

Hydrothermal Liquefaction of Marine Biomass: An Integrated Process

*¹İrem Deniz, ²Özlem Yeşil Çelikaş, ³Fazilet Vardar Sukan
¹Manisa Celal Bayar University, Faculty of Engineering, Manisa
 irem.deniz@cbu.edu.tr

²Department of Bioengineering, Faculty of Engineering, Ege University, Bornova-Izmir, Turkey

³Department of Bioengineering, Faculty of Engineering, Ege University, Bornova-Izmir, Turkey

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Abstract

Ethanol production from lignocellulosic materials has been extensively studied in order to substitute fossil fuels. The aim of the study was to investigate the potential of *Pocidonia oceanica* residues as a potential source of fermentable sugars for bioethanol production in an integrated process. By consecutive supercritical CO₂ and supercritical water (SCW) hydrolysis with a solid:liquid ratio of 0.08 (w/v) at 400 °C, cellulose and lignin were retained in solid phase whereas a reducing sugar value of 14.1 g/L was quantified in the liquid phase. Supercritical CO₂ extraction acted as a pretreatment method in order to loosen the lignin structure and SCW hydrolysis was responsible for both releasing some of the hemicellulose in the matrix. The results are very promising in terms of deployment of the utilization processes to industrial scale applications, thereby proposing an alternative solution to the landfill of *P. oceanica* residues.

Keywords: Marine Biomass, Hydrothermal Liquefaction, Supercritical CO₂, Supercritical Water Hydrolysis, Reducing Sugar.

1. INTRODUCTION

Environmental issues related to the energy resources have been discussed all over the world over the past few decades but have grown as its importance become more explicit recently with the development of ecological sustainability policies [1]. The studies to substitute fossil fuel are focused on ethanol production and particularly from lignocellulosic materials as they are abundant and constitute higher cellulosic and hemicellulosic contents up to 80-95 % [2, 3].

The bioconversion of the lignocellulosic materials to bioethanol includes two processes: hydrolysis of cellulose to fermentable sugars and fermentation of the sugars to ethanol. The aim of the first step is to reduce the cellulose crystallinity, increase porosity and especially remove lignin. However this process is generally carried out with dilute acid solutions which are toxic, corrosive, hazardous and require reactors that are resistant to corrosion [4]. In dilute acid hydrolysis, depolymerisation of lignin is performed [5]. Cellulose and hemicelluloses in the biomass are liquefied in basic sugar forms and this sugar containing broth can be used for further applications such as paper pulp, polymeric materials, scaffold for tissue culturing, biodispersants, resins, carbon fibers, composites, H₂ and CO production and also chemical intermediates to replace those derived from oil [6]. Recently research efforts have

been dedicated to develop obtaining sugar rich fractions in liquid form which allow further usage of sugars.

Pocidonia oceanica is a highly available seagrass in Turkey and its functionalities for several biological activities were demonstrated with a high amounts of holocellulose (65-75 %) which make it a unique candidate for both utilization of sugars and subsequently bioethanol production with a biorefinery approach [7]. Therefore an efficient separation of cellulose and leaving lignin in biomass could potentially enhance the economics of bioethanol production from liquid phase.

Supercritical fluid applications are considered to be 'green' alternatives to the corrosive acids and organic solvents, which are used industrially at present [8]. The behavior of a lignocellulosic material in supercritical systems was investigated before, where water dissolved hemicelluloses completely, leaving lignin in the solid phase without introduction of chemicals to the process [9].

The objective of this study was to investigate the effect of temperature and solid:liquid ratio (w/v) for obtaining a sugar rich fraction of *P. oceanica* by employing supercritical water hydrolysis without adding a catalyst or a chemical reagent. The response was based on maximization of the sugar content in the liquid phase.

*Corresponding Author: ¹Manisa Celal Bayar University, Faculty of Engineering, Manisa, irem.deniz@cbu.edu.tr

Subsequently, amounts of reducing sugars in the liquid phase were quantified in order to propose a holistic approach for utilization of *P. oceanica* residues as a feedstock, rather than to be deposited for landfill.

2. MATERIALS AND METHODS

2.1. Materials

Posidonia oceanica leaves were harvested from Izmir, Turkey. These wastes were washed with distilled water in order to eliminate sand and contaminants and then dried to a constant weight at room temperature. The dried sample was chopped into small pieces and passed through a 0.2-0.5 cm sieve and stored at 4 °C.

