

Scientific paper

Effect of Chemical Pre-treatments on Bioethanol Production from *Chlorella minutissima*

Burcu Şerbetçioğlu Sert, Benan İnan and Didem Özçimen*

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

* Corresponding author: E-mail: ozcimen@yildiz.edu.tr

Received: 01-08-2017

Abstract

In recent years, algal bioethanol production comes into prominence as a trend towards sustainable development. Due to being sustainable energy source and environmental friendly, bioethanol production from algae is becoming increasingly popular all over the world. However, yield of bioethanol production from algae is lower than first generation feedstock's currently, and needs to be improved. In order to increase bioethanol yield, pre-treatments should be performed as cell disruption process on algal biomass. For this reason, researchers investigate the most appropriate pre-treatment method and its parameters for high yield bioethanol production from algae. In this study, cultivated *Chlorella minutissima* was utilized for bioethanol production. Effects of pre-treatment method (dilute acid and alkaline), chemical concentration, pre-treatment temperature and pre-treatment time on bioethanol yield were investigated. It was found that, the highest bioethanol yield was obtained as 18.52% with acid pre-treatment at pre-treatment temperature of 100 °C and pre-treatment time of 60 minutes.

Keywords: Bioethanol, biofuel, biomass, Chlorella minutissima, microalgae

1. Introduction

Increase in the world population and emerging industry cause an increase in energy demand which are met by fossil fuels. However, fossil fuel resources are exhausting from day to day, and this decline in the reserves increases the price of petroleum fuels because of political impacts. Negative effects of petroleum fuels on environment, increased greenhouse gas emissions and global warming cause countries to take action on this matter. In order to reduce the environmental problems, increase socio-economic development and provide sustainable energy, utilizing renewable energy technologies such as solar, wind, hydro, and biomass, has been considered. As a renewable energy source, biofuel is one of the promising alternatives to the fossil fuels. Today, bioethanol has been identified as the most widely used biofuel for transportation worldwide.2 Bioethanol is produced from sugars and starch-rich raw materials such as corn, wheat and sugarcane. It can be blended with gasoline in different ratios, and there are specially designed engines which can use 100% bioethanol.2 Bioethanol has excellent fuel properties for spark ignition internal combustion engines because of its high octane and heat of vaporization. In comparison with gasoline, these properties make ethanol more efficient as a pure fuel.3 Currently, bioethanol production is mostly carried out with sugarcane and corn as first generation bioethanol feedstock. Still, it is merely meet the current demand, and there are many conflicts and debates about their sustainability due to the depletion of water sources and the use of arable land to produce biomass for bioethanol production.^{4,5} Although lignocellulosic biomass is an alternative source for first generation bioethanol feedstocks, it requires intensive labor and a high capital cost for processing.5 Algae are considered as third generation biofuel feedstock and capable of generating more organic carbon per hectare than terrestrial plants. Except Asia, algae avoid the food versus fuel argument since they are not a major food source. Biomass production of algae is 5–10-times greater than that of land-based plants due to their more photosynthetic efficiency.⁵ Algae grow rapidly and can be easily grown in various aquatic environments such as fresh water, saline water or municipal waste water. Microalgae don't need structural biopolymers such as hemicellulose and lignin which are necessary for terrestrial plants. This simplifies the process of bioethanol production from microalgae.7 Microalgae which have high amount of starch such as Chlorella, Dunaliella, Chlamydomonas, Scenedesmus are very useful for bioethanol production. Like as other bioethanol feedstocks, algae are pre-treated with various methods before fermentation process. Although there are different pre-treatment methods for different biomass sources such as physical, chemical, physio-chemical and biological pre-treatments, chemical pre-treatments are the most used techniques for pre-treatment of algal biomass.8 Chemical pre-treatments are easy to perform, and good conversion yields can be achieved with these pre-treatments in a short time. According to the BP statistical review of world energy-2016 report, ethanol production of the world is higher than biodiesel production.10 In the last decade, the increase of the production of ethanol is quite remarkable, and due to the global warming and high oil prices, it is considered that the ethanol production will continue to increase. Although there are a lot of studies on biodiesel production from different type of microalgae species in the literature, researches on bioethanol production from microalgae are less. In this study, cultivation of C. minutissima was carried out in labscale reactor, and the growth of microalgae was monitored with optical density analysis. Obtained algal biomass was analyzed with various analytical methods. During the bioethanol production from C. minutissima, in order to compare the effect of pre-treatment methods on bioethanol yield, acid and alkaline pre-treatments were performed. Effects of concentration, pre-treatment temperature and pre-treatment time on bioethanol yield were also investigated. There is not any study which is on the bioethanol production from C. minutissima and the comparison of chemical pre-treatment methods in the literature. The results of this study will contribute to the further studies and industrial applications by determining the efficient pre-treatment conditions for bioethanol production from microalgae.

