Bioresource Technology 153 (2014) 165–172

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

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.

ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 12 November 2013
Accepted 25 November 2013
Available online 1 December 2013

Keywords:
Halophytes
Hydrothermal pretreatment
Bioethanol
Enzymatic hydrolysis
Saccharomyces cerevisiae

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).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.11.071

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