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RESEARCH ARTICLE

A comparison of bioethanol and biochar production from various algal biomass samples and sweet sorghum energy crop

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ABSTRACT

In this study, bioethanol and biochar production from various algal biomass samples (*Chlorella minutissima, Chlorella vulgaris, Nannochloropsis oculata* microalgae *and Laminaria digitata, Codium fragile, Ulva lactuca* macroalgae*)* and an energy crop sample (sweet sorghum bagasse) were investigated. In bioethanol production, acid pre-treatments were performed with 2 N H_2SO_4 solution at the temperature of 100°C, and pre-treatment time of 60 minutes. Fermentation was carried out in erlenmeyer flasks which were placed in a shaking incubator set to the 150 rpm at the temperature of 30°C for 48 hours. The highest bioethanol yield was obtained as 44% by utilizing sweet sorghum. Biochar production was carried out at a heating rate of 20°C min^{-1}, 250 ml min^{-1} nitrogen flow rate and temperature of 400°C in a carbonization retort. The characterization of biomass samples were performed, and all biofuel yields were compared. Although sweet sorghum as an energy crop has higher bioethanol production potential than micro and macroalgae sources, especially macroalgae can be a remarkable solution of waste utilization as a new generation feedstock of biosorbent and biochar production.

Keywords: Biochar, bioethanol, biomass, algae, sweet sorghum

1. INTRODUCTION

Nowadays high amount of energy demand and carbon emissions which cause global warming lead the world to use renewable energy. Especially bioethanol and biodiesel production have increased rapidly due to meet this energy requirement. Bioethanol can be produced from sugar-based feedstocks which are also food source and lignocellulosic biomass which are not used for food requirements. Crop plants like sweet sorghum and algae which are third generation biofuel feedstock are seen as alternative feedstock for bioethanol production [1].

Sweet sorghum is a C4 crop and has a high photosynthetic efficiency [2]. It has high biomass and sugar yield and it is resistant to drought, salinity and alkalinity, it can remain dormant during these conditions [1], [2]. Therefore, it can be cultivated in both irrigated and non-irrigated environments [3-4]. Due to this characteristic, it can grow everywhere and there are no competition between food growing and biofuel feedstock growing for lands. It contains soluble carbohydrates such as glucose, fructose and sucrose and insoluble carbohydrates like cellulose and hemicelluloses [1]. In addition to these carbohydrates, sweet sorghum juice contains some nutrients for microorganisms which produce ethanol and this can increase the process yield [5].

High sugar content of sweet sorghum makes it an interesting feedstock for bioethanol production. There are remains obtained after extraction of its juice and it should be utilized in different ways in concept of waste management and also to reduce energy cost in the process. In large scale production of bioethanol from sweet sorghum, high amount of sweet sorghum remains can become available raw material for another usage [6].

As third generation biofuel feedstock, algal biomass becomes very popular in biodiesel production in the last decade because of its high lipid content [7]. On the contrary, recent studies show the increase in bioethanol production from algal biomass instead of

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biodiesel production, considering the world's renewable energy trends. Macroalgae which are often located in coastal areas can be a good solution for both disposal of waste and economically viable bioethanol production. While microalgae mostly stand out as biodiesel feedstock with the ability of high lipid production and photosynthetic efficiency in the last decade, macroalgae are utilized for biogas or bioethanol production due to their high carbohydrate content [8].

In the concept of using renewable energy, many researchers investigate different biofuels such as biochar, bio-oil and syngas which is obtained by the conversion of biomass. Biochar is a carbon rich product which produced from biomass in absence of oxygen by thermal decomposition and it can be used as fuel due to its high fixed carbon content and high calorific value, a soil amendment, a soil carbon sequestration and a raw material for activated carbon production [9][10][11]. In order to reduce the biofuel production cost and apply waste management, it is important to utilize waste products or biomass which considered as waste for biofuel productions.

