/acid}_{\text{extract}}\left(\frac{\text{g}}{100\text{gTS}}\right) = \frac{C_{\text{extract}} * V_{\text{extract}} * \frac{1\text{g}}{1000\text{mg}}}{\text{TS}} * 100 \quad (6)$$

where C_{extract} is the concentration of a measured component (sugar or organic acid) analyzed by the HPLC [mg/ml]; V_{extract} is the volume of the extract [ml], and TS is the total solids content measured [g]. The recovery factor (R_f) of the individual sugars

was calculated according to Eq. (7). To account for any sugar degradation occurring during acid hydrolysis, an evaluation was conducted on a reference sample, which was prepared by adding spiked solution (solution with a predefined sugar concentration). With this, the recovery factor was obtained, which was used as a correction factor, as explained in Eq. (8).

$$R_f = \frac{C_{h+s(\text{measured})}}{C_{s(\text{added})} + C_{h(\text{measured})}} \tag{7}$$

where C_{h+s} is the sugar in acid hydrolysate with standard addition [g/100 g DM]; C_s is the sugar standard added [g/100 g DM], and C_h is the sugar in acid hydrolysate without standard addition [g/100 g DM].

$$C_{\text{corrected}} = \frac{C_{h(\text{measured})}}{R_f} \tag{8}$$

Concentration of the polymeric sugars was calculated from the corresponding monomeric sugar concentration (e.g., glucose to glucan or xylose to xylan conversion), applying an anhydrous-correction of 0.88 (or 132/150) for C5 sugars (xylose and arabinose) and a correction of 0.90 (or 162/180) for C6 sugars (glucose, galactose, and mannose), as shown in Eq. (9).

$$C_{\text{anhydro}} = C_{\text{corr}} * \text{Anhydrous correction} \tag{9}$$

Acid insoluble lignin (Klason lignin) content in the "as received" sample was calculated using Eq. (10).

$$\%\text{AIL}_{\text{received sample}} = \frac{(W_b - W_a)}{W_s} * 100\% \tag{10}$$

where, %AIL is the acid insoluble lignin (%), W_b is the weight of residue *before* drying at 105 °C (g), W_a is the weight of residue *after* drying at 105 °C (g) and W_s is the weight of a sample (g).

Sugar recovery calculations

Mass balance of all the important components (glucose, xylose, arabinose, and ash) was performed using Eqs. (11),(12),(13),(14). 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.

$$\text{DM in (g)} = \frac{\text{DM}_{\text{raw}} * W_{\text{ib}} * C_{\text{raw}}}{100\%} \tag{11}$$

where

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

$$\text{DM out(g)} = \frac{\text{DM}_p * W_f * C_f}{100\%} \tag{12}$$

where

W_f weight of the fiber fraction after the pretreatment [g]
DM_p total solids of the pretreated *Salicornia bigelovii* [%]
C_f content of the specific component in the fiber fraction after the pretreatment measured by strong acid hydrolysis [g/gDM]

$$\text{Fiber fraction recovery}\% = \frac{\text{Dry mass out(g)}}{\text{Dry mass in(g)}} * 100\% \tag{13}$$

$$\text{Liquid fraction recovery}\% = \frac{\text{Amount of the component in the filtrate(g)}}{\text{Dry mass in (g)}} * 100\% \tag{14}$$

Pulp's pretreatment severity factor

The pretreatment severity was calculated for all pretreatments using (Eq. (11)) (Overend et al. 1987), which showed that temperature has a greater effect than the residence time (exponential versus linear) on overcoming recalcitrance of the biomass to enzymatic hydrolysis.

$$\log(Ro) = \log\left(t\exp\left(\frac{T-100}{14.75}\right)\right) \tag{15}$$

where

T pretreatment temperature [°C]
T pretreatment time [min]
R_o severity factor (Cybulska et al. 2014b).

Statistical analysis

Mean comparisons over all treatments have been performed using Tukey's honestly significant difference (HSD) method calculated with a critical q value at $p < 0.05$. The comparisons were performed for fibers composition, sugar recovery in the pretreatment process, and ethanol yield in the fermentation of the pretreated fibers.