2.2. Supercritical Water Hydrolysis

The supercritical water (SCW) biomass conversion system used in this study was a batch-type reaction vessel with an working volume of 100 cm³. The meshed biomass and water were fed with a ratio of 1.2:15 (w:v) to the hydrolysis reactor. The gas in the vessel was replaced with nitrogen gas and then it was heated at the set temperature. After cooling to room temperature, water-soluble portion was retrieved by filtration. The filtrate was used for reduced sugar analysis, and filter was dried to constant weight in an oven (Memmert, Germany) at 70°C. Pulp yield was calculated by the amount of the obtained solid after hydrolysis per the amount of the solid biomass.

2.3. Reducing Sugar Analysis

Total reducing sugars in the residue was determined colorimetrically by applying dinitrosalicylic acid (DNS) (Merck, Darmstadt, Germany) method using glucose as the standard [10].

2.4. Statistical Analyses

All results are the means of two samples and analysis of variance (ANOVA) was used to compare the results and yields. Statistical significance level was 5% ($p < 0.05$).

3. RESULTS AND DISCUSSION

In terms of bioethanol production from liquid fraction of lignocellulosic biomass, supercritical water treatment is considered to be a promising pretreatment and temperature is important for liquefaction of hemicellulose content in hydrolysis process. In order to present a holistic utilization approach, reducing sugar contents of liquid fractions were analyzed while the other components were left to remain in the solid phase.

Investigation of the effect of temperature on reducing sugar contents indicated that the increase of temperature from 300 to 600 °C yielded a decrease in reducing sugar concentrations from 6.9 to 3.3 g/L where 39.2% higher value was recorded at 300 °C compared to the value obtained at 400 °C (Figure 1). It is worth mentioning that, pure cellulose conversion to water soluble sugars was observed to increase sharply at high temperatures in the range 302-405°C [11]. The reaction mechanism of cellulose was reported to change from heterogeneous to homogeneous hydrolysis at around the critical point of water with respect to the shrinking rate of cellulose that increased with temperature [3] which also supports the statement that cellulose dissolves into water at near and supercritical water conditions [12] and in agreement with the highest concentration of reducing sugar obtained at 300 °C in this study. Regarding conversion selectivity, the solubilized cellulose formed by the supercritical water pretreatment was converted to glucose and cellobiose in a selectivity of about 84 % at about 30 min [13]. Another study indicated that half of the cellulose was converted to organic acids after hydrolysis at 320°C and 25 MPa but with a reaction time of only 9.9 s due to the rapid change in the polarity of water transforming from reaction condition to room temperature where solid cellulose-like residues were formed [3], whereas no solid cellulose-like residues were observed in our study. It was also reported that hydrolysis dissolved hemicelluloses completely at higher temperatures from alfalfa fiber with no chemicals used [14].

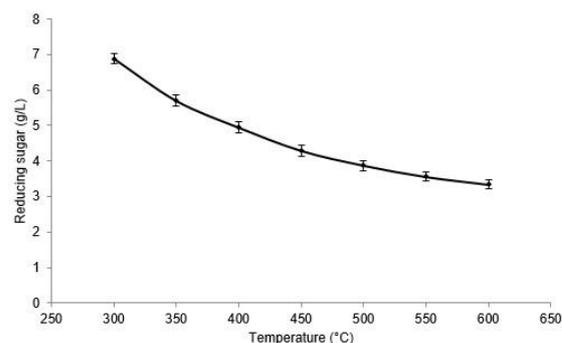


Figure 1. The effect of temperature on reducing sugar contents of the liquid fractions from hydrolysis of *P. oceanica*.

Solid to water ratios ranging between 0.04-0.12 (w/v) were tested in terms of reducing sugar contents and the results obtained are depicted in Figure 2. As the solid content increased two fold from 0.04 to 0.08, the reducing sugar concentration was increased 48.8 % ($p < 0.05$). However as the solid content was further increased, the increase in the concentration (7.7 %) was not statically significant ($p > 0.05$). Most recently, the influence of the corn slurry was

investigated for batch systems and at loading of 456 granular starch hydrolyzing units per gram of substrate, a yield of reducing sugars was obtained as 1.6 g/L [15].