In the literature, there are many studies which investigate bioethanol and biochar production from different biomass sources. It is especially important to compare biofuel yields of algae and energy crops to be able to decide true raw material for biofuel production. For this reason, bioethanol and biochar production from various algal biomass samples (*Chlorella minutissima, Chlorella vulgaris, Nannochloropsis oculata* microalgae and *Laminaria digitata, Codium fragile, Ulva lactuca* macroalgae*)* and an energy crop sample (sweet sorghum bagasse) were carried out to compare biofuel yields.

2. EXPERIMENTAL

2.1. Materials

In the experiments, *Chlorella minutissima, Chlorella vulgaris, Nannochloropsis oculata, Ulva lactuca, Laminaria digitata, Codium fragile* algae and sweet sorghum bagasse were used as raw materials (Fig 1.). Microalgae species were cultivated in Bioengineering Department of Yıldız Technical University. In the cultivation step, BBM culture media and F/2 media were used for marine and fresh water microalgae species. *Ulva lactuca* and *Codium fragile* were obtained from coastal regions of Marmara Sea, and *Laminaria digitata* was collected from the coastal regions of Mediterranean Sea. Sweet sorghum was obtained from Adana region of Turkey. As for the pre-treatments, H_2SO_4 (98% concentrated, Merck) was used. Luria Broth (LB) medium (Merck) was supplied to use in fermentation step, and 96% purity ethanol (Merck) was used for gas chromatography analysis.

2.2. Preparation and Characterization of Biomass Samples

Microalgae cultures were cultivated in 500 mL Erlenmeyer flasks in BBM and F/2 mediums at pH 7.8 in a shaking incubator set to 150 rpm at 25±3°C under continuous illumination. As for macroalgae samples, they were washed with tap water to remove impurities such as sand, shellfish and other materials. Microalgae samples were centrifuged and dried with macroalgae samples in an oven for 24 h at 70°C. Dried samples were stored in a clean air-tight container. Harvested sweet sorghum plants were cut to the smaller pieces and then they were smashed in a mixer for creating larger area for fermentation and carbonization.

Fig 1. Biomass samples used in biofuel production

YL 6100 gas chromatography (GC) was used to evaluate bioethanol concentration. Samples from fermentation process were taken and prepared for GC instrument for further analysis. Firstly, samples were filtered using 0.45 µm filters to avoid blocking in column. The GC contains flame ionization detector (FID) and 30 m x 0.32 mm x 0.25 µm ZB-FFAP column. The temperature of injector, detector and oven were maintained at 150°C, 250°C and 100°C, respectively. Hydrogen was used as carrier gas. Bioethanol concentration was calculated using calibration curve that was prepared by the different concentration of bioethanol standards (0.1% -10% (v/v)).

Proximate analysis of the raw materials were carried out using the thermogravimetric analyzer (TA Instrument, SDT Q600) [12]. The functional groups of the raw materials were determined by FTIR spectroscopy (Bruker, Alpha).

2.3. Bioethanol Production

Biomass samples were pre-treated with dilute acid to degrade the biomass for fermentation process. Acid pre-treatments were performed with 2 N H_2SO_4 solution at the temperature of 100°C, and pre-treatment time of 60 minutes. After pre-treatments, samples in flasks were cooled down to the room temperature. The liquid from pre-treatment was neutralized before the fermentation. pH was maintained at 4.8 by alkaline/acid solutions. *Saccharomyces cerevisiae* yeast was chosen for the fermentation process of bioethanol production. The yeast was cultured in Luria Broth medium. Yeast suspension was aseptically transferred to 150 ml of sterilized LB medium and cultured in an incubator set to 150 rpm at the temperature of 30°C for 24 h, and 3% (v/v) of *Saccharomyces cerevisiae* was inoculated to the working medium. Fermentation was carried out in Erlenmeyer flasks which were placed in a shaking incubator set to the 150 rpm at the temperature of 30°C for 48 hours. Aliquots of 5 ml were taken to determine the concentration of ethanol by gas chromatography (GC) analysis.