Results

Fresh biomass

The fresh unwashed biomass of *Salicornia sinus-persica* was found to contain 22.42% dry matter (DM), of which 47.07% was ash. Mass distribution of each fraction (juice and pulp) was measured after juicing the feedstock; the liquid fraction was found to represent 67.78 ± 6.57% of the biomass. The wet pulp fraction of fresh *S. sinus-persica* was found to contain 38.88 ± 2.10% DM, of which 13.19 ± 1.15% was ash and 2.08% total nitrogen (representing 13.00% protein applying the factor 6.25 (Islam and Adams 2000)).

Chemical characterization of fresh juice

Dry matter content of the juice was found to be 13.53 ± 0.55%, of which 61.12 ± 3.60% was ash. Fresh juice composition (in % of DM of juice) is shown in Fig. 1, indicating that less than 40% of the DM of the juice was composed of protein, sugars, and organic acids; the rest was ash.

Composition analysis of fresh untreated juice showed about 1.0–1.5% free sugar, consisting of glucose, fructose, and arabinose (i.e., 8.78 ± 1.02 g/L glucose, 3.90 ± 0.79 g/L fructose and xylose combined, and 0.35 ± 0.18 g/L arabinose).

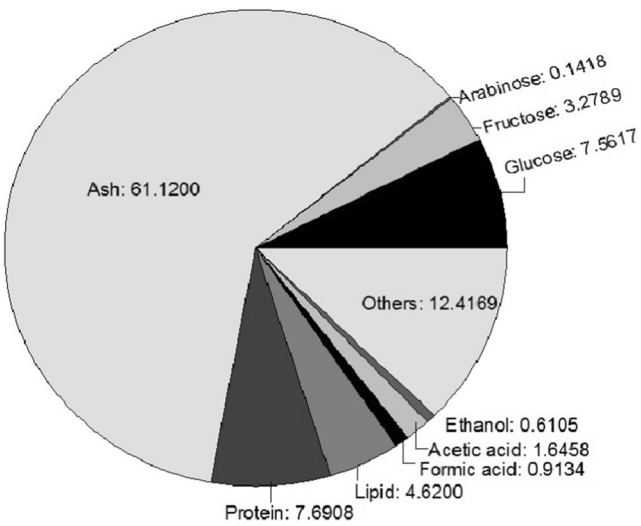

Fig. 1 Salicornia fresh juice chemical composition (% of dry mass DM)

Organic acids and ethanol were detected in negligible concentrations. Acid hydrolysis of Salicornia juice resulted in an increase in fructose, xylose, and arabinose concentrations (5.83 ± 0.12 g/L fructose, + xylose, and 3.32 ± 0.02 g/L arabinose), where the glucose concentration did not change significantly (8.29 ± 0.08 g/L).

Fresh juice fermentation

Untreated fresh juice fermentations were conducted in 1.0 L bioreactors, by inoculating *S. cerevisiae* in a concentration of 2.0 g/L. Product formation and sugar utilization during fermentation are shown in Fig. 2. Experiments showed that fresh Salicornia juice could obtain ethanol yields exceeding 70% on available C6 sugar (glucose and fructose). Other products were generated at later stages, such as lactic acid and acetic acid.

Fiber fraction (pulp)

Chemical characterization of the raw pulp and pretreated fiber fractions

The raw pulp of fresh *Salicornia sinus-persica* contained 15.63 ± 0.50 g/100 g DM glucose, 10.68 ± 0.57 g/100 g DM fructose and xylose combined, and 11.08 ± 0.35 g/100 g DM arabinose. Glucan content in the pulp fraction was improved by increasing the pretreatment temperature. The highest glucan content (i.e., 30.54 ± 1.77 g glucose/100 g DM) was obtained at a severity of 3.06, by a treatment achieved at 170 °C for 10 min (Fig. 3). However, Tukey's HSD test has revealed no significant differences among all treatments. The ash content of the untreated pulp is higher than that for the treated pulp, which is mostly true for higher severity treatments based on the mean comparisons. The results also show that the Klason lignin content significantly ($p > 0.05$) increased with rising pretreatment temperature.

Sugar recovery

Sugar recovery was calculated for the pretreated pulps (Fig. 4). Cellulose recovery was above 75% in all pretreatments, with the highest obtained value for biomass pretreated at a severity of 2.40 (94.6 ± 4.57%). Glucan recovery decreased to 77.49 ± 2.35% and to 75.66 ± 1.69% when the pretreatment severity increased to 2.77 and 3.06, respectively. Recovery of hemicellulose was relatively high for all pretreatments. Tukey's test confirmed those observations.

