

A Comparative Study of Bioprocess Performance for Improvement of Bioethanol Production from Macroalgae



This work is licensed under a Creative Commons Attribution 4.0 International License

B. İnan and D. Özçimen*

Faculty of Chemical and Metallurgical Engineering,
Department of Bioengineering, Yildiz Technical University,
Davutpasa Campus, 34220 Esenler-Istanbul, Turkey

doi: 10.15255/CABEQ.2018.1499

Original scientific paper
Received: October 4, 2018
Accepted: March 13, 2019

In the last decade, studies that have focused on biodiesel production from algal biomass have been replaced with bioethanol production from algae, because bioethanol production from algae seems more promising when assessed on economic terms. Most coastal areas are covered with macroalgae, which are considered as a waste, and thus become a great problem for the municipality. Instead of their disposal, they can be alternatively utilized for bioethanol production. In this study, macroalgae located in the coastal regions of the Marmara Sea were collected and utilized for bioethanol production, and effects of the concentration of pre-treatment chemicals, pre-treatment temperature, and pre-treatment time on bioethanol yield were investigated. The highest bioethanol yields for dilute acid and alkaline pre-treatments were obtained under the conditions of 2 N sulfuric acid and 0.15 N potassium hydroxide solutions at the pre-treatment temperature of 100 °C and pre-treatment time of 60 minutes.

Keywords:

bioethanol, biomass, bioprocess, macroalgae, *Ulva lactuca*

Introduction

Starting with the oil crisis in the mid-1970s, finding new energy sources became a hot topic due to the increasing energy demand. In addition to this demand, effects of using petroleum-based fuels on the environment and global warming caused countries to take serious precautions in the last decade¹. As an alternative for gasoline, bioethanol has gained much attention during this period since the use of biomass as feedstock provides sustainability.

Today, three different generations of feedstock are presented for bioethanol production. The first-generation feedstock comprises food materials with high carbohydrate content such as wheat, corn, and sugar beet². However, increasing debate on food vs. fuel leads to utilization of waste lignocellulosic materials as second-generation feedstock for bioethanol production³. Despite contributing to waste management, difficulties in the pre-treatment process of lignocellulosic materials and its high cost show the need for alternative raw materials and improved pre-treatment techniques. As third-generation biofuel feedstock, algal biomass has become very popular in biofuel production in the last decade because of its simple structure, and high car-

bohydrate and lipid content⁴. Macroalgae often located in coastal areas can be a good solution for both disposal of waste and economically viable bioethanol production. Although they resemble other plants by appearance, they are different from terrestrial plants by their features of morphological and physiological characters and chemical compositions^{5,6}. While microalgae mostly stand out as biodiesel feedstock with the ability of high lipid production and photosynthetic efficiency, macroalgae are utilized for biogas or bioethanol production due to their high carbohydrate content⁷. In contrast to the structure of the microalgal biomass, macroalgae consist of substances such as carrageenan, laminaran, mannitol, and alginate, which can be utilized in various sectors. Macroalgae are separated from microalgae and lignocellulosic material having low lipid content and less or no lignin in their structure³. Therefore, this simple structure simplifies the pre-treatment stage of the bioethanol production process⁸.

Macroalgae accumulations on coastal areas are an environmental problem. City councils in coastal areas are required to remove them to preserve their Blue Flag Beach category and maintain appropriate conditions for tourism⁹. Smetacek *et al.* reported that in 2011, the disposal of 100,000 t of *Ulva* from Brittany's coasts was ordered to mitigate its impact on local tourism and the costs of disposal ranged

*Corresponding author:
ozcimen@yildiz.edu.tr

from US\$ 10–150 per ton¹⁰. According to Maceiras *et al.*, in Galicia, large amounts of macroalgae are collected along the coast, approximately 100,000 t per year, and most of them are treated as waste and thrown into landfills¹¹.

For efficient bioethanol production, a pre-treatment process is necessary. The objectives of an effective pre-treatment are obtaining fermentative carbohydrates directly or later by hydrolysis, preventing the loss or degradation of obtained sugars, limiting the formation of toxic materials that inhibit ethanol production, reducing energy requirement of the process, and minimizing the production cost. Pre-treatment process contributes substantially to the cost of ethanol production. Although there is no technique that can be considered as the best option, research and development is carried out to improve the performance and reduce the cost. Pre-treatments can be divided into physical, chemical, physico-chemical, and biological pre-treatments. These processes are carried out according to the type of feedstock due to the composition of the raw material, which has different cellulose, hemicellulose, and lignin content. Among the pre-treatment methods, alkaline pre-treatment and acid pre-treatment are the most commonly preferred chemical pre-treatment techniques. Alkaline pre-treatments are conducted under low process temperature and pressure in comparison with other techniques. However, they have limited efficiency, which appears as a disadvantage. In addition, some alkalis can turn into non-recoverable salts, and solubility of hemicelluloses and cellulose is low when compared with acid pre-treatment. In contrast, acid pre-treatments increase the accessibility of cellulose for enzymatic digestion. Acid pre-treatments can be divided as dilute acid pre-treatments and concentrated acid pre-treatments. Using concentrated acid is a less preferred method because of the high amount of inhibiting products and corrosion of the equipment. Acid pre-treatment is already used for the pre-treatment of lignocelluloses and first-generation feedstock such as corn. Although this method has some challenging problems, its efficiency in the conversion of sugars to bioethanol has overcome its disadvantages^{12–15}.