2.4. Biochar Production

In this experiment, a split furnace (Protherm, ASP 11/100/500) was used as carbonization retort. The split furnace is in dimensions of 51 cm × 40 cm × 50 cm and pipe diameter is 10 cm. The temperature control is provided by the Honeywell DC1010 PID controller. The experimental set-up was shown in Fig 2. Experimental conditions for carbonization process were selected as temperature of 400°C, heating rate of 20°C min^{-1}, retention time of 30 min and nitrogen flow rate of 250 ml min^{-1}.

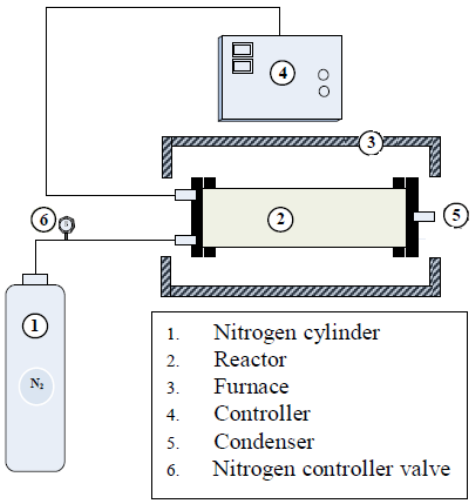

Fig 2. A schematic sketch of the carbonization experimental set-up

3. RESULTS

3.1. Characterization of Biomass Samples

Proximate analysis results of algae and sweet sorghum were shown in Table 1. As it can be seen in Table 1, *U. lactuca* has maximum moisture content (12.27%), *C. minutissima* has minimum moisture content (4.71%). Besides, moisture content of microalgae was found as lower than macroalgae. Ash content of *N. oculata* was found higher than other biomass samples due to the absorption of salts from seawater. The amounts of volatile substances of algal samples were very high, which was an expected result, because of the high carbohydrate content of macroalgae and high lipid content of microalgae [13]. The FTIR results of the raw materials used in the experiments were shown in Fig 3. When the obtained spectrums were examined, it was seen that the structure of the raw materials (especially algae) was similar, yet, the content ratios of the samples were different. The absorption between the range of 3300 and 3200 cm^{-1} can be assigned to the stretching vibrations of the OH groups which might be responsible for the moisture content [14][15] and this peak also appeared in three spectrum. Aliphatic C-H stretching at 2930 cm^{-1} and 2917 cm^{-1} caused by cycloparaffin structure [16]. The peaks at 1635 and 1626 cm^{-1}, which were determined in all raw materials, were presence of the protein content [17]; and the peaks at the range of 1100-1000 cm^{-1} in all biomass species, were determined because of the presence of the carbohydrate content [18].

3.2. Bioethanol Production

In order to make a comparison between results of the literature studies and this study, results were given in the Table 2. In this study, the highest bioethanol yield was obtained with sweet sorghum bagasse (SW) as 44%. This was an expected result, because of the carbohydrate content of sweet sorghum bagasse. Studies showed that, various sweet sorghum samples were resulted with 90-42% bioethanol yields by using different pre-treatment methods such as physical, acid and enzymatic. In this study, only 2 N acid pre-treatment was applied to the sweet sorghum samples. When considered the literature studies, it can be said that, acid+enzymatic pre-treatment is more efficient method than only acid pre-treatment. Bioethanol yield of *U. lactuca* was the second highest among them, and the lowest bioethanol yields were obtained with *L. digitata* and *C. fragile*. It was seen that bioethanol yields of microalgal samples were quite similar. According to the literature studies, carbohydrate content of *U. lactuca* generally constitutes of 55-60% of the biomass [19]. On the other hand, although carbohydrate content can be changed in seasons, 38-50% were reported for *L. digitata* and *C. fragile,* respectively [19][20]. When these samples were analyzed for carbohydrate content, the contents were found as 63%, 44%, and 36% for *U. lactuca, L. digitata* and *C. fragile.* This may be the reason of the bioethanol yield difference between these macroalgal samples. As in stoichiometric aspects, it can be commented that, the higher carbohydrate content will increase the bioethanol yield. In comparison with other macroalgal biomass, similar results were obtained in this research. As for the microalgal samples, bioethanol yields of *C. vulgaris, C. minutissima* and *N. oculata* were quite similar. Harun et al. reported that the highest bioethanol production from *Chlorococcum infusionum* was found as 26.13% under the conditions of 0.75% NaOH pre-treatment at the temperature of 120°C for 30 min [21]. In another study, bioethanol yield of *C. reinhardtii* was found as 29.10% after 3% H_2SO_4 pre-treatment in 110°C for 30 min by using modified yeast in fermentation. These studies show that, higher concentrations of acid pre-treatment can reduce bioethanol yield due to the formation of toxic molecules such as furaldehyde, acetate and hydroxymethylfuraldehyde which inhibit the fermentation [22]. In addition to this, concentration of bioethanol can increase in progress till there is no left convertible carbohydrate in the fermentation medium. The results obtained from this study are in agreement with these studies for bioethanol production.

