
Halophytes for the Production of Liquid Biofuels

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Abstract

We discuss the potential of using halophytes as a source for producing liquid biofuels. We review the potential pathways for converting oilseeds into biodiesel and bio-derived synthetic paraffinic kerosene and presents some preliminary data on biomass composition and pretreatment of the halophyte *Salicornia bigelovii*. Six samples of *S. bigelovii* cultivated at three fertilizer levels (F1: 1 gN/m², F2: 1.5 gN/m² and F3: 2 gN/m²) and two salinity levels (S1: 10 ppt and S5: 50 ppt salt) were analyzed with regard to chemical composition and bioethanol potential. Chemical characterization showed that *S. bigelovii* contained, 16.31–55.67 g/100gTS (total solids) of carbohydrates, 5.42–16.60 g/100gTS of lignin, 27.85–66.37 g/100gTS of total extractives (including extractable ash), and 2.18–9.68 g/100gTS of structural ash, depending on the plant fraction and cultivation conditions. Enzymatic hydrolysis of the pretreated samples revealed high glucose recoveries of up to 90 % (of glucose in raw *S. bigelovii*) corresponding to ethanol yield of 111 kg ethanol/dry ton *S. bigelovii*.

1 Introduction

As the use of fossil fuels increases, the greenhouse gas emissions from their burning also increases, pushing us to look for alternative fuels that can be developed from plant biomass sources. One of the drawbacks of using plants for biofuels is that they

can compete for land and freshwater resources with conventional food crops [1, 2]. If second generation biofuel crops can be developed that use marginal land and water sources, then the competition with food production is minimized [1]. Halophytes, which are able to grow in salinized soils using saline water, have been proposed for use as bio-energy crops [3, 4]. Halophytes have been explored for the production of both lignocellulosic biomass

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for ethanol production [3], as well as for the production of biodiesel and hydro-processed fuels from oilseeds [4, 5]. Typical limitation on the use of halophyte biomass for biofuels is the high ash content of the straw.

As part of an integrated seawater aquaculture/agriculture project being developed in Abu Dhabi, we are investigating the potential of using oilseeds for the production of liquid biofuel, as well as investigating the potential of using the residual biomass to produce ethanol. The halophyte oilseed literature is reviewed, while recent data is presented on the characterization of biomass of the annual halophyte *Salicornia bigelovii*, which is a potential candidate crop species for this project.

2 Oils

2.1 Biodiesel

Oils from halophyte oilseeds can be converted to biodiesel via trans-esterification. Halophytes are reported to be the potential candidates for biodiesel production and the fatty acid methyl esters of the oil of many such are comparable to oils currently used for the production of biodiesel [4]. In another report, the oil content of six halophytes varied between 22 and 25 % and there was generally high composition of unsaturated fatty acids [6]. The annual succulent saltmarsh plant *Salicornia bigelovii*, has been cultivated in Mexico using seawater, and mean seed yields were 2 t/ha, with seeds yielding 28 % oil [7]. These yields are comparable to soybean yields grown on freshwater. Biodiesel derived from halophyte oil should be able to be used to supplant petroleum-based diesel for ground transportation.

2.2 Bio-derived Synthetic Paraffinic Kerosene (Bio-SPK)

Though biodiesel and ethanol derived from biomass feedstock are technologically proven fuel alternatives for some modes of ground transport, both are limited from becoming aviation fuel substitutes. The aviation sector cannot use ethanol

and biodiesel due their reduced specific energy relative to jet fuel [8]. However, the aviation industry is looking for a renewable drop in fuel that can substitute for jet fuel [5]. Recently a process has been developing to convert vegetable oils into paraffins that can substitute for petroleum based jet fuels [9]. Oils are converted to the shorter chain diesel-range paraffins using a proprietary process, which removes oxygen molecules from the oil and converts olefins to paraffin's by reaction with hydrogen. A second reaction then cracks and isomerizes the diesel range paraffins, to paraffins and iso-paraffins with carbon numbers in the jet range [9]. This process is agnostic with respect to the composition of the oil, so ostensibly a wide range of halophyte oils could be used to produce Bio-SPK.

2.3 Residue Biomass

Significant amount of lignocellulosic biomass is produced in halophyte cultivation where up to 90 % of the *S. bigelovii* plant is the straw/bush fraction [7]. The lignocellulosic part of the halophyte can be converted into biofuels by biological conversion using enzymatic hydrolysis and microbial fermentation. Lignocellulosic biomasses often contain significant amounts of alkali metals e.g. potassium and sodium [10], which represent a challenge in utilizing biomass for high temperature energy processes such as combustion, resulting in problems with fouling, slagging, and corrosion [11]. For halophyte crops such as *S. bigelovii*, the sodium content of stems and spikes has been reported to be 6–12 % of the dry biomass and the total ash content to be up to 30 % of dry biomass [12]. The aim of this study was to examine if *S. bigelovii* straw could be a suitable biomass for second generation bio-ethanol production.