In this study, *Ulva lactuca* macroalgae were collected as waste from coastal regions of the Marmara Sea. Dilute acid and alkaline pre-treatments were performed on algal biomass to compare their effects on bioethanol yield. Effects of the concentration of pre-treatment chemicals, pre-treatment temperature, and pre-treatment time on bioethanol yields were also investigated, and cell disruption from chemical pre-treatments on the macroalgal biomass were observed.

Although there are various studies on bioethanol production from algae, there is no study on the comparison of chemical pre-treatment methods for bioethanol production bioprocess from *Ulva lactuca* macroalgae collected from the coastal areas of the Marmara Sea. Considering the world's renewable energy trends and waste valorization, presenting the feasibility of the pre-treatment methods for improving bioethanol production yield from macroalgal waste will provide original contributions to further studies and industrial bioprocess applications.

Material and methods

Materials

Ulva lactuca obtained from coastal regions of the Marmara Sea was used as algal feedstock. Sulfuric acid (98 % concentrated) and potassium hydroxide pellets (KOH) were used for pre-treatments, and 96 % grade of ethanol was used for quantitative determination of bioethanol. Phenol and D-glucose were used for determination of carbohydrate content. Luria-Bertani Broth (LB) medium was used for the yeast growth. All chemicals were supplied from Merck. Cellulase (C8546) and α -amylase (A4551) were supplied from Sigma-Aldrich to use in hydrolysis of macroalgal slurry for bioethanol production.

Preparation of raw material

U. lactuca samples were washed with tap water to remove impurities like sand, shellfish, and other materials, and then dried in an oven at 70 °C for 24 h. Dried samples were ground to improve the pre-treatment efficiency, and stored in a clean air-tight container.

Chemical pre-treatments

In order to investigate the effects of chemical pre-treatments on bioethanol yield, algal biomass was subjected to dilute acid and alkaline pre-treatment in flasks. To see the effects of pre-treatment chemical concentration, pre-treatment temperature, and time on bioethanol production from *U. lactuca* clearly, experimental parameters were chosen according to the literature studies. Macroalgal biomass was pre-treated with 0.5 N, 1 N, 2 N, 3 N, and 5 N H₂SO₄ at 100 °C for 60 minutes. Alkaline pre-treatments were carried out with 0.1 N, 0.15 N, 0.2 N, 0.25 N, and 0.35 N KOH at 100 °C for 60 minutes. To investigate the effect of pre-treatment temperature and pre-treatment time on bioethanol yield, experiments were conducted at 80 °C, 100 °C, 120 °C, and 140 °C in an oven for 15, 30, and 60 minutes, respectively. Macroalgal slurry after the

pre-treatment was cooled to room temperature. The liquid from pre-treatment was neutralized before fermentation. The pH was maintained at 4.8 by alkaline/acid solutions.

After determining the most effective factors for pre-treatment of macroalgal waste, enzymatic hydrolysis of macroalgal slurry was also applied after chemical pre-treatment. Enzymatic hydrolysis was carried out with cellulase and α -amylase enzymes for 24 h at 45 °C rotated at 150 rpm in an incubator. Enzyme loadings were 2 mg enzyme g⁻¹ algae dry matter.

Fermentation

Saccharomyces cerevisiae (YSC1, type I baker's yeast) supplied from Sigma-Aldrich, was chosen for the fermentation process of bioethanol production. The yeast was cultured in Luria Broth medium maintained at pH 4.8 by 1 M citrate solution. Yeast suspension was aseptically transferred to 150 mL of sterilized LB medium, and cultured in an incubator set to 150 rpm at 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 150 rpm at temperature of 40 °C for 48 hours. Aliquots of 5 mL were taken to determine the concentration of ethanol by gas chromatography (GC) analysis.

Analytical methods

Phenol-sulfuric acid method was used to analyze sugar concentration of macroalgae¹⁶. Lipid analysis and protein analysis were carried out with Soxhlet Ethanol Extraction and Lowry methods, respectively¹⁷. Proximate analysis of macroalgae was also performed according to ASTM-E 1755-01 and ASTM-D E872-82 standards. Properties of *U. lactuca* are given in Table 1.

In order to analyze the concentration of macroalgal bioethanol, GC was used. Analyses were performed with YL Instruments 6100 GC consisting of a flame ion detector (FID) and ZB-FFAP column, 30 m x 0.32 mm x 0.25 μ m ID. The injector, de-

tector, and oven temperatures were maintained at 150 °C, 200 °C, and 100 °C respectively. Hydrogen was used as the carrier gas. Samples were filtered with 0.45 μ m filters to prevent any damage to column before the analysis. The bioethanol concentration was quantified using a calibration curve prepared by injecting different concentrations of commercial ethanol as standard (0.1–10 %, v/v). Bioethanol yields were calculated based on dry matter. Data are presented as the mean of double replicates; the mean and standard deviation of the data are given in Figs. 1–4.