Enzymatic convertibility of pulp fibers in SSF

Increasing the pretreatment time from 30 to 60 min, while maintaining the temperature at 121 °C showed about 30% decrease in ethanol yields in both water and pretreatment

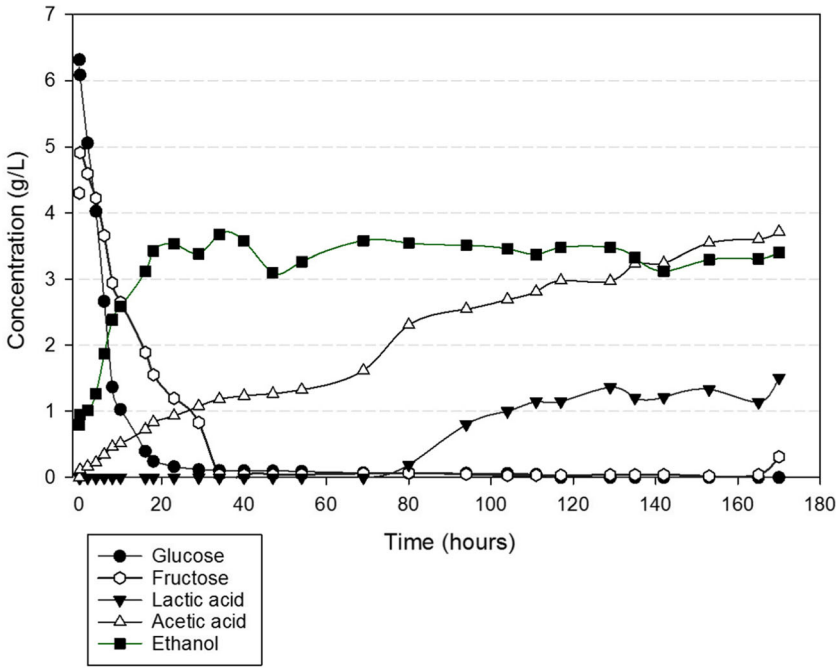

Fig. 2 Product formation and sugar utilization during fermentation with *S. cerevisiae* in fresh juice of *Salicornia sinus-persica*

media. The convertibility of the biomass pretreated at 120 °C for 10 min was very low; it was comparable to what was obtained by the fresh, untreated biomass (about 6.83% ethanol yield). The yield increased eightfold when the pretreatment temperature increased from 120 to 150 °C (comparable to yields obtained by longer residence-time pretreatments) and almost 12-fold when increased to 170 °C, to reach a yield of 76.91 ± 3.03% (Fig. 5). The best convertibility of 76.91 ± 3.03% was found at the highest process severity of 3.06 (170 °C, 10 min).

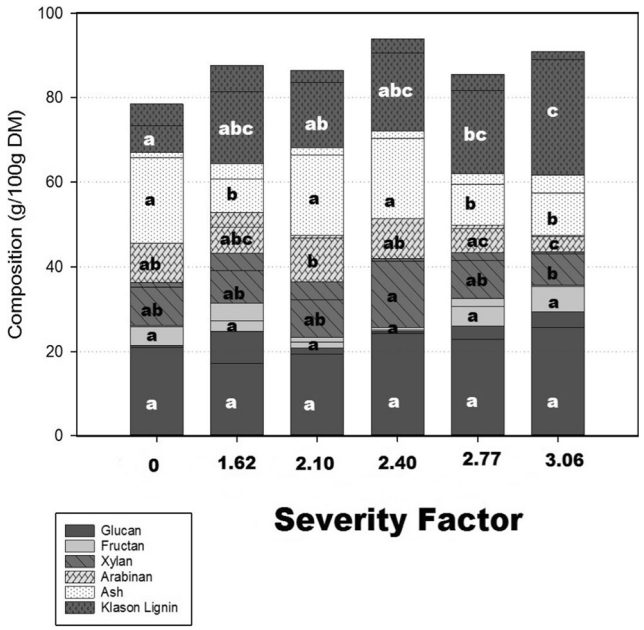

Fig. 3 Fiber composition after low-severity pretreatment at different temperatures (*letters* denote significant differences among treatments based on Tukey's HSD)

Pretreated pulp fermentation in water medium versus pretreatment liquid medium

Adding the pretreatment liquid instead of water as a fermentation medium (with 5% DM loading), showed a substantial increase in the ethanol yields obtained by pulp pretreated at 120 °C for 10 min (ethanol yield increased from 6.97 ± 1.54% to 85.46 ± 0.94%) (Fig. 6.). However, a decrease in the obtained ethanol yield was observed for all other pretreatments. The highest decrease was observed for the treatment done at