Table 1. Proximate analysis of biomass samples

Biomass	*Moisture (%)*	*Volatile Matter (%)*	*Fixed Carbon (%)*	*Ash (%)*
Ulva lactuca	12.27	66.31	13.99	7.43
Laminaria digitata	10.89	55.85	24.95	8.31
Codium fragile	9.04	65.45	13.64	11.87
Chlorella vulgaris	6.77	76.68	11.62	4.93
Chlorella minutissima	4.71	75.29	10.55	9.38
Nannochloropsis oculata	4.87	68.06	12.88	14.19
Sweet sorghum	8.27	74.49	11.54	5.77

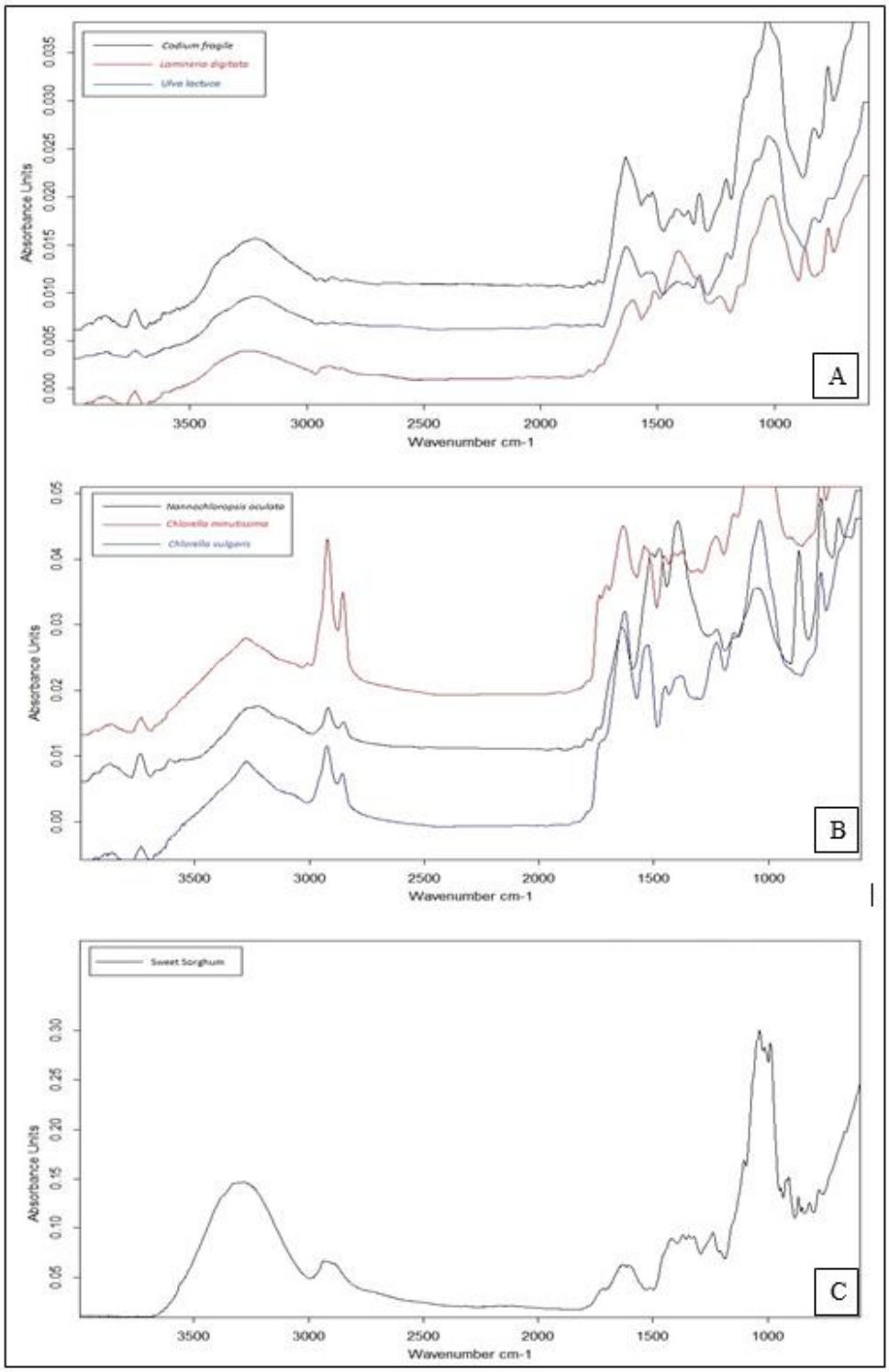

Fig. 3. FTIR spectrums of (A) macroalgae, (B) microalgae and (C) sweet sorghum

Table 2. Studies for bioethanol production from different biomass samples

Pre-treatment Method	*Sample*	*Time (h)*	*Ethanol yield (%)*	*Ref.*
Physical	SW-Stem	24	90.5	[6]
Physical	SW-Juice	40	50	[23]
Acid and enzymatic	SW-Stalk	24	42-45	[9]
Acid and enzymatic	SW-Bagasse	168	89.4	[10]
Alkaline	*C. infusionum*	72	26.00	[21]
Acid	*C. reinhardtii*	24	29.10	[24]
Hydrothermal and enzymatic	*Schizochytrium sp*	72	5.51	[25]
Acid	*C. humicola*	50	48	[26]
Physical	*L. japonica*	36	11.3	[27]
Acid and enzymatic	*G.verrucosa*	96	43	[28]
Acid	*G. corneum*	48	5.8	[29]
Alkaline	*N. oculata*	48	2.96	[30]
Acid	*U. lactuca*	48	26.52	*This study*
Acid	*Laminaria sp.*	48	9.88	*This study*