Results and discussion

Effect of acid and alkaline concentration on bioethanol yield

In this study, in order to investigate the effect of concentrations of acid and alkaline used in pre-treatment process on bioethanol yield, the experiments were performed at temperature of 100 °C, pre-treatment time of 60 min under different H₂SO₄ concentrations of 0.5 N, 1 N, 2 N, 3 N, and 5 N, and KOH concentrations of 0.1 N, 0.15 N, 0.2 N, 0.25 N, and 0.35 N, respectively. Because of low lignin content, diluted solutions were prepared in order to make the conditions for the process as mild as possible. The results of these experiments are given in Figs. 1–2. It was observed that bioethanol yields of acid pre-treated algal biomass were in the range of 2.31–6.19 % and 6.64–24.48 % after 24 h and 48 h, respectively. In contrast, bioethanol yields of alkaline pre-treated algal biomass were in the range of 1.22–2.01 % and 1.59–11.47 % after 24 h and 48 h, respectively. According to the results obtained from the experiments, increasing acid concentration led to an increase in bioethanol yield up to 2 N acid pre-treatment, while a decrease was observed when algal biomass was pre-treated with 3 N and 5 N H₂SO₄. The highest bioethanol concentration was obtained with 2 N H₂SO₄ pre-treatment.

It was assumed that increased acid concentration might have caused the formation of inhibiting products, such as acetic acid, formic acid, furfural, and hydroxymethylfurfural for fermentation process. Harun *et al.* studied the effect of acid pre-treatment process on microalgal biomass by applying 1–10 % (v/v) acid pre-treatment, and it was found that the highest bioethanol yield was obtained from microalgal biomass with 1 % acid pre-treatment application¹⁸. It was also reported that, even at low concentrations, toxic molecules such as furfuraldehyde, acetate, and hydroxymethylfurfuraldehyde, inhibit fermentation of glucose via *S. cerevisiae*¹⁹. Miranda *et al.* investigated the effect of acid pre-treatment in the range of 0.05–10 N acid concentrations on

Table 1 – Chemical composition and proximate analysis of *U. lactuca*

| Component | Content (%) | Component | Content (%) |
|---------------|-------------|--------------------|-------------|
| Carbohydrates | 63.1 | Ash | 13.6 |
| Proteins | 17.3 | Moisture | 12.3 |
| Lipids | 6.1 | Volatile substance | 67.4 |
| | | Fixed carbon | 6.4 |

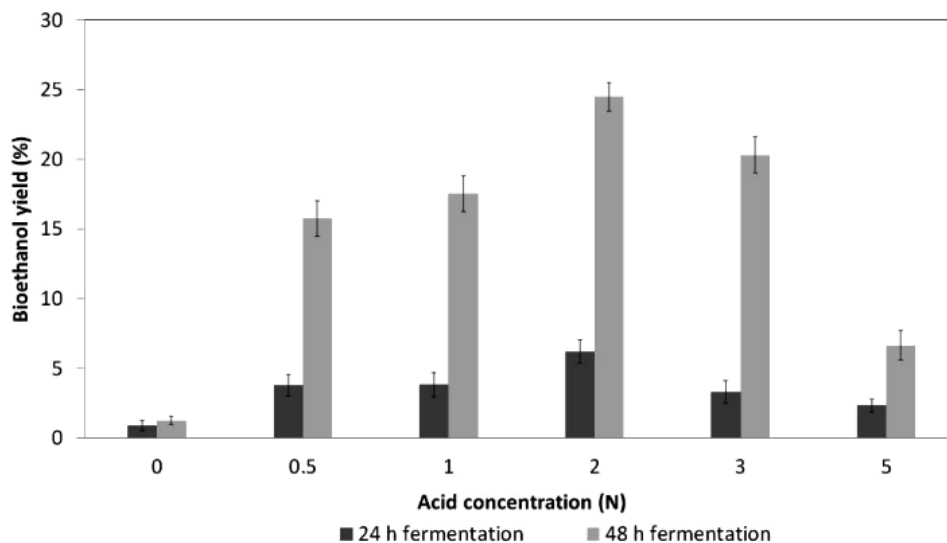


Fig. 1 – Bioethanol yields obtained from acid pre-treated macroalgae (pre-treatment temperature: 100 °C, pre-treatment time: 60 min)

