An Optimization Study of Lipid Extraction from *Chlorella minutissima* for Biodiesel Production

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Abstract: Nowadays, researches on microalgal biodiesel production are focused on to improve the process efficiency and reduce the operational costs. One of the most expensive steps in microalgal biodiesel production is lipid extraction of microalgae. In this study, *C. minutissima* microalgae was produced in photobioreactor, and then solvent extraction of microalgal lipids were investigated. Box-Behnken design (BBD) was used to study the effect of extraction temperature, solvent/biomass ratio and residence time of solvent on the oil yield and to optimize lipid extraction. The maximum extraction yield was found to be 75% under the conditions of at the temperature of 70°C, 30:1 solvent/biomass ratio and 8 h. This study showed that the most effective parameter on extraction yield was temperature among three parameters. The results showed that optimized process conditions improved the extraction yield and it is a cost-effective way to produce biodiesel efficiently.

Keywords: *Chlorella minutissima,* microalgae, biodiesel, lipid, extraction.

1. INTRODUCTION

People have begun to look for economic, renewable and environmentally friendly resources and processes, due to the continuous growth of the global population and the fact that the world's resources can no longer handle this growth. In this context, microalgal biomass is one of the sources with the greatest potential. Microalgae are photosynthetic microorganisms which are easy to grow and can be used in many different fields such as food, energy and cosmetics with biorefinery approach [1,2]. Some microalgae species produce valuable compounds to adapt to ambient conditions and are able to withstand very extreme stress conditions. These compounds are extracted from microalgae and used in important industrial fields such as pharmaceuticals, cosmetics and supplementary foods [3]. In addition to that, microalgae are used in biodiesel production because of the high oil content in them. When the literature research on microalgae is assessed, it is seen that the most commonly used microalgae is *Chlorella* species.

Chlorella minutissima is a microalgae that grows in fresh water and is a photosynthetic microorganism with a diameter of 2-10 µm [4]. This type of microalgae has a significant potential in biodiesel production since it contains high amount of lipids. In addition to this, it is a very suitable microorganism for large scale production due to its high temperature tolerance, shear stress resistance and low adhesion to the bioreactor surface [5]. For these reasons, the extraction of structures such as lipids found in *Chlorella minutissima* microalgae has a potential to be evaluated in biodiesel production.

The most commonly used methods in oil extraction from microalgae are solvent extraction method, expeller pressing, supercritical fluid extraction method and ultrasound extraction [6, 7]. Mechanical extraction methods such as expeller pressing are very effective methods in large scale production, but they are very costly to install and operate [8]. Supercritical fluid extraction method provides a very high purity and concentration of lipids, but, similar to mechanical extraction methods, the installation and operating costs of supercritical fluid extraction are very high [9].

The solvent extraction is a widely used method in the extraction of microalgal oils. High yields of lipids can be obtained with this conventional method. In addition to the solubility of microalgal oil in solvents, these solvents also cause cell disruption. Thus, the lipid content in the cell dissolves in the solvent and extraction process is achieved in high efficiency [10]. Compared to other methods, the installation and operating costs of this method are very low. However, the long process and the use of harmful solvents are the disadvantages of this method [11]. Furthermore, the solvents to be used and the proportion of these solvents vary depending on the lipid content. Therefore, optimization of process conditions is very important in lipid extraction from microalgae. In this study, lipid extraction from *Chlorella minutissima* microalgae was performed and biodiesel was produced from the obtained lipids. This microalgae specie has been used in experiments because it can be produced easily on a large scale and the lipid content is very high to be utilized in biodiesel production. In this context,

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firstly *Chlorella minutissima* was cultivated in photobioreactors and characterized. Then, the effects of process parameters on extraction efficiency were statistically analyzed using Box-Behnken factorial design. Finally, biodiesel production was carried out from the obtained lipids. Box-Behnken factorial design is a practical technique for optimization and development of experimental processes. By means of this experimental design technique, a second-order response surface model equation is obtained by using dependent and independent variables and the effects of the variables can be interpreted [12]. In this study, three independent variables such as temperature, n-hexane to biomass ratio and residence time of solvent were selected for the investigation of the effects of these variables on extraction efficiency.