The processing of lignocellulosic biomass to sugars for fermentation to ethanol and other products requires specifically designed pretreatment processes. In general, it involves the breaking up of the naturally resistant carbohydrate-lignin shield that limits the accessibility of enzymes to cellulose and hemicelluloses [13–15]. The pretreated slurry then undergoes enzymatic

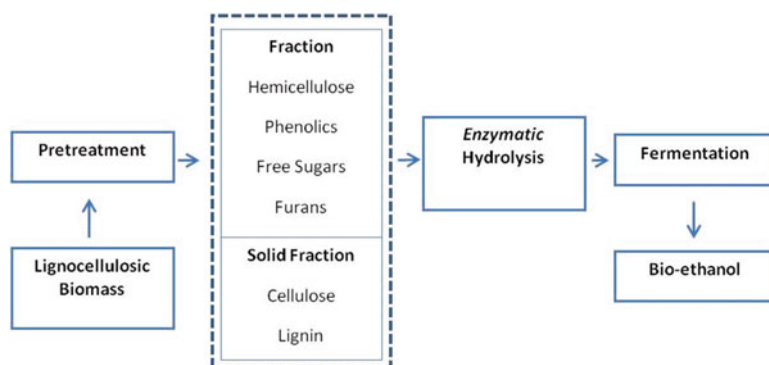


Fig. 1 Processes needed to convert lignocellulosic straw of *S. bigelovii* into bioethanol

hydrolysis where carbohydrates are hydrolyzed to monomeric sugars which can be fermented in the microbial process to e.g. bio-ethanol (Fig. 1).

In this study six samples of *S. bigelovii* cultivated at three fertilizer levels (F1: 1 gN/m², F2: 1.5 gN/m² and F3: 2 gN/m²) and two salinity levels (S1: 10 ppt and S5: 50 ppt salt) were analyzed to determine chemical composition and bioethanol production potential.

3 Materials and Methods

Biomass composition characterization was performed according to National Renewable Energy Laboratory protocol (NREL/TP-510-42618), using pre-hydrolysis with concentrated sulfuric acid (72 %) at 30 °C followed by a dilute acid hydrolysis (4 % sulfuric acid) at 121 °C. The sugar content in the hydrolyzate was analyzed using High Performance Liquid Chromatography (HPLC), while acid-insoluble lignin was quantified gravimetrically. The biomass was extracted with water and ethanol prior to the acid hydrolysis (using a Soxhlet apparatus) to remove the extractives.

Preliminary trials of the hydrothermal pretreatment have been carried out at the Technical University of Denmark. The process was performed at 10 % 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 % sulfuric acid). Processing time was maintained at 10 min. The raw material used for pretreatment was washed with fresh

water to bring down the salt content of the plant to avoid corrosion of the equipment. The fibers have been subjected to enzymatic hydrolysis according to National Renewable Energy Laboratory protocol (NREL/TP-510-42629), 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. Glucose released during the enzymatic hydrolysis was quantified using HPLC. Liquid fractions (hydrolyzates) obtained from the pretreatment process were hydrolyzed with sulfuric acid (to degrade residual oligosaccharides) and analyzed for glucose content using HPLC.

4 Results

4.1 Halophyte Compositional Analysis

The six samples were fractionated into stem and seed spikes for chemical characterization (Table 1).

The analyses showed that *S. bigelovii* contained 27.85–66.37 g/100gTS (total solids) of total extractives (including extractable ash), 16.31–55.67 g/100gTS of carbohydrates, 5.42–16.60 g/100gTS of lignin and 2.18–9.68 g/100gTS of ash incorporated in the plant matrix (structural ash), depending on the plant fraction and cultivation conditions. The results show clearly that *S. bigelovii* seed spikes have a significantly different composition than the stems ($P < 0.01$).

Table 1 Chemical composition of the different samples of *S. bigelovii* cultivated at three fertilizer levels (F1: 1 gN/m², F2: 1.5 gN/m² and F3: 2 gN/m²) and two salinity levels (S1: 10 ppt and S5: 50 ppt)