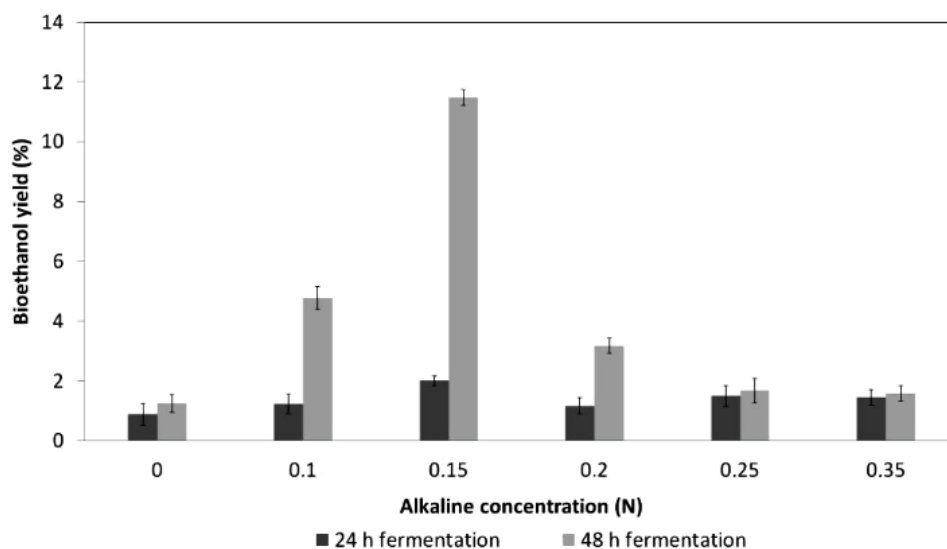


Fig. 2 – Bioethanol yields obtained from alkaline pre-treated macroalgae (pre-treatment temperature: 100 °C, pre-treatment time: 60 min)

Scenedesmus obliquus microalgae, and obtained the highest bioethanol yield when 2 N acid pre-treatment was used. They reported that acid pre-treatments at concentrations higher than 2 N caused a decrease in bioethanol yields²⁰. Larsson *et al.* also observed a decrease in bioethanol yield of soft wood with increasing concentrations of dilute acid pre-treatment, and found that the decrease occurred due to the formation of formic acid, which is a toxic molecule for fermentation²¹.

Sudhakar *et al.* investigated bioethanol production from spent seaweed biomass and performed hydrolysis of spent biomass with 0.1 %, 0.5 %, and 1 % concentrations of sulfuric acid. High sugar yield was obtained with 0.5 % and 1 % sulfuric acid pre-treatment from brown seaweed spent biomass and red seaweed spent biomass, respectively²².

The highest bioethanol concentration was obtained with 0.15 N KOH pre-treatment. In the literature, the highest bioethanol yield was achieved under the conditions of 0.75 % (w/v) NaOH pre-treatment¹². Similar to the effect of increasing acid concentration, increasing alkaline concentration caused a decrease in bioethanol yield due to fermentation-inhibiting products after exceeding a certain concentration of alkaline pre-treatment²³. Wang *et al.* reported that increasing concentration of alkaline pre-treatment caused a decrease in the sugar yields of coastal Bermuda grass²⁴. Nevertheless, an increase in the glucose yield of switch grass was obtained with increasing concentrations of alkaline pre-treatment²⁵. It could be that the different effects observed from alkaline pre-treatments of various raw materials are due to the complex struc-

tures of the raw materials. The results obtained from this study are in agreement with literature studies on bioethanol production given previously. According to the results of this study, it was found that acid pre-treatment was more efficient than alkaline pre-treatment for the production of bioethanol from algal biomass. Since the highest bioethanol concentration was obtained with 2 N H_2SO_4 pre-treatment, an enzymatic hydrolysis was applied to see the difference of the bioethanol yield obtained from samples which were hydrolyzed with only acid and acid+enzyme. Bioethanol yields of the enzymatically hydrolyzed samples were determined as 8 % and 32 % after 24 h and 48 h, respectively. This increase in bioethanol yield is in agreement with the literature studies. Wu *et al.* reported that the amount of released sugars of *Gracilaria sp.* increased from 200 $mg\ g^{-1}$ to 277 $mg\ g^{-1}$ by applying acid/enzyme hydrolysis in comparison with only acid pre-treatment experiment²⁶.

Also in our study, we found that enzymatic hydrolysis after acid pre-treatment increased bioethanol yield at lab-scale. Although a slight increase was observed with the enzymatic step, the use of enzyme in a large-scale production of bioethanol may not be preferred considering high enzyme price and extra process costs.

Effect of pre-treatment temperature on bioethanol yield

In order to investigate the effect of pre-treatment temperature on bioethanol yield, acid pre-treatment using 1 N and 5 N H_2SO_4 solutions, and alkaline pre-treatment using 0.15 N and 0.25 N KOH solutions for 60 min at temperatures of 80 °C, 100 °C, 120 °C, and 140 °C were performed. Since the

effects of 0.5 N and 1 N H_2SO_4 solutions were quite similar, only 1 N H_2SO_4 solution was used. Data obtained from these experiments are given in Fig. 3. An increase in bioethanol yield was observed in both 1 N and 5 N H_2SO_4 pre-treatments up to the temperature of 120 °C. However, the pre-treatments conducted at temperature of 140 °C caused a decrease in bioethanol yields. It could be said that bioethanol yields increased up to a certain temperature, and then decreased at conditions of high pre-treatment temperatures. The possible reason for this might be the fast disruption of structure and conversion of sugars to furan aldehydes¹⁸. In a study where *Yarrowia lipolytica* was used as biomass, acid pre-treatment with the ratios of 1:8, 1:10, 1:12, and 1:15 w/w was performed at temperatures between 90–150 °C for 60 min. It was reported that between the temperatures of 90–120 °C, an increase was observed in glucose yield, then a decrease occurred in glucose yield²⁷. In another study, maize was pre-treated with dilute acid at temperatures between 120–140 °C for 60 min. It was observed that the yield of monomeric sugars decreased at high temperatures²⁸. It could be indicated that pre-treatments conducted at 140 °C might have caused the degradation of sugars of macroalgal biomass to furan aldehydes in this study. Karimi and Karimi also reported that increasing pre-treatment temperatures can increase the HMF, furfural, and acetic acid concentrations in the liquid fraction when pre-treated with sulfuric acid²⁹.