2. MATERIAL AND METHODS

2.1. Biomass Cultivation and Characterization

In this study, *Chlorella minutissima* microalgae obtained from Algal Biotechnology Laboratory of Yıldız Technical University, TURKEY, was selected for optimization of extraction and biodiesel production. The cultures were maintained in BG-11 medium which is composed of $NaNO_3$: 1.5 g/L; K_2HPO_4: 0.04 g/L; $MgSO_4 \cdot 7H_2O$: 0.075 g/L; $CaCl_2 \cdot 2H_2O$:0.036 g/L; citric acid:0.006 g/L; ferric ammonium citrate: 0.006 g/L; EDTA (disodium salt):0.001 g/L; Na_2CO_3: 0.02 g/L and 1 mL trace element solution (composition: H_3BO_3: 2.86 g/L; $MnCl_2.4H_2O$:1.81 g/L; $ZnSO_4.7H_2O$:0.222 g/L; $NaMoO_4.2H_2O$:0.39 g/L; $CuSO_4.5H_2O$:0.079 g/L; $Co(NO_3)2 \cdot 6H_2O$:0.0494 g/L) at pH 7.0 ± 1. The microalga was grown in 20 L photobioreactor exposed to 24-hour white light fluorescent light illumination (8000 lx), at the temperature of 25 ± 1^0C. After cultivation, microalgae were centrifuged for 10 minutes at 5000 rpm, dried at temperature of 60°C for 18 hours and stored. In order to determine the biochemical content of microalgae, Lowry method, phenol-sulphuric acid method and direct solvent extraction method were used to quantify the protein, total carbohydrate and lipid content, respectively [13,14]. Since Lowry and phenol-sulphuric acid methods are colorimetric methods, UV-visible spectrophotometer (PG Instruments, T60) was used for determination of the protein and total carbohydrate content. Bovine Serume Albumin and glucose solutions were used as standard in protein and carbohydrate analyzes, respectively. All chemicals used for microalgae cultivation and biochemical characterization experiments were provided from Merck Millipore (Darmstadt, Germany).

2.2. Lipid Extraction and Statistical Analysis

C. minutissima microalgae biomass was ground and pestle to obtain it in the form of powder. n-hexane was chosen as the solvent for the lipid extraction of the dried *C. minutissima* microalgae. In the extraction processes, two grams of dried and ground *C. minutissima* were used. Using Eq. (1), the lipid percentage in the initial sample was calculated with the following equation [15]:

$$Lipid\ yield = \left(\frac{M_{lipid}}{M_{biomass}} \right) \times 100 \quad (1)$$

In this study, Box-Behnken design (BBD) was used to observe the effects and interactions of three numerical factors. BBD consisting of 15 experimental runs was implemented in the Design-Expert® Software Version 10 (Stat-Ease Inc., Minneapolis, MN, USA). Table **1** shows the experimental design, run order, and the observed response (microalgae lipid yield) were randomized to minimize the effects of three factors and 15 treatments run generated [15]. The experimental variables consisting of extraction temperature (°C, X_1), n-hexane to biomass ratio (v/w, X_2), and extraction time (h, X_3) and their levels were given as:

X_1: 70°C (superior level), 50°C (central level), 30°C (inferior level);

X_2: 30/1 (superior level), 20/1 (central level), 10/1 (inferior level);

X_3: 10 h (superior level), 8 h (central level), 6 h (inferior level).

X_1, X_2 and X_3 are the normalized values of the experimental variables in the regression equation.

2.3. Biodiesel Production

Transesterification reaction was carried out under the conditions of 9:1 methanol:microalgal oil mole ratio in the presence of 1.5 wt.% KOH catalyst in oil content in a 250 mL beaker at the temperature of 60°C. After the transesterification reaction, two phased mixture that was composed of methyl ester-glycerine, was cooled in an ice bath. Then, in order to separate glycerin and methyl ester, centrifugation was performed at 5000 rpm for 5 min. Gas chromatography device was used to determine the content of microalgae oil. Analyses were performed with YL Instruments 6100 GC gas chromatography device. The gas chromatography device contains a flame ionization detector and a 30 m

× 0.32 mm × 0.25 µm ZB-FFAP column. The column temperature program starts at 50°C and reaches to 175°C at 15°C /min, then temperature increases to 230°C at 5°C /min. The injector temperature was 230°C and the flow rate was set at 1.8 mL/min. The detector temperature was kept at 280°C. Hydrogen gas was used as the carrier gas. Methyl heptadeconate (C17: 0) was used as internal standard and the samples were made ready for gas chromatography by mixing with methyl heptadeconate and n-heptane [16].