Acid	*C. fragile*	48	8.72	*This study*
Acid	*C. vulgaris*	48	15.20	*This study*
Acid	*C. minutissima*	48	14.46	*This study*
Acid	*N. oculata*	48	14.22	*This study*
Acid	SW-Pulp	48	44	*This study*

Table 3. Studies for carbonization process of different biomass samples

Species	*Conditions*	*Char yield (%)*	*Ref.*
Macroalgae			
Ulva flexuousa	450 °C	50	[31]
Cladophora sp.	550 °C, 60 min	31.0	[32]
Mix macroalgae species	500 C	29-36	[33]
Saccharina japonica	450^{0}C	33	[34]
Gracilaria gracilis	400-600 °C	26-32	[35]
Ulva lactuca	400°C	58	*This study*
Laminaria digitata	400°C	49	*This study*
Codium fragile	400°C	45	*This study*
Microalgae			
Arthrospira platensis	550 °C, 60 min	31.0	[32]
Chlamydomonas reinhardtii	350 °C, 20 min	44.0	[36]
Chlorella vulgaris	350 °C	31.0	[37]
Nannochloropsis sp.	400, 500, and 600 °C, 60 min	24.8-33.5	[38]
Tetraselmis sp.	500 °C, 1 h	~20	[39]
Chlorella vulgaris	400°C	34	*This study*
Chlorella minutissima	400 °C	32	*This study*
Nannochloropsis oculata	400°C	35	*This study*
Sweet Sorgum			
Raw sweet sorghum	427	21,4	[6]
Sweet sorghum bagasse	500	13,4	[6]
Sweet sorghum bagasse	500	28,8	[40]
Sweet sorghum bagasse	400 C	34	*This study*