As shown in Fig. 3, bioethanol yield increased with the increasing temperatures up to 100 °C, and then a slight decrease was observed at the temperature of 120 °C after 0.25 N KOH pre-treatment. Conversely, bioethanol yield continued to increase up to 120 °C after 0.15 N KOH pre-treatment. In a

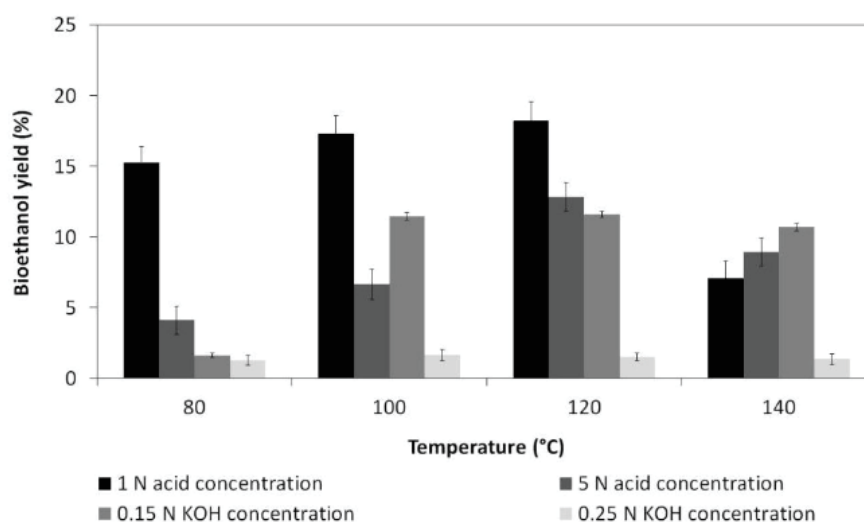


Fig. 3 – Effect of pre-treatment temperature on bioethanol yield (0.15 N and 0.25 N KOH, 1 N and 5 N H_2SO_4 , pre-treatment time: 60 min)

study on bioethanol production from *Chlorococcum infosionum*, it was reported that bioethanol yields were obtained as 21.26 % and 23.37 %, at temperatures of 80 °C and 120 °C for 60 min after pre-treatment of 0.75 % (w/v) NaOH solution, respectively. On the other hand, bioethanol yield decreased with increasing pre-treatment temperature after pre-treatment with 2 % (w/v) NaOH solution¹⁸. In conclusion, the results in our study are in agreement with these studies on bioethanol production in the literature. It could be said that various ratios of different parameters can show synergetic effects and it is very difficult to assess one parameter independently³⁰.

Effect of pre-treatment time on bioethanol yield

In order to investigate the effect of different pre-treatment times on bioethanol production, the pre-treatment process was carried out with 1 N and 5 N H₂SO₄ solutions, and 0.15 N and 0.25 N KOH solutions at 100 °C, with the pre-treatment time of 15, 30, and 60 minutes. Influence of various pre-treatment times on bioethanol yield is given in Fig. 4. As may be seen in Fig. 4, an increase in bioethanol yields was observed with increasing pre-treatment time for both 1 N and 5 N acid pre-treatments. In a study where *Sargassum* spp. microalgae was used under the conditions of 1–3 % H₂SO₄ acid pre-treatment applications between the reaction times of 10–110 minutes, increasing glucose yields were observed³¹. In another study, corn-cob was pre-treated with 1 % HCl solution for 20–40 minutes at 100–130 °C. It was reported that increasing glucose yields were achieved with increasing pre-treatment time³². It could be said that

the results for pre-treatment time in our study are in agreement with the studies mentioned previously.

The highest bioethanol yields were obtained after 60 min and 15 min under the conditions of 0.15 N and 0.25 N KOH pre-treatment, respectively. Although the results of bioethanol yields were similar for 15–30 min of pre-treatments under the conditions of 0.15 N KOH pre-treatment, it was observed that an increase occurred after 60 min. Nevertheless, bioethanol yield decreased after 15 min in the 0.25 N KOH pre-treatment. Harun *et al.* reported that bioethanol yield increased from 12.88 % to 21.26 % at the temperature of 80 °C with the pre-treatment time of 30 and 60 min after treatment with 0.75 % (w/v) NaOH solutions. However, a decrease from 26.13 % to 23.37 % was observed at the temperature of 120 °C under the same pre-treatment time. Small increases and decreases were reported at 80 °C and 120 °C for pre-treatment time of 30 and 60 min after the treatment of 2 % (w/v) NaOH solutions¹². In the pre-treatment of switch grass, an increase was reported at 120 °C, for 15, 30, and 60 min after the treatment of 0.5 %, 1 % and 2 % (w/v) NaOH solutions²⁵. Based on these results, an increase after 60 min with the pre-treatment of 0.15 N KOH was expected. Increase in bioethanol yields of switch grass has been reported with the increase in concentrations of chemicals and pre-treatment time due to it having a more complex structure than macroalgae. Nevertheless, due to the low lignin content of macroalgae, there is an excess degradation possibility for macroalgal biomass with the increase in concentrations of chemicals and at high pre-treatment temperatures, with a consequent