2. Experimental Section

2. 1. Materials

C. minutissima microalgae was cultivated in Bioengineering Department of Yıldız Technical University. In the cultivation step, BBM culture media was prepared with 0.075 g K₂HPO₄, 0.014 g KH₂PO₄, 0.075 g MgSO₄7H₂O, 0.09 g NaNO₃, 0.025 g CaCl₃ · 2H₂O, 0.025 g NaCl, 0.05 g EDTA–Na₄, 0.00498 g FeSO₄7H₂O, 0.01142 g H₃BO₃, 0.232 mg MnCl₂ · 4H₂O, 1.41 mg ZnSO₄ · 7H₂O, 0.252 mg CuSO₄ · 5H₂O, 0.192 mg NaMoO₄ · 5H₂O. All of these chemicals were supplied from Merck. As for the pre-treatments and analytical measurements, KOH (Merck) and H₂SO₄ (98% concentrated, Merck), and 96% purity ethanol (Merck), phenol (Sigma-Aldrich) and D-Glucose (Sigma-Aldrich) were used, respectively. LB Broth (Merck) was supplied to use in fermentation step.

2. 2. Biomass Cultivation

Cultures of the *C. minutissima* were firstly cultivated in 500 mL Erlenmeyer flasks in BBM medium at pH 7.8 in

a shaking incubator set to 150 rpm at 25 \pm 3 °C under continuous illumination. In logarithmic phase of the cultures, 10% (v/v) inoculum was transferred to the 20 L photobioreactor, and cultivation was carried out with working volume of 15 L at 27 °C and pH 8.3. A pump was used for aeration of the culture medium. Bioreactor was exposed to six pink-fluorescent lamps continuously (4500 lux). Growth of the culture was monitored by optical density measurement. Samples were taken from the photobioreactor and analysed daily. The conductivity of the medium and pH were measured as 256 mS/cm and 8.2, respectively. Harvested algae were centrifuged and dried in an oven for 24 h at 70 °C.

2. 3. Pre-treatment of Microalgal Biomass

Dried microalgal biomass was pre-treated to degrade cellulosic cell wall for accessing fermentable carbohydrate components. Acid pre-treatments were performed with 0.5 N, 1 N, 2 N, 3 N and 5 N H₂SO₄ solutions at different temperatures (100 °C, 120 °C and 140 °C) and pre-treatment times (15, 30 and 60 minutes). Pre-treatment conditions were chosen according to the previous studies in the literature. In order to compare the effect of pre-treatment type on bioethanol yield, alkaline pre-treatments were also carried out. Alkaline pre-treatments were conducted with various concentrations of KOH solutions (0.5%, 0.75%, 1%, 1.5% and 2% (w/v)) at the different pre-treatment temperatures (80 °C, 100 °C and 120 °C) and pre-treatment times (15, 30 and 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.

2. 4. Bioethanol Production

The day before the fermentation, *S. cerevisia* yeast was cultured in flask with LB medium at the temperature of 40 °C and 150 rpm shaking speed. 3% (v/v) of yeast was inoculated to the pre-treated samples and fermentation was carried out in an incubator set to 150 rpm and 40 °C for 48 h. 5 ml were taken from the samples to determine the concentration of bioethanol by gas chromatography (GC) analysis.

