J. of Supercritical Fluids 68 (2012) 117–122

Contents lists available at SciVerse ScienceDirect

The Journal of Supercritical Fluids

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

An integrated process for conversion of *Zostera marina* residues to bioethanol

Muge Pilavtepe[a], Sayit Sargin[a], Melih Soner Celiktas[b], Ozlem Yesil-Celiktas[a,*]

[a] Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova, Izmir, Turkey
[b] Solar Energy Institute, Ege University, 35100 Bornova, Izmir, Turkey

ARTICLE INFO

Article history:
Received 5 April 2012
Received in revised form 24 April 2012
Accepted 25 April 2012

Keywords:
Biorefinery
Seaweed
Zostera marina
Supercritical fluid extraction
Lignocellulosic
Hydrolysis
Simultaneous saccharification and fermentation
Bioethanol

ABSTRACT

Zostera marina is an aquatic plant forming wide grasslands and considered as the lungs of the marine ecosystems. However, the residues reaching the coastlines create nuisance and high costs are required for their disposal. The objective was to investigate the potential of *Z. marina* residues as a source of secondary metabolites and feedstock in order to propose alternative solutions to the landfill. The supercritical CO_2 extract had a total phenol value of 55.4 mg GAE/g extract and a radical scavenging capacity of 71.4%. Considering the raffinate phase, 3% higher hemicellulose content was reached after supercritical CO_2 treatment. Enzymatic hydrolysis revealed 31.45% and the yield of simultaneous saccharification and fermentation was 8.72% corresponding to a productivity of 0.273 kg/(m^3 h). An integrated process is proposed, where supercritical fluid extraction can act both as the main process to obtain solvent-free pharmaceutical compounds and a pretreatment method in order to loosen the lignin structure, thereby liberating some of the hemicellulose in the matrix.

1. Introduction

Modern societies are increasingly concerned by environmental issues. Apart from industrial pollution, considerable quantities of solid wastes are produced by marine sources. In this context, onshore residues of various seaweeds pose a significant environmental problem in the coasts of Mediterranean countries. In particular, pollution sources have to be properly identified and sustainable approaches have to be developed through implementation of new processes capable of converting the waste materials into value-added products.

Seaweeds are marine flowering plants forming wide grasslands involved in the oxygenation of seawaters, fauna protection and littoral erosion prevention. Among the seaweeds, *Zostera marina* is a widespread specie forming meadows that contribute to the productivity of coastal ecosystems by generating substantial amounts of organic matter, while providing a habitat for many organisms [1]. But leaves and part of rhizomes detach off the marine plant during spring and reach the beaches [2]. Onshore residues represent a great environmental, economical, social and hygienic problem in all coastal zones of Mediterranean Basin because of the great disturbance to the bathers and population due to the bad smell rising from the uncontrolled decomposition of the organic matter [3].

In literature, various approaches have been proposed for utilizing the residues of *Zostera* species. In one of the studies, *Zostera noltii* was considered as a candidate for water decontamination and its ability to remove cupric ions from dilute aqueous solutions was investigated [4]. Structural characterization and cytotoxic properties of an apiose-rich pectic polysaccharide obtained from the cell wall of *Z. marina* was reported [5]. Additionally, *Z. marina* residues collected from Arcachon beach in southwestern France were evaluated for ethanol production [6].

Therefore, the objectives of this study were to determine the chemical properties of *Z. marina* residues, to optimize the process parameters in acid and enzymatic hydrolyses and to evaluate bioethanol potential by applying simultaneous saccharification and fermentation with the ultimate aim of presenting an alternative and holistic approach for utilization of *Z. marina* residues as a feedstock, rather than to be deposited for landfill.

2. Experimental

2.1. Feedstock

Z. marina residues were collected from beaches nearby Izmir at the west coast of Turkey between May 2010 and February 2011. The leaves were washed with tap water, dried at room temperature,

* Corresponding author. Tel.: +90 232 343 4400; fax: +90 232 374 4289.
E-mail addresses: ozlem.yesil.celiktas@ege.edu.tr, ozlemyesil@gmail.com (O. Yesil-Celiktas).

0896-8446/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.supflu.2012.04.019

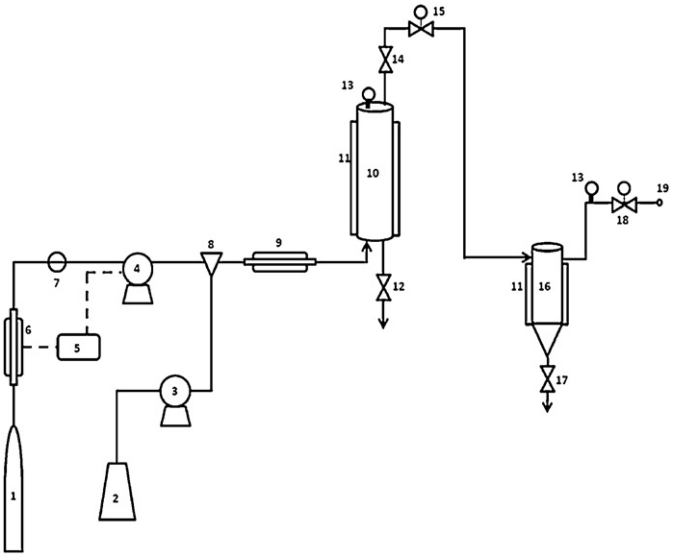

Fig. 1. The diagram of supercritical fluid process used (1, CO_2 supply in; 2, co-solvent reservoir; 3, co-solvent pump; 4, CO_2 pump; 5, cooling bath; 6, cooling heat exchanger; 7, flow meter; 8, mixer; 9, heat exchanger; 10, extraction vessel; 11, heat jacket; 12, bleed valve; 13, gauge; 14, on-off valve; 15, automated back pressure regulator (BPR); 16, fraction collector; 17, drain valve; 18, manual BPR; 19, vent).

ground by using a conventional grinder and stored in the dark at +4 °C.