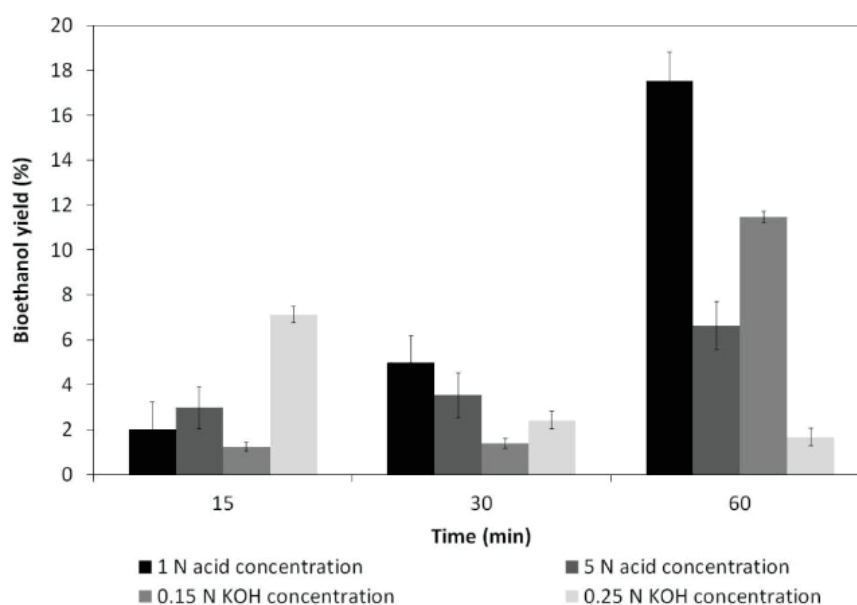


Fig. 4 – Effect of pre-treatment time on bioethanol yield (1 N and 5 N H₂SO₄, 0.15 N and 0.25 N KOH, pre-treatment temperature: 100 °C)

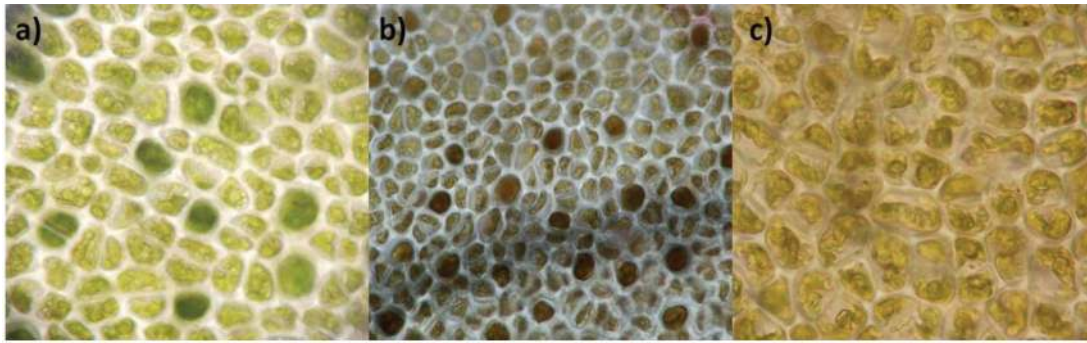


Fig. 5 – Microscopic images of a) macroalgal biomass, b) acid pre-treated macroalgae, and c) alkaline pre-treated macroalgae

reduction in bioethanol yield. Thus, it could be said that an increase in toxicity and a decrease in bioethanol yield had occurred after the treatment of 0.25 N KOH solution with increasing pre-treatment time.

Disruption of macroalgal biomass by chemical pre-treatments

The structural changes caused by various pre-treatments were observed using a light microscope (Olympus 100x). Microscopic images of disruption before and after 0.15 N KOH pre-treatment and 2 N H₂SO₄ pre-treatment are presented in Fig. 5. As may be seen in Fig. 5, the breakdown of macroalgal cell walls was observed with alkaline pre-treatment. A change in the macroalgal cells color was clearly observed in acid-pre-treated samples. This color change was caused by the interaction between sulfuric acid and cellulosic material of the macroalgae structure and acid caused disruption of cellulosic compounds. Although there are not many reports on the disruption of algal biomass, Harun *et al.* investigated the effects of chemical treatments on microalgal cells, and disruptions were observed clearly in the presented microscopic images, and confirmed that the chemical treatments broke the cell walls of algae^{12,18,33}.