3. RESULTS AND DISCUSSIONS

3.1. Microalgal Growth

Growth of *C. minutissima* was monitored by optical density measurement (Figure **1**). It was seen that microalgae grew for 20 days, and then the number of microalgal cells started to decrease by entering the death phase. Specific growth rate was calculated as 0.11 day^{-1} and the doubling time of the microalgal cells was calculated as 6,3 days in photobioreactor environment. Although there are studies in which microalgae have less doubling time than 6 days, it is a remarkable growth because the aeration may overwhelm stress in photobioreactor environment and microalgae may need more time to multiply [17]. According to the biochemical analysis, carbohydrate and protein content of *C. minutissima* was found to be 32% and 22.52%, respectively. In the literature, similar findings on biochemical content of *C. minutissima* were reported. Burcu *et al*. stated that, *C. minutissima* cultured in photobioreactor has 33.05% and 24.69% carbohydrate and protein content, respectively [17]. Margarites and Costa studied nitrogen stress on biochemical composition of *C. minutissima,* and it was reported that on the case of using BG-11, carbohydrate content was in the range of 15-54% of the dry biomass

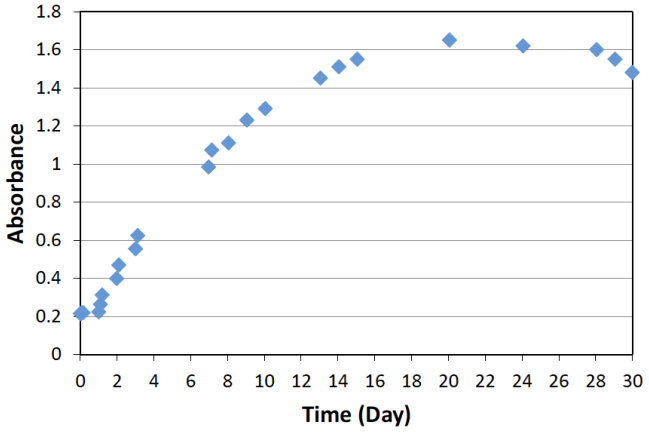

Figure 1: Growth curve of *C. minutissima.*

[18]. Illman *et al*. studied effect of nutrient stress on biochemical composition of various *Chlorella* strains. It was reported that, among *Chlorella* strains, control group of *C. minutissima* has 24% and 42% protein and carbohydrate content, respectively [19].

3.2. Statistical Analysis of Lipid Extraction Experiments

In this study, BBD was used to provide information regarding the interior of the experiment region and it was utilized to observe the effects and interactions of the extraction temperature, solvent:biomass ratio and extraction time on the lipid yield. In Table **1**, experimental results of extraction of *C. minutissima* oil were presented. Regression equation was obtained to evaluate the lipid yield and to understand the effects of experimental variables on the yield. The design matrix was examined statistically to define and measure the main effects quantitatively by using the analysis of variance (ANOVA) technique. For an approximate calculation of the lipid yield values of *C. minutissima* with this statistical analysis, regression Eq. (1) was developed:

$$Y_{lipid\ (\%)} = +51.67+15.42X_1+5.10X_2+2.19X_3-0.4150X_1X_2-0.2075X_2X_3+4.89X_1^2-1.56X_2^2+0.9374\ X_3^2 \quad (1)$$

The coefficient of determination for regression Eq. (1) was found as 0.99. According to the regression equation, it was found that the coefficient of temperature was the highest among all the variables, and therefore its effect is the strongest. The reaction time and solvent/biomass ratio also affected the lipid yield positively. The interactive effect of X_1X_2 (temperature and solvent/biomass ratio) and X_2X_3 (solvent/biomass ratio and extraction time) had small coefficient values. X_1X_3 (temperature and extraction time) has no effect on extraction yield.

The ANOVA method compares the variances within and between the groups. The reliability of the model is confirmed with this technique. ANOVA parameters for the model equation are presented in Table **2**. A very low probability value ($p < 0.01\%$) implies that the model is significant. Moreover, a high value of correlation coefficient shows a close agreement between predicted value and actual value of response. The determination coefficient (R^2) controls the suitability of the model. As the value of R approaches 1, it demonstrates better correlation between observed and predicted values [20]. The determination coefficient value was determined as R^2 = 0.99 and it represented that only