3.3. Biochar Production

In order to make a comparison between the results of the some literature studies and this study, results were given in the Table 3. As it can be seen in the Table 3, the biochar yields of macroalgae were higher than those of microalgae. The main reason for this is the volatile matter ratios were lower in macroalgae. In addition, macroalgae collected from the seas have structures such as silica and lignin which degrade at very high temperatures. So, this has led to a higher yield of the solid. The microalgae used in this study have no silica and lignin content, because they are single cell microorganism and grown in fresh and marine water. In addition, due to high lipid content of microalgae was degraded in the temperature range of 250-350°C, the percentage of solid product was lower than the macroalgae.

As a result of the literature study, it was seen that, the yield of biochar obtained from micro- and macroalgae ranged from 20.0-63.0% to 8.1-62.4%, respectively [41]. Ronsse et al. [42], reported that the yield of algal biochar is lower than those of other lignocellulosic biomass. In addition, algal biochar yield was inversely proportional to temperature and residence time.

As a result of the carbonization process, char yields of *U. lactuca, L. digitata* and *C. fragile* were found as 58%, 49% and 45%, respectively. This result seems to be similar to the other studies in the literature. Bird et al. [31] calculated the yield of char obtained from *Ulva flexuosa* at the temperature of 450 °C as 50%. Yanik et al [33] found that the yields of chars produced from macroalgae (*Laminaria digitata, Fucus serratus* and *mix macroalgae species*) which collected from Black Sea, were 29-36% at the temperature of 500°C. Choi et al. [34] calculated to biochar yield that produced from *Saccharina japonica* as 33% at 450°C.

The char yields obtained from the carbonization of *C. vulgaris, C. minutissima* and *N. oculata* in our study were calculated as 34%, 32% and 35%, respectively. Wang et al. [37], in their study, found that *C. vulgaris* had a char yield of 31% at temperature of 350°C. Adamczyk and Sajdak [43] calculated that char yield of *Nannochloropsis sp.* at temperature of 400°C was about 55%; Aysu and Sanna [38] also calculated as 33.5%. The difference between two yields can be explained with their ash contents.

In our experiments, biochar yield of sweet sorgum bagasse was found as 33%. In the literature, Yin et al [40] found that the solid yield obtained from pyrolysis of sweet sorghum bagasse was 28.8% at temperature of 500°C. Piskorz et al. [6] used raw sorghum and sorghum bagasse in pyrolysis experiments and calculated their biochar yields as 21.4% and 13.4%, respectively. As can be seen in these studies, biochar production from sweet sorghum can be varied by pyrolysis conditions and raw materials used.

4. DISCUSSION AND CONCLUSIONS

Today, demand for fossil fuels cannot be met with current reserves and increasing oil prices with economic and political crisis and effects of global warming are led countries to use renewable energy sources. Currently, biodiesel and bioethanol are the lead biofuels. However, the high operational cost of the production of these biofuels led the researchers to utilize different raw materials and waste products to reduce the cost. In this study, sweet sorghum bagasse, *U. lactuca, L. digitata, C. fragile, C. vulgaris, C. minutissima* and *N. oculata* were utilized for bioethanol and biochar production. Bioethanol and biochar yields of these biomass samples were compared. As the highest bioethanol yield was obtained as 44% by utilizing sweet sorghum; the highest char yield was obtained from *U. lactuca* as 58%. As can be seen that, ethanol production potential from sweet sorghum has higher than algal biomass due to its high carbohydrate content. On the contrary, biochar production yields of algal biomass gave better results than sweet sorghum bagasse. Although sweet sorghum as an energy crop has higher bioethanol production potential than micro- and macroalgae sources, especially macroalgae can be a remarkable solution of waste utilization as a new generation feedstock of biosorbent and biochar production.

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