2. 5. Analytical Methods

During the microalgae cultivation, optical density measurement of the culture was used to monitor the algal growth. Optical density was measured by using UV-visible spectrophotometer at 680 nm. Productivity of *C. minutissima* was expressed as the specific growth rate (μ) and doubling time (t_d) by using the Equation 1–2 from the cell density change during specific time period of exponential phase.¹¹

$$\mu = \frac{\ln \frac{N(t)}{N(0)}}{t} \tag{1}$$

$$td = \frac{\ln 2}{\mu} \tag{2}$$

Carbohydrate analysis, lipid analysis and protein analysis were carried out with Phenol-sulfuric acid method, Soxhlet Ethanol Extraction and Lowry method, respectively.

Characterization of *C. minutissima* was also performed with Fourier Transform Infrared spectroscopy (FTIR spectroscopy) and proximate analysis. Proximate analysis was performed with TA instrument (Q600 SDT) according to ASTM-E 1755-01 and ASTM-D E872-82 standards. FTIR spectroscopy was carried out with the instrument of Thermoscientific (Nicolet 6700) and functional groups were determined in the wavenumber range of an IR spectrum of 600–4000 cm⁻¹.

YL 6100 GC gas chromatography was used to evaluate bioethanol concentration. Samples from fermentation process (at 24 h and 48 h) 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 gas chromatograph contains flame ionization detector (FID) and 30 m \times 0.32 mm \times 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)). The mean and standard deviations of the data were calculated, and data were presented as the mean of three.

3. Results and Discussion

3. 1. Growth of C. minutissima Microalgae

Growth of C. minutissima was monitored by optical density measurement. Specific growth rate was calculated as 0.0879 day⁻¹ and the doubling time of the microalgal cells was calculated as 7.8 days in photobioreactor environment. Although there are studies in which microalgae have less doubling time than 7 days, it is a remarkable growth because the aeration may overwhelm stress in photobioreactor environment and microalgae may need more time to multiply according to Kawaroe et al.¹² Lim et al. also reported that doubling time of microalgae can be up to 6-7 days in 100 ml of flask.13 Results of chemical composition and proximate analysis of C. minutissima were given in the Table 1, and FTIR analysis results were given in the Table 2 and Fig 1. As can be seen from the FTIR results given in Table 2, aliphatic CH stretching at 2922 cm⁻¹ is caused by cycloparaffin structure.14 C=C ring stretching in bands between 1400 and 1600 cm⁻¹ indicate presence of alkenes.15 Bands show that symmetrical and asymmetrical C-H stretching linked to -CH, group, derived from aliphatic hydrocarbons and saturated aliphatic cyclic hydrocarbon. 16 1743 cm $^{-1}$ band (C = O) indicates the presence of lipids, fatty acids and ester groups. The region from 1200 to 900 cm $^{-1}$ signifies a sequence of bands due to C–O, C–C and C–O–C stretching vibrations of polysaccharides.

 Table 1. Chemical composition and proximate analysis of C. minutissima

Biochemical Analysis	Content (%)	Proximate Analysis	Content (%)
Carbohydrate	33.05	Ash	9.39
Protein	24.69	Moisture	4.71
Lipid	42.26	Volatile substance Fixed carbon	75.63 10.04

Table 2. FTIR analysis of C. minutissima

Wave number (cm ⁻¹)	Functional Groups
3275	-OH stretching
2922	Aliphatic CH stretching
1743	C=O strecthing
1643	Aromatic C=C ring stretching
1537	Aromatic C=C ring stretching
1462	CH stretching in methyl lipids
1398	Aliphatical CH ₃ deformation
1274	Aromatic CO – stretching
1257	Aliphatic C-N stretching
1037	Aliphatic ether C-O and alcohol C-O stretching
765	4 adjacent H deformation