Conclusion

The macroalgal wastes that accumulate on the coastal areas are an environmental nuisance. These wastes are starchy materials that are a suitable substrate for producing bioethanol. Although they are potential bioethanol feedstocks, bioethanol production from untreated macroalgal wastes is inefficient. In this regard, pre-treatment methods were investigated for bioethanol production from macroalgal wastes in this study. The highest bioethanol yield of 11.47 % was obtained when alkaline pre-treatment was performed at 100 °C with 0.15 N KOH solution for 60 minutes. On the other hand, the highest

bioethanol yield of 24.48 % was obtained when acid pre-treatment was performed at 100 °C with 2 N of sulfuric acid solution for 60 minutes, which was almost twice higher than alkaline pre-treatment.

Although research on algal biotechnology mainly focuses on algal biodiesel production, there is a growing interest in bioethanol production from algae. For years, algae have been evaluated for biodiesel production due to their high lipid content, but algal biomass is also a raw material for bioethanol production, because of its cellulosic structure without the need for other applications. We showed the feasibility of these methods and the effect of different parameters for improving bioethanol production yield from macroalgal biomass. Even though these methods are useful for bioethanol production from algae, as shown in the study, the enzymatic step may increase the bioethanol yield. Therefore, further studies should be carried out to obtain economic production costs while achieving higher bioethanol yields using enzymatic hydrolysis besides chemical pre-treatment.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from the Yıldız Technical University Scientific Research project (2014-07-04-YL02) provided for this work.

References

1. Nigam, P. S., Singh, A., Production of liquid biofuels from renewable resources, *Prog. Energy Combust. Sci.* **37** (2011) 52.
doi: <https://doi.org/10.1016/j.pecs.2010.01.003>
2. Terzioğlu, P., Yücel, S., Rababah, T. M., Özçimen, D., Characterization of wheat hull and wheat hull ash as a potential source of SiO₂, *BioResources* **8** (2013) 4406.
3. Daroch, M., Geng, S., Wang, G., Recent advances in liquid biofuel production from algal feedstocks, *Appl. Energy* **102** (2013) 1371.
doi: <https://doi.org/10.1016/j.apenergy.2012.07.031>