<i>S. bigelovii</i> sample	Glucan (g/100 g DM)	Xylan (g/100 g DM)	Arabinan (g/100 g DM)	Total sugars (g/100 g DM)	Lignin (g/100 g DM)	Structural ash (g/100 g DM)	Extractives (g/100 g DM)
Stem, S1, F1	16.55	11.18	3.34	31.07	16.60	6.27	37.22
Stem, S1, F2	27.12	22.63	5.93	55.67	14.08	2.34	27.85
Stem, S1, F3	22.31	17.80	4.48	44.59	15.58	2.18	29.10
Stem, S5, F1	20.11	18.04	2.31	40.47	12.19	2.35	37.32
Stem, S5, F2	20.54	18.91	4.62	44.07	13.56	3.42	36.65
Stem, S5, F3	18.08	15.38	3.82	37.27	10.49	5.04	42.95
Spike, S1, F1	9.02	7.39	6.61	23.02	5.42	7.99	57.54
Spike, S1, F2	7.79	7.34	5.26	20.39	7.29	5.64	59.25
Spike, S1, F3	8.92	7.72	6.39	23.03	9.37	7.04	54.13
Spike, S5, F1	7.01	5.39	5.69	18.09	7.97	6.86	66.37
Spike, S5, F2	7.72	7.34	4.85	19.91	7.76	8.31	63.10
Spike, S5, F3	6.58	5.53	4.20	16.31	7.44	9.68	65.03



Fig. 2 Liquid fraction and digestible fiber fraction produced by pretreatment of *S. bigelovii*

Fertilizer level was found to have a significant influence ($P < 0.05$) on the carbohydrates, extractives and ash, while salinity was a significant factor for ash and extractives content in both fractions of the plants ($P < 0.05$). The results suggest that *S. bigelovii* stems cultivated at low salinity (10 ppt) and medium fertilizer grade (1.5 g N/m²) contain the highest carbohydrate content and are of high value for biofuels production, while seed spikes can be more suitable for extracting value-added active components (having a high extractives content).

4.2 Pretreatment and Enzymatic Hydrolysis

Fractionation of the dry biomass produced a liquid fraction containing extracted sugars (primarily pentose sugars) and a digestible fiber fraction (Fig. 2). The highest concentrations of monomeric sugars of 7 g/l were extracted in to the liquid phase when using the acid catalyst. Significantly less monomeric sugars were extracted without the catalyst (Table 2).

Table 2 Sugar monomers extracted into the liquid fraction during pretreatment

Pretreatment conditions	210 °C/no catalyst	200 °C/catalyst
Glucose	0.05 ± 0.00	0.79 ± 0.25
Extracted to the hydrolyzate [g/L]		
Xylose	0.92 ± 0.04	4.69 ± 0.25
Extracted to the hydrolyzate [g/L]		
Arabinose	0.00 ± 0.00	1.56 ± 0.05
Extracted to the hydrolyzate [g/L]		
Total sugar concentration	0.97 ± 0.05	7.05 ± 0.56
In the hydrolyzate [g/L]		

Table 3 Summarized results of the hydrothermal pretreatment and enzymatic hydrolysis

Pretreatment conditions	210 °C/no catalyst	200 °C/catalyst
Glucose recovery in fibers [% of glucose in raw <i>S. bigelovii</i>]	89.49 ± 6.96	80.63 ± 4.70
Glucose yield from the fibers [kg/ton <i>S. bigelovii</i> (dry biomass)]	217.9 ± 1.69	196.3 ± 1.14
Theoretical ethanol yield from the fibers [kg/ton <i>S. bigelovii</i> (dry biomass)]	111.10	100.10

Enzymatic hydrolysis of the pretreated fiber samples revealed that all of the treatment conditions produced highly digestible cellulose-rich pulp. The highest glucose recoveries were observed for samples pretreated at 210 °C (with no catalyst) and 200 °C (with the catalyst) (Table 3). These results suggest ethanol yield of 100–111 kg ethanol/dry ton *S. bigelovii*, which is about half of the typical lignocellulosic residue (e.g. corn stover, which has ethanol potential of 230 kg/dry ton) [16].

5 Discussion and Conclusions

The most promising approach to developing biofuels from halophytes would be a system whereby multiple products would be obtained from a single species or farming operation. For example, in the case of *S. bigelovii*, oil could be used for biodiesel or Bio-SPK production. Seed meal could be used for animal feed [7]. The straw could be used to produce ethanol. Other value added products might be derived from the biomass conversion to ethanol. Preliminary trials on the pretreatment and enzymatic hydrolysis of *S. bigelovii* revealed that the biomass has potential as a lignocellulosic bioethanol feedstock. High glucose recoveries show that this biomass can be efficiently pretreated without the use of

chemicals, making the process more sustainable. Since *S. bigelovii* contains less cellulose (up to 27 % in the stems, 20–24 % in the mixed plant) than typical lignocellulosic materials (e.g. corn stover cellulose content is up to 45 % [17], the overall ethanol potential is lower for the *S. bigelovii*. This result could be increased by separating the spikes from the stems prior to the pretreatment (mixed plant was used in this study), since cellulose is mainly contained in the stem fraction of the plant. Furthermore, pentose sugars could be utilized in a pentose-hexose co-fermentation using genetically modified microorganisms. The pretreatment and fermentation research should be continued to fully explore bioethanol potential of this plant. In this way multiple products and revenue streams are obtained from a single crop, and greater economic and energy efficiency is achieved.

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