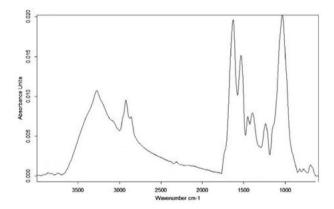


Fig 1. FTIR spectrum of C. minutissima

3. 2. Effect of Acid and Alkaline Concentrations on Bioethanol Production

In order to investigate the effect of acid concentration on bioethanol yield, acid pre-treatments were performed with 0.5 N, 1 N, 2 N, 3 N and 5N $\rm H_2SO_4$ solutions

under the temperature of 100 °C and pre-treatment time of 60 min. As can be seen in the Fig 2, bioethanol yields were obtained between 2.92-4.78% for 24 h and 5.26-18.52% for 48 h fermentation time. It was found that bioethanol yield increases up to a certain acid concentration. The highest bioethanol yield was obtained under 1 N H₂SO. acid concentration. Above 1 N H₂SO₄ bioethanol yields decreased with 2 N, 3 N and 5 N acid pre-treatments. It is considered that toxic components such as furaldehyde, acetate and hydroxymethylfuraldehyde may occur due to the effect of acid pre-treatment, and they inhibit the fermentation.¹⁷ Decrease in bioethanol yield in high acid concentrations of pre-treatment was observed. In the study of pre-treatment of Sargassum spp. with 1.0-5.0% (m/v) H₂SO₄ it was found that the best result was obtained with 3.4-4.6% acid pre-treatment, and bioethanol yield decreased with increasing acid concentrations.¹⁸ In another study, Gracilaria sp. was pretreated with 0.05, 0.1, 0.3 and 0.5 N H₂SO₄ and the highest bioethanol yield was achieved with 1 N acid pre-treatment.19

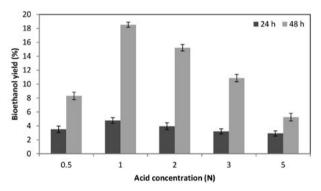


Fig 2. Bioethanol yields obtained from acid pre-treated (at 100 °C, 60 min) microalgae

Experimental data obtained under the conditions of 0.5%, 0.75%, 1%, 1.5%, and 2% (w/v) KOH solutions at 100 °C and 60 min were given in the Fig 3. It was determined that bioethanol yield was changed between 1.01-1.92 % for 24 h, and %1.43-6.11% for 48 h fermentation time. The highest bioethanol yield was obtained with 0.75% (w/v) KOH solution pre-treatment. Like as acid pre-treatment, a decrease was also observed with increasing alkaline concentrations above this concentration. In the literature, the highest bioethanol yield was achieved by pretreating Chlorococcum infusionum microalgae under the conditions of 0.75% (w/v) NaOH pre-treatment.20 Also, this can be seen in alkaline pre-treatment of *Ulva lactu*ca macroalgae collected from Marmara Sea. It was reported that, bioethanol productivity increased up to a certain concentration, and then it was started to decrease.21 The results obtained from this study are in agreement with these studies for bioethanol production. According to the results, it can be said that, acid pre-treatment is more efficient method than alkaline pre-treatment for algal biomass.

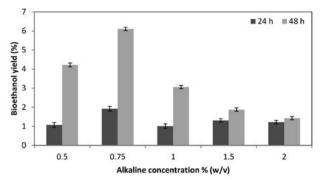


Fig 3. Bioethanol yield obtained after from alkaline pre-treated (at $100\,^{\circ}\text{C}$, $60\,\text{min}$) microalgae

3. 3. Effect of Pre-treatment Temperature on Bioethanol Production

Results obtained from the experiments performed at 100 °C, 120 °C and 140 °C with 1 N and 5 N H₂SO₄ solutions for 60 min, were given in Fig 4. As it was shown in the figure, bioethanol yield increased up to 120 °C with both 1 N and 5 N pre-treatments. On the other hand, pre-treatments conducted at 140 °C resulted with a decrease in bioethanol yield. Bioethanol yield determined after pre-treatments carried out at high temperatures, increases up to a certain temperature and then, it decreases. This is due to the pre-treatments which change direct solubility of sugars and disrupt the structure of sugar based components.²² Generally, it is reported that, raw materials which contain lignin and hemicellulose, were pretreated at the temperatures above 160 °C with 0.5-1.5% (v/v) acid solutions.²³ In another study, corn fibers were pretreated with sulfuric acid at the temperatures of 120 °C and 140 °C and 60 min pre-treatment time. It was observed that, yield of monomeric carbohydrates decreases with increasing pre-treatment temperatures.²⁴ It is indicated in the literature, experiments should be performed under low pre-treatment temperature and acid concentrations when lignin content of raw materials are not high. Since structure of microalgae is simpler than other materials, it is assumed that monomeric carbohydrates are decomposed to toxic compounds at high temperatures.