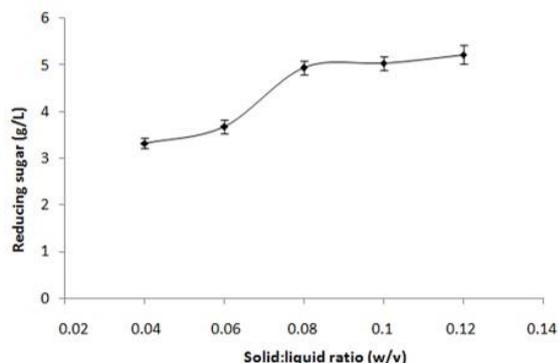


Figure 2. The effect of solid:liquid ratios (w/v) on reducing sugar contents of the liquid fractions from hydrolysis of *P. oceanica*.

The success of hydrolysis is directly related to the temperature during the process, therefore different temperatures (300-600 °C) were studied. Pulp yield increased with the increasing temperature until 400 °C (Table 1). At higher temperatures pulp yields decreased dramatically. Similar results were obtained in other studies, where higher temperatures resulted in less pulp yield in the solid phase [16].

Table 1. Effect of reaction temperature on gas volume and the hydrolysis of *P. oceanica* with a solid:liquid ratio of 0.08 (w/v).

Temperature (°C)	Pulp yield (%)
300	18.5
350	20.2
400	20.3
450	15.1
500	16.8
550	18.9
600	17.9

Solid to water ratios ranging between 0.04-0.12 (w/v) were tested and the results obtained in this set of experiments are shown in Table 2. As the solid to liquid ratio increased, pulp yield also increased in the solid phase. However, the pulp yield decreased about 36.09 % at higher solid to liquid ratios compared to the critical value of 0.08 (w/v) (Table 2). Similarly, it was shown that at higher water densities of about 1011 kg/m³ at 400 °C, liquefaction of biomass was very low [9].

Table 2. Effect of solid to liquid ratio (w/v) on the hydrolysis of *P. oceanica* (temperature 400°C).

Solid:liquid ratio (w/v)	Pulp yield (%)
0.04	9.2
0.06	14.1
0.08	20.3
0.10	19.1
0.12	18.1

Moreover, *P. oceanica* was subjected to supercritical CO₂ extraction and the remaining residue after this step was processed at high pressure hydrolysis in order to investigate the effect of high pressure pretreatment (Table 3). Indeed, 14.1 g/L reducing sugar was solubilized in liquid phase which was 2.9 fold higher than the value recorded for the liquid phase of the untreated samples. These results suggest the loosening of lignin structure by high pressure and possibly facilitation of the release of holocelluloses which can serve as an alternative technique to overcome the main problem of lignin barrier when dealing with lignocellulosic biomass for ethanol production [17, 8].

Supercritical CO₂ was reported to selectively hydrolyze sugar extract with experiments conducted at 360-374 °C and 225 bar. Moreover, it was mentioned that supercritical water and supercritical CO₂ acted upon glucan to selective hydrolysis of sugars in biomass [18]. In another study, the extraction of secondary metabolites from *Zostera marina* as studied and supercritical CO₂ extraction was reported to act as a pretreatment method in order to loosen the lignin structure, thereby liberating some of the hemicellulose in the matrix [17]. However, in contrast to the increase in terms of reducing sugar content in the liquid phase, the gas yields were decreased due to the conservation of total carbon amount when supercritical CO₂ pretreated sample was used.

Table 3. Analysis of solid, liquid and gas phases of *P. oceanica* samples by supercritical CO₂ pretreatment prior to supercritical water hydrolysis.

Property	Amount
Solid Phase	
Pulp yield (%)	11.1
Liquid Phase	
Reducing sugar (g/L)	14.1

4. CONCLUSIONS

This study focused on an alternative approach for the utilization of *P. oceanica* residues which create nuisance at the coastlines. Supercritical water was used to produce a liquid fraction comprised of soluble sugars and a solid insoluble fraction. Obtained fermentable sugar serves as a polymeric

material in deriving high-value intermediates and chemicals whereas hexose and pentose sugars are feedstocks for bioethanol fermentation. The results of this study also indicated that supercritical CO₂ extraction process acted as a pretreatment method for increasing the availability of hemicelluloses and celluloses. Under optimized conditions, 14.1 g/L reducing sugar was obtained by consecutive supercritical CO₂ and SCW hydrolysis where insoluble content remained in solid phase which shows the potential of *P. oceanica* residues to be utilized for not only as polymeric materials, but also for bioethanol production on industrial scale. Consequently, results of this research can be of great value for mitigating the increasing coastal pollution and contamination.

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