4. Gaurav, K., Srivastava, R., Singh, R., Exploring biodiesel: Chemistry, biochemistry, and microalgal source, *Int. J. Green Energy* **10** (2013) 775.
doi: <https://doi.org/10.1080/15435075.2012.726673>
5. Bruton, T., Lyons, H., Lerat, Y., Stanley, M., Rasmussen, M. B., A review of the potential of marine algae as a source of biofuel in Ireland, Dublin: Sustainable Energy Ireland-SEI, 2009.
6. Jung, K. A., Lim, S. R., Kim, Y., Park, J. M., Potentials of macroalgae as feedstocks for biorefinery, *Bioresour. Technol.* **135** (2013) 182.
doi: <https://doi.org/10.1016/j.biortech.2012.10.025>
7. Özçimen, D., Gülyurt, M. Ö., İnan, B., Algal Biorefinery for Biodiesel Production, in Fang, Z. (Ed), *Bio-diesel-Feedstocks, Production and Applications*, InTech, Croatia, 2012, pp. 25–58.
8. John, R. P., Anisha, G. S., Nampoothiri, K. M., Pandey, A., Micro and macroalgal biomass: A renewable source for bioethanol, *Bioresour. Technol.* **102** (2011) 186.
doi: <https://doi.org/10.1016/j.biortech.2010.06.139>
9. URL: <http://ec.europa.eu/environment/life/project/Projects/index> (13.03.2017).
10. Smetacek, V., Zingone, A., Green and golden seaweed tides on the rise, *Nature* **504** (2013) 84.
doi: <https://doi.org/10.1038/nature12860>
11. Maceiras, R., Cancela, Á., Sánchez, Á., Pérez, L., Alfonsin, V., Biofuel and biomass from marine macroalgae waste, *Energy Sources, Part A Recover. Util. Environ. Eff.* **38** (2016) 1169.
doi: <https://doi.org/10.1080/15567036.2013.862584>
12. Harun, R., Jason, W. S. Y., Cherrington, T., Danquah, M. K., Exploring alkaline pre-treatment of microalgal biomass for bioethanol production, *Appl. Energy* **88** (2011) 3464.
doi: <https://doi.org/10.1016/j.apenergy.2010.10.048>
13. Carvalheiro, F., Duarte, L. C., Gírio, F. M., Hemicellulose biorefineries: A review on biomass pretreatments, *J. Sci. Ind. Res. (India)* **67** (2008) 849.
14. Prasad, S., Singh, A., Joshi, H. C., Ethanol as an alternative fuel from agricultural, industrial and urban residues, *Resour. Conserv. Recycl.* **50** (2007) 1.
doi: <https://doi.org/10.1016/j.resconrec.2006.05.007>
15. Agarwal, A. K., Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines, *Prog. Energy Combust. Sci.* **33** (2007) 233.
doi: <https://doi.org/10.1016/j.pecs.2006.08.003>
16. Dubois, M., Gilles, K., Hamilton, J. K., Rerbers, P., Smith, F., Colorimetric method for determination of sugars and related substances, *Anal. Chem.* **28** (1956) 350.
doi: <https://doi.org/10.1021/ac60111a017>
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* **193** (1951) 265.
18. Harun, R., Danquah, M. K., Influence of acid pre-treatment on microalgal biomass for bioethanol production, *Process Biochem.* **46** (2011) 304.
doi: <https://doi.org/10.1016/j.procbio.2010.08.027>
19. Delgenes, J. P., Moletta, R., Navarro, J. M., Effects of lignocellulose degradation products on ethanol fermentation of glucose and xylose by *S. cerevisiae*, *Z. mobilis*, *P. stipitis*, and *C. shehate*, *Enzym. Microb. Technol.* **19** (1996) 220.
doi: [https://doi.org/10.1016/0141-0229\(95\)00237-5](https://doi.org/10.1016/0141-0229(95)00237-5)
20. Miranda, J. R., Passarinho, P. C., Gouveia, L., Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol production, *Bioresour. Technol.* **104** (2012) 342.
doi: <https://doi.org/10.1016/j.biortech.2011.10.059>
21. Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant, N. O., The generation of fermentation inhibitors during dilute acid hydrolysis of softwood, *Enzyme Microb. Technol.* **24** (1999) 151.
doi: [https://doi.org/10.1016/S0141-0229\(98\)00101-X](https://doi.org/10.1016/S0141-0229(98)00101-X)
22. Sudhakar, M. P., Merlyn, R., Arunkumar, K., Perumal, K., Characterization, pretreatment and saccharification of spent seaweed biomass for bioethanol production using baker's yeast, *Biomass Bioenergy* **90** (2016) 148.
doi: <https://doi.org/10.1016/j.biombioe.2016.03.031>
23. Vincent, M., Pometto, A. L., Van Leeuwen, J. H., Ethanol production via simultaneous saccharification and fermentation of sodium hydroxide treated corn stover using *Phanerochaete chrysosporium* and *Gloeophyllum trabeum*, *Bioresour. Technol.* **158** (2014) 1.
doi: <https://doi.org/10.1016/j.biortech.2014.01.083>
24. Wang, Z., Keshwani, D. R., Redding, A. P., Cheng, J. J., Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass, *Bioresour. Technol.* **101** (2010) 3583.
doi: <https://doi.org/10.1016/j.biortech.2009.12.097>
25. Wang, Z., Li, R., Xu, J., Marita, J. M., Hatfield, R. D., Qu, R., Cheng, J. J., Sodium hydroxide pretreatment of genetically modified switchgrass for improved enzymatic release of sugars, *Bioresour. Technol.* **110** (2012) 364.
doi: <https://doi.org/10.1016/j.biortech.2012.01.097>
26. Wu, F. C., Wu, J. Y., Liao, Y. J., Wang, M. Y., Shih, I. L., Sequential acid and enzymatic hydrolysis in situ and bioethanol production from *Gracilaria* biomass, *Bioresour. Technol.* **156** (2014) 123.
doi: <https://doi.org/10.1016/j.biortech.2014.01.024>
27. Tsigie, Y. A., Wu, C. H., Huynh, L. H., Ismadji, S., Ju, Y. H., Bioethanol production from *Yarrowia lipolytica* Polg biomass, *Bioresour. Technol.* **145** (2013) 210.
doi: <https://doi.org/10.1016/j.biortech.2012.11.091>
28. Noureddini, H., Byun, J., Dilute-acid pretreatment of distillers' grains and corn fiber, *Bioresour. Technol.* **101** (2010) 1060.
doi: <https://doi.org/10.1016/j.biortech.2009.08.094>
29. Karimi, S., Karimi, K., Efficient ethanol production from kitchen and garden wastes and biogas from the residues, *J. Cleaner Prod.* **187** (2018) 37.
doi: <https://doi.org/10.1016/j.jclepro.2018.03.172>
30. İnan, B., Utilization of Algae for Bioethanol Production, MSc Thesis, 2014, Yildiz Technical University, Istanbul, Turkey.
31. Borines, M. G., de Leon, R. L., Cuello, J. L., Bioethanol production from the macroalgae *Sargassum* spp., *Bioresour. Technol.* **138** (2013) 22.
doi: <https://doi.org/10.1016/j.biortech.2013.03.108>
32. Zu, S., Li, W., Zhang, M., Li, Z., Wang, Z., Jameel, H., Chang, H., Pretreatment of corn stover for sugar production using dilute hydrochloric acid followed by lime, *Bioresour. Technol.* **152** (2014) 364.
doi: <https://doi.org/10.1016/j.biortech.2013.11.034>
33. Spiden, E. M., Scales, P. J., Yap, B. H. J., Kentish, S. E., Hill, D. R. A., Martin, G. J. O., The effects of acidic and thermal pretreatment on the mechanical rupture of two industrially relevant microalgae: *Chlorella* sp. and *Navicula* sp., *Algal Res.* **7** (2015) 5.
doi: <https://doi.org/10.1016/j.algal.2014.11.006>