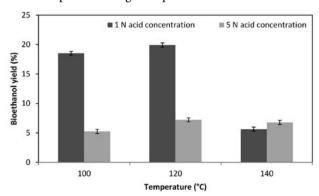


Fig 4. Effect of different acid pre-treatment temperatures on bioethanol yield

Unlike acid pre-treatments, bioethanol yield increased up to 100 °C, then decreased at 120 °C with 0.75% (w/v) KOH alkaline pre-treatment, however, it increased up to 120 °C with 1.75% (w/v) KOH alkaline pre-treatment (Fig 5). It is in agreement with the study carried out with *Chlorococcum infosionum* microalgae which were pretreated with 0.75% (w/v) NaOH solution for 60 min at 80 °C and 120 °C. In that study, bioethanol yields were obtained as 21.26% and 23.37%, respectively. In higher concentrations of NaOH, yield was determined as 23.75% at 80 °C, and it decreased to 18.74% at 120 °C.²⁰ Therefore, effect of different values of different parameters in the same time is variable and it can be said that it is difficult to assess a parameter alone independently.

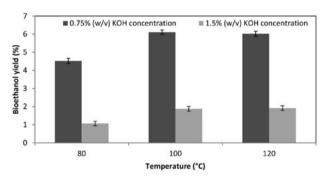
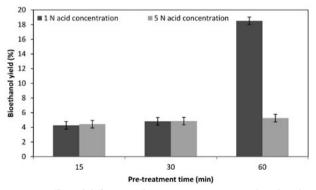


Fig 5. Effect of different alkaline pre-treatment temperatures on bioethanol yield

3. 4. Effect of Pre-treatment Time on Bioethanol Production

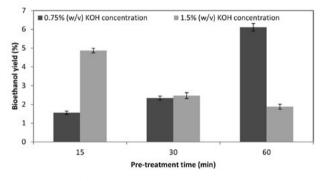
In order to investigate the effect of different pre-treatment times on bioethanol production yield, 1 N and 5 N $\rm H_2SO_4$ acid pre-treatments were carried out at the temperature of 120 °C in the pre-treatment times of 15, 30 and 60 minutes. According to Fig 6, increasing pre-treatment time increases bioethanol yield in both 1 N and 5 N acid pre-treatments. In the literature, the highest bioethanol yield was obtained for *Scenedesmus obliquus* microalgae under 2% $\rm H_2SO_4$ pre-treatment at 121 °C for 20 min.²⁵ In



 ${f Fig}$ 6. Effect of different acid pre-treatment times on bioethanol yield

another study, corn cob was pretreated with 1% HCl solution for 20–40 min at 100–130 °C, and it was observed that bioethanol yield increased with increasing pre-treatment time. This effect was also seen in the study of pretreating *Kappaphycus alvarezii* macroalgae with 1–1.5–2% H₂SO₄, at 121 °C for 20, 40 and 60 min. It was found that the highest productivity was achieved with 1% H₂SO₄ solution and 60 min pre-treatment time. Solution 23