Table 1: Experimental Conditions and Lipid Yield of *C. minutissima* Microalgae

Run	X_1 Temperature (°C)	X_2 Solvent/biomass ratio	X_3 Reaction time (h)	Y Lipid yield (%)
1	-1	-1	0	34.17
2	1	-1	0	65.83
3	-1	1	0	45.00
4	1	1	0	75.00
5	-1	0	-1	40.83
6	1	0	-1	71.67
7	-1	0	1	43.33
8	1	0	1	74.17
9	0	-1	-1	42.50
10	0	1	-1	53.33
11	0	-1	1	49.17
12	0	1	1	59.17
13	0	0	0	51.67
14	0	0	0	52.50
15	0	0	0	50.83

Table 2: ANOVA Results of Statistical Evaluation for the Extraction Yield

Sources of variations	Degree of freedom	Sum of squares	Mean square	F-value	Probability
Regression model	9	2253.43	250.38	146.85	0.0001
Error	5	8.53	1.71		
Corrected total	14	2261.95			

about 1% of the total variation was not explained by the respective model. Besides that, the value of the adjusted determination coefficient was found very high as adj. $R^2 = 0.98$.

3.3. The Effect of Temperature on the Extraction Yield

In this study, effect of temperature on extraction yield was investigated by evaluating three different temperature values. It was seen that the lipid extraction yield increased with increasing temperature. The highest lipid yield was obtained under the conditions of 70°C, 30/1 solvent:biomass ratio and 8 h. However, the parameters that affected the extraction yield had synergic effects. Because of this reason, effect of temperature is usually evaluated with other parameters such as particle size, solvent type and extraction time. Mani *et al*. studied the extraction of moringa seed kernel oil using response surface method. According to their results, the oil yield decreases, when the particle size and extraction temperature increases. The reason for this was using larger particle size samples which have more resistance to penetrate the solvents into the samples, and caused low oil yield in the experiments. On the contrary, as extraction temperature increases, the diffusivities of the solute (oil) and solvent increases, resulting in high oil yield. However, since the density of the solvent is very low at higher extraction temperature, the oil yield decreased. Because of this, it was reported that heating of solvents above their boiling points did not improve the oil yield. In addition to that, it was stated that the oil yield increased as the extraction time increased, but the combined effect of particle size and extraction temperature on oil yield was higher than that of extraction time [21].

Similar to our results, Bhutada *et al*. reported that the extraction yield of the Moringa seed oil increased with the increasing temperature due to the increase of solubility and diffusivity which improves the mass transfer [22].

Suganya and Renganathan also investigated the effect of temperature on the extraction yield. According to their results, lipid extraction yields of *Ulva lactuca* increased when the temperature increased from 35°C to 55°C. The highest lipid extraction yield was obtained as 9.75% at the temperature of 55°C. It was reported that the solubility of the solvent was found to be increased with the increase in diffusion rate [23].

Although higher temperature improves the dissolution capacity of solvents as well as the solubility and diffusion rate of solids, higher extraction temperatures cause higher operation costs [24].

Silitonga *et al*. studied the optimization of extraction of lipid from *Isochrysis galbana* microalgae by evaluating the process parameters. The effect of temperature on the extraction of microalgal lipid yield was investigated in the range 50–70°C. It was found that the temperature increase improves lipid yield until optimum however, further increase was found to enhance the capacity of solvents to dissolve the lipid [15].

3.4. The Effect of Solvent/Biomass Ratio on the Extraction Yield

Effect of solvent/biomass ratio is another important parameter that affects the oil extraction yield. Sayyar *et al*. investigated the process parameters such as solvent type, temperature, solvent/biomass ratio, extraction time and particle size on jatropha oil extraction yield. It was found that the total amount of extracted oil using hexane increased from 40.0-47.3% by increasing solvent/biomass ratio from 4:1-6:1 (v/w). It was reported that increasing solvent/biomass ratio up to a specific limit will increase the extraction yield since the concentration gradient between the solid and the liquid phase becomes greater which favors good mass transfer [25].

Baboli and Kordi also indicated that the oil extraction yield was improved by increasing the extraction time, extraction temperature and solvent/biomass ratio [26]. Similar results were obtained by Meziane and Kadi. It was reported that, increasing the solvent/biomass ratio has increased olive oil extraction yield which was expected since the concentration was driving force in the transfer mechanism between the solvent and biomass in the process [27].

Suganya and Renganathan also studied the influence of solvent-to-solid ratio from 3:1 to 7:1 on oil extraction. It was reported that, as the solvent-to-solid ratio increased from 3:