2.2. Chemicals

CO_2 (99%) was taken from Habas, Izmir, Turkey. Sulfuric acid, acetic acid, phenol, D(+)glucose monohydrate, $(NH_4)_2HPO_4$, yeast extract, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, K-Na tartrate, sodium metabisulfite, sodium hydroxide, citric acid monohydrate, trisodium citrate dihydrate and Folin Ciocalteu were from Merck (Darmstadt, Germany). Bacto peptone was purchased from BD (USA) and 3,5-dinitrosalicylic acid from Sigma–Aldrich (Steinheim, Germany), whereas Baker's yeast was supplied from Dr. Oetker (Izmir, Turkey). Cellic CTec2 was kindly donated by Novozyme. Nanopure water used in the analysis was prepared by using in-house nanopure water system (Sartorius Arium 611, Sartorius-Stedim, Goettingen, Germany).

2.3. Supercritical CO_2 extraction

Supercritical CO_2 extraction was carried out at SFE 100 System (Thar Instruments, Inc., UK, 2006). The extractor was filled with about 10 g of ground *Z. marina* residues. Liquefied CO_2 was introduced into the sample cartridge through a piston pump with a cooling jacket. Both the pressure and temperature of the cartridge were automatically reached and maintained by a control unit. The pressure was set to 250 bar, temperature to 80 °C, ethanol as co-solvent to 20%, while the flow rate was adjusted as 15 g/min during dynamic extraction [7]. Extracts were collected from the separator after releasing CO_2 from the system (Fig. 1).

2.4. Antioxidant assay

2.4.1. Total phenol assay

The total phenols in the extracts were determined by Folin–Ciocalteu method. Briefly, 100 μl aliquot of the extract was added into a tube containing Milli-Q water (final volume 10 ml). Then 500 μl of Folin–Ciocalteu's reagent was added and the solution was stirred vigorously by vortex and left to stand for 5 min. Finally, 1.5 ml of saturated sodium carbonate solution was added, stirred vigorously for the last time and left to stand at room temperature for an hour. The absorbance was determined spectrophotometrically at 760 nm [8]. The total phenols were determined in duplicates, and the results are expressed as mean values and given as gallic acid equivalent (GAE) per gram of extract.

2.4.2. Radical scavenging activity

The extracts were dissolved in 4 ml of methanol (final concentration 250 μg/ml) and then added to 0.5 ml of 1 mM methanolic solution of DPPH• (Sigma). The contents were stirred vigorously for 15 s, and then left to stand at room temperature for 30 min. The decrease in colorization was measured spectrophotometrically at 517 nm [9]. The radical scavenging activity (RSA) was calculated using the equation as follows:

$$\%\,\mathrm{RSA} = 100 \times \left(1 - \frac{A_E}{A_D}\right)$$

where A_E is the absorbance of the solution containing the antioxidant extract and A_D the absorbance of the DPPH• solution.

2.5. Chemical analysis

All chemical analyses were conducted both for the untreated *Z. marina* residue and the raffinate phase that remained after the supercritical CO_2 extraction.

2.5.1. Elemental analysis

Samples were analyzed for carbon (C), hydrogen (H) and nitrogen (N) content by Leco Tru Spec (USA). CHN-S was calibrated according to the ASTM-D5373 and ASTM D-4239 standards.

2.5.2. Lignin, holocellulose and α-cellulose analyses

The lignin was analyzed by gravimetry after treatment with 72% sulfuric acid [10]. Holocellulose which is comprised of cellulose and hemicellulose was also determined by gravimetry after the reaction of about 5 g sample with 1.5 g sodium chlorite, 0.5 ml acetic acid and 160 ml of distilled water at 80 °C [11]. α-Cellulose is defined as the residue that is insoluble in 17.5% NaOH when the treatment is conducted under specified conditions. In total 75 ml of 17.5% NaOH was added to 3 g of bleached cellulose sample by stirring and left on a water bath for 30 min. The sample is filtered under vacuum, washed with distilled water, subsequently treated with 2 N acetic acid and filtered once more. Finally, the cellulose content was determined gravimetrically [12] and the hemicellulose was calculated based on the difference between holocellulose and cellulose.

2.5.3. Reducing sugar estimation

Reducing sugar concentration was analyzed using the dinitrosalicylic acid (DNS) method and the absorbance was measured at 540 nm [13].

2.5.4. Quantification of total carbohydrates

The total carbohydrate was determined with the phenol–sulphuric acid method and the absorbance was measured at 490 nm [14].

2.6. Pretreatment of raffinate phase

2.6.1. Optimization of acid hydrolysis

Studies on acid pre-treatment of the feedstock was conducted by investigating two different parameters; acid concentration (0.05, 0.10, 0.50, 1.0, 2.0%, v/v) and pre-treatment time (30, 60, 90 min). About 5 g of biomass from raw material and raffinate phases were