Results of the experiments conducted at 100 °C for 15, 30 and 60 min with 0.75 and 1.5% (w/v) KOH solution were given in Fig 7. As can be seen in Fig 7, the highest yield was obtained under the conditions of 0.75% (w/v) KOH solution for 60 min. On the other hand, the highest yield was obtained with 15 min using 1.5% (w/v) KOH solution. Results of bioethanol yields were similar for 15-30 min pre-treatments under the condition of 0.75% (w/v) KOH pre-treatment, however, it was observed that an increase was occurred after 60 min. On the other hand, bioethanol yield decreased after 15 min pre-treatment time with 1.5% (w/v) KOH pre-treatment. In the study which performs alkaline pre-treatment on Chlorococcum infusionum microalgae, bioethanol yield increased from 12.88% to 21.26% at 80 °C with the pre-treatment time of 30 and 60 min after the treatment of 0.75% (w/v) NaOH solution. However a slight decrease was seen at 120 °C under the same pre-treatment time. After the treatment of 2% (w/v) NaOH solutions, small increases and decreases were observed at the temperatures of 80 °C and 120 °C for 30 and 60 min.20



 ${\bf Fig~7}.$ Effect of different alkaline pre-treatment times on bioethanol yield

4. Conclusion

In this study, *C. minutissima* was cultivated for bioethanol production, and applications of acid and alkaline pre-treatments were conducted before the fermentation, and effects of solution concentration, pre-treatment time and pre-treatment temperature on bioethanol yield were investigated. When acid pre-treatment was performed at 100 °C with 1 N $_2$ SO $_4$ for 60 minutes, the highest bioethanol concentration was obtained as 18.52% which was almost three times higher than alkaline

pre-treatment. Nowadays, algae are mostly utilized for biodiesel production due to their high lipid content. However, high operational costs lead the investigators to find new production methods or utilize algal biomass completely with biorefinery aspects. Algae have considerable carbohydrate content that cannot be ignored. Bioethanol production from algae is a technology ongoing in the last decade and open to development. Nevertheless, innovative and efficient fermentation processes and pre-treatment techniques are still needed to make ethanol production preferable.

5. Acknowledgement

The authors acknowledge financial support from the Yıldız Technical University Scientific Research project (2015-07-04-YL03) provided for this work.

6. References

- 1. D. Özçimen, A. Ersoy-Meriçboyu, *Fuel Process. Technol.* **2008**, *89*(*11*), 1041–1046. **DOI:**10.1016/j.fuproc.2008.04.006
- A. Cesaro, V. Belgiorno, *Energ*, 2015, 8(8), 8121–8144.
 DOI:10.3390/en8088121
- 3. J. Yu, T. Tan, *Fuel Process. Technol.* **2008**, *89*(*11*), 1056–1059. **DOI**:10.1016/j.fuproc.2008.04.008
- 4. M. Vohra, J. Manwar, R. Manmode, S. Padgilwar, S. Patil, *J. Environ. Chem. Eng.* **2014**, *2*(1), 573–584.
 - DOI:10.1016/j.jece.2013.10.013
- R. Bibi, Z. Ahmad, M. Imran, S. Hussain, A. Ditta, S. Mahmood, A. Khalid, Renewable Sustainable Energy Rev., 2017, 71, 976–985. DOI:10.1016/j.rser.2016.12.126
- 6. J. M. M. Adams, G. Bleathman, D. Thomas, J.A. Gallagher, *J. Appl. Phycol*, **2017**, 1–7.
- R. P. John, G. S. Anisha, K. M. Nampoothiri, A.Pandey, *Bioresour. Technol.* 2011, 102(1): 186–193.
 - DOI:10.1016/j.biortech.2010.06.139
- 8. J. Zhao, L. Xia, Fuel Process. Technol. **2010**, 91(12), 1807–1811. **DOI:**10.1016/j.fuproc.2010.08.002
- J. Hill, E. Nelson, D. Tilman, S. Polasky, D. Tiffany, *Proc. Natl. Acad. Sci.* 2006, 103(30): 11206–11210.
 DOI:10.1073/pnas.0604600103
- 10. BP Statistical Review of World Energy June 2016, https://www.bp.com/content/dam/bp/pdf/energy-economics/statis-