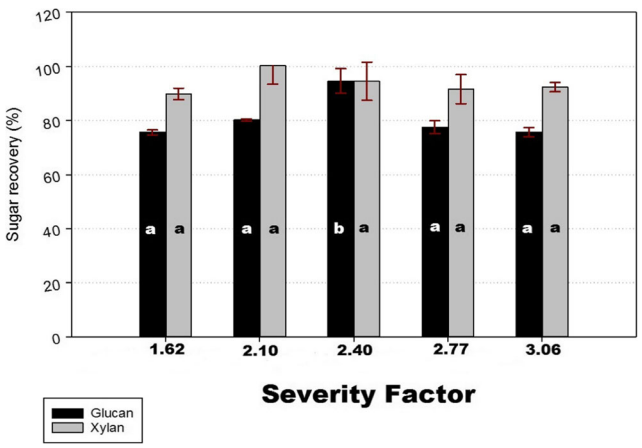

Fig. 4 Glucan and xylan (%) for pulp pretreated at five different severities (*letters* denote significant differences among treatments based on Tukey's HSD)

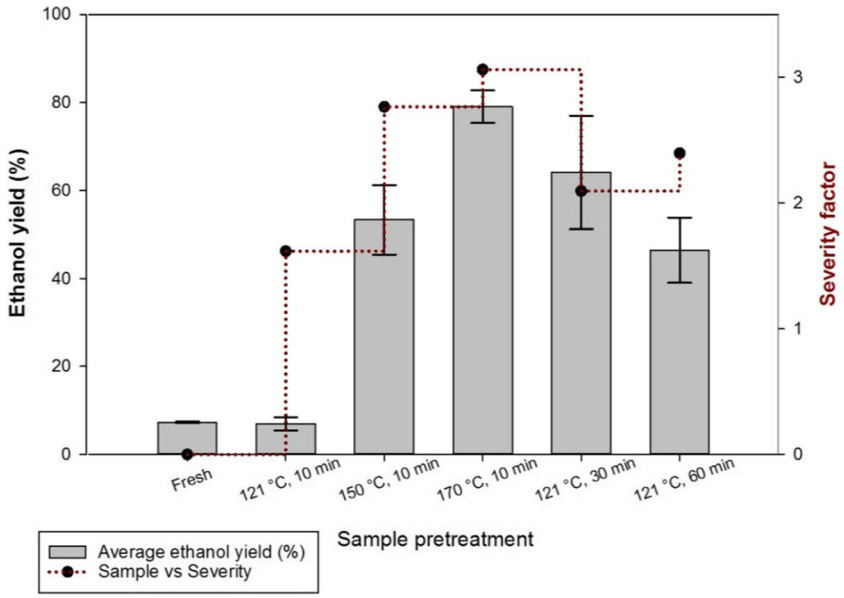

Fig. 5 Ethanol yield and severity factor for pulp pretreatments

170 °C; yields decreased from almost 80% to about 64% (Fig. 6). Tukey's test was performed for the mean comparisons for each experiment (separately for water-diluted and filtrate-diluted samples). Ethanol yield was found to be significantly higher for severity factors over 2.00 when water was used as the fermentation medium, and no significant differences were observed among the ethanol yields when filtrate was used as the fermentation medium.

Discussion

The results of this paper indicated that succulent green biomass such as *Salicornia sinus-persica* could give high yields in an ethanol process applying green fractionation followed by very low severity hydrothermal pretreatment and, at the same time, eliminating the use of fresh water in the process.

Fresh biomass

The measured nitrogen content of the fresh biomass (2.08%) was comparable to what was reported for the halophyte *Salicornia gaudichaudiana* with total nitrogen of 2.2 ± 0.26% (Barbarino and Lourenço 2009).

Juicing the biomass showed that about 70% of the fresh biomass is liquid; this, as a fact, helps in water preservation in the system of salicornia-based biorefinery. More than 60% of the dry matter content of the juice fraction was composed of ash, resulted from the high salt content of this biomass, both structural and extractable. The sugar composition of the fresh untreated juice was very little (1.0–1.5% free sugar), indicating that juice fermentation will not be producing tangible amounts of ethanol. Nevertheless, it was important to evaluate the effect of the high salt content on the process of fermentation by yeast. Exposing the Salicornia juice to acid hydrolysis resulted in an increase in mostly the C5 sugar concentrations; this increase could be explained by hydrolysis of soluble fibers and residual pulp in the juice.

The raw pulp of fresh *Salicornia sinus-persica* showed sugar content that is comparable to the lignocellulose composition of the mature (dry) plant (Cybulska et al. 2014b). As expected, glucan content in the pulp improved by increasing the pretreatment temperature, due to the improved extraction of hemicellulose and ash into the liquid fraction. Due to the washing taking place during pulp treatment, the untreated pulp showed higher ash contents compared to the treated pulp.

Fresh juice fermentation

Untreated fresh juice fermentations (as shown in Fig. 2.) showed that glucose was completely consumed in 18 h of fermentation. Fructose was being consumed in parallel to glucose however, at a slower rate, and was completely consumed after 34 h yielding the maximum ethanol concentration of 3.68 g/L. Arabinose in the juice (~0.5 g/L) was being consumed between 69 and 80 h of fermentation. Around this point, lactic acid started being produced to reach a concentration of 1.5 g/L after 170 h of fermentation. Acetic acid was continuously produced throughout the fermentation process. The concentration doubled from 1.18 g/L (at 34 h) to 2.31 g/L (at 80 h) and reached the highest concentration (3.71 g/L) after 170 h of fermentation. Production of acetic acid is probably obtained due to consumption of pentoses in this non-sterile medium (yeast is not capable of converting pentoses, hence no ethanol was formed during this period). Throughout juice fermentation with *S. cerevisiae*, glucose is consumed at a higher

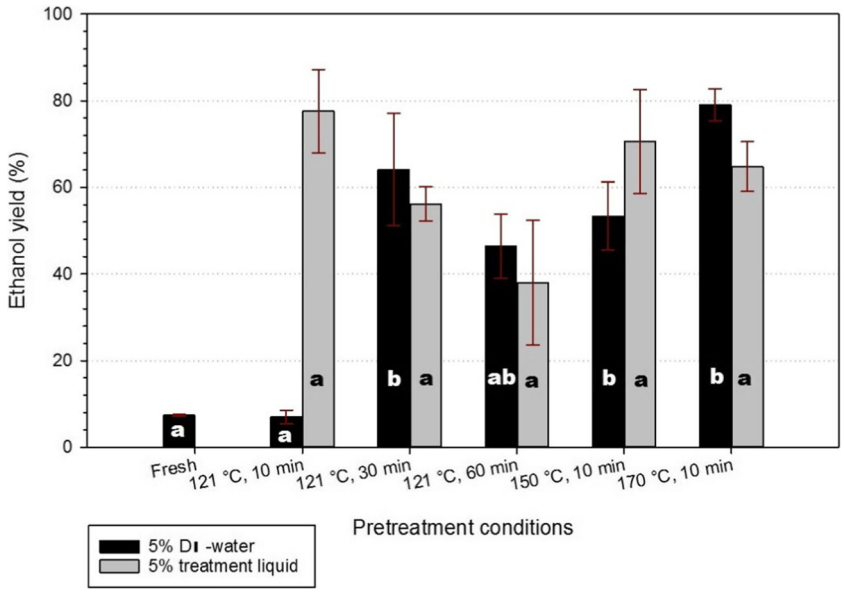

Fig. 6 Ethanol yield obtained by pretreated pulp suspended once in DI water and in pretreatment liquid (*letters* denote significant differences among treatments based on Tukey's HSD)

rate than fructose, thus, fructose tends to be the main sugar source during late stages of alcoholic fermentation (Guillaume et al. 2007). Wang et al. (2004) discussed that *S. cerevisiae* tends to be glucophilic, although some strains have a clear preference for fructose. All in all, fresh Salicornia juice could obtain ethanol yields exceeding 70% on available C6 sugar (glucose and fructose), indicating that the high salt composition does not result in yeast inhibition.

Fiber fraction (pulp) pretreatment

Sugar recovery

With the highest pretreatment severity of 3.06, relatively high cellulose recoveries (above 75%) were obtained in all pretreatments. Glucan recovery increased to reach its highest value at a pretreatment severity of 2.40, where it dropped at higher-severity pretreatments (Fig. 4.). Tukey's test confirmed that the glucan recovery was significantly higher for the pulps pretreated at a severity of 2.40, being exactly in the middle of the severity range tested. No significant differences were observed for the rest of the treatments for glucan recovery, and for all of the treatments for hemicellulose recovery.