- tical-review-2016/bp-statistical-review-of-world-energy-2016-full-report.pdf, (assessed: February 01, 2017)
- 11. F. Widdel, Theory and measurement of bacterial growth. Di Dalam Grundpraktikum Mikrobiologie, **2007**, 1–11. Retrieved from https://www.mpi-bremen.de/Binaries/Binary13037/Wachstumsversuch.pdf, (assessed: February 15, 2017)
- 12. M. Kawaroe, J. Hwangbo, D. Augustine, H. A. Putra, Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux), **2015**, *8*(*5*).
- 13. D. K. Lim, S. Garg, M. Timmins, E.S. Zhang, S.R. Thomas-Hall, H. Schuhmann, P.M. Schenk, PLoS One, **2012**, *7*(*7*), 1–13 **DOI**:10.1371/journal.pone.0040751
- 14. J. A. Vieira Costa, M. G. de Morais, in A. Pandey, D. Lee, Y. Chisti, and C. Rsocol (Ed.): Biofuels From Algae, Elsevier, USA. **2014**, pp.1–22.
- 15. T. Aysu, M. M. Küçük, *Energ.* **2014**, *64*, 1002–1025. **DOI:**10.1016/j.energy.2013.11.053
- 16. G. Y. Elbeyli, PhD Thesis, Yıldız Technical University, **2005,** Istanbul, Turkey.
- J. W. Lee, J. Y. Kim, H. M. Jang, M. W. Lee, J. M. Park, *Bioresour. Technol.* 2015, 182: 296–301
 DOI:10.1016/j.biortech.2015.01.116
- M. G. Borines, R. L. de Leon, J. L. Cuello, *Bioresour. Technol.* 2013, 138: 22–29. DOI:10.1016/j.biortech.2013.03.108
- F. C. Wu, J. Y. Wu, Y. J. Liao, M. Y. Wang, L. Shih, *Bioresour. Technol.* 2014, *156*: 126–131
 DOI:10.1016/j.biortech.2014.01.024
- R. Harun, W. S. Y. Jason, T. Cherrington, M. K. Danquah, *Appl Energy.* 2011, 88, 3464–3467.
 DOI:10.1016/j.apenergy.2010.10.048
- Inan, B, Master's Thesis. Yıldız Technical University, 2014, Istanbul, Turkey.
- 22. R. Harun, M. K. Danquah, *Process Biochem.*, **2011**, 46(1): 304–309. **DOI:**10.1016/j.procbio.2010.08.027
- P. L. Hargreaves, C. A. Barcelos, A. C. Augusto da Costa, N. Pereira Jr., *Bioresour. Technol.* 2013, 134: 257–263.
 DOI:10.1016/j.biortech.2013.02.002
- H. Noureddini, J. Byun, Bioresour. Technol. 2010, 101(3): 1060–1067. DOI:10.1016/j.biortech.2009.08.094
- J. Lee, P. Li, J. Lee, H. J. Ryu, K. K. Oha, *Bioresour. Technol.* 2013, 127:119–125. DOI:10.1016/j.biortech.2012.09.122
- S. Zu, W. Li, M. Zhang, Z. Li, Z. Wang, H. Jameel, H. Chang, *Bioresour. Technol.* 2014, 152: 364–370.
 DOI:10.1016/j.biortech.2013.11.034

Povzetek

Zaradi okolju prijazne tehnologije postaja v zadnjih letih po vsem svetu vse bolj popularna proizvodnja bioetanola iz algne biomase. Žal so izkoristki v primerjavi z drugimi tradicionalnimi surovinami slabi, zato jih je potrebno izboljšati. Podobno kot pri prvi in drugi generaciji surovin za proizvodnjo bioetanola se izvajajo različne vrste predpriprav algne biomase. Na tem področju raziskovalci proučujejo primerno metodo in ustrezne parametre za visoke izkoristke. V predstavljeni raziskavi je bila za optimiranje predpriprave uporabljena biomasa alge *Chlorella minutissima* v kislem in bazičnem mediju. Proučevani so bili vplivi koncentracije kemikalij, temperature in časa na izkoristek proizvodnje etanola. Rezultati so pokazali, da je bil najvišji izkoristek dosežen s predpripravo v kislem mediju.