Enzymatic convertibility of pulp fibers in SSF

Enzymatic convertibility of cellulose (examined by SSF) was significantly improved after treating the pulp hydrothermally.

The residence time indicated a great influence on releasing sugars, as was observed in pretreatments done at 120 °C (applying different residence times) when compared to the pretreatment done at 150 °C. Cellulose convertibility obtained by

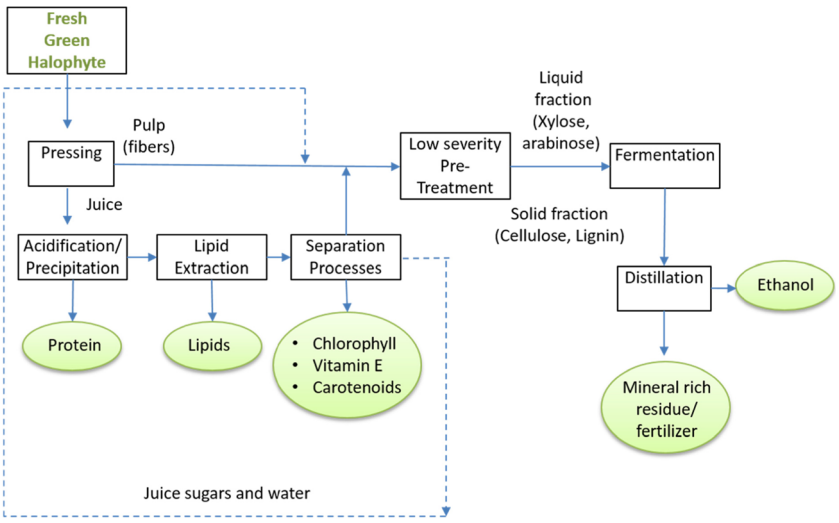

Fig. 7 Biorefining of fresh green Salicornia biomass into a range of value added products and bioethanol

pulp pretreated at 120 °C for 30 and 60 min was similar to what was obtained by pulp treated at 150 °C for 10 min, despite the increase in the severity factor as shown in Fig. 5.

In spite of the positive impact obtained by longer residence time, this factor has to be optimized, as was observed for the pretreatments done at 120 °C. About 30% decrease in ethanol yields was detected when doubling the residence time from 30 to 60 min. On the other hand, thee convertibility of the biomass pretreated at 120 °C for 10 min was very low.

Pretreated pulp fermentation in water medium versus pretreatment liquid medium

Using the pretreatment liquid as a fermentation medium instead of water increased the ethanol yields significantly for the pulp treated at the lowest severity (1.62), as presented in Fig. 6. The increase at low process severity indicated that the soluble sugars were still available for fermentation in the pretreatment liquids. The slight decrease in the ethanol yield obtained at severity above 2.00 indicated inhibitory effects produced by adding the pretreatment liquid under the described pretreatment conditions. This confirmed that higher pretreatment severity results in sugar degradation and hence inhibitor formation, as detected for the pretreatment done at 170 °C, where yields decreased from almost 80% to about 64% when the pulp was suspended in the pretreatment liquid (Fig. 6).

In summary, green, succulent biomass of *Salicornia sinus-persica* was subjected to wet-fractionation followed by hydrothermal pretreatment and SSF. The sugar composition of the juice was low (1.0–1.5%), but the fresh pulp showed significant sugar content (~50% *w*/w). Wet fractionation yielded 70% juice and 30% pulp. Direct fermentation of the fresh juice using *S. cerevisiae* showed no salt inhibition of the yeast and ethanol yields of ~70% were achieved. Cellulose convertibility of mildly pretreated pulps in DI water was found to be significantly higher for severity factors over 2.00 with the best ethanol yield of 76.91 ± 3.03% was found at 3.06. This study shows great potential for green harvested succulent halophyte to be used for biorefining into platform chemicals. It holds especially great promise in arid coastal areas, where fresh water is sparse, as the juice fraction can act as the water source in processing of the biomass. Furthermore, valuable components can be extracted from the fresh juice prior to acting as water phase in the pulp pre-treatment, adding to the economics of the biofuels process (Fig. 7).

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study does not contain any studies with human participants or animals performed by any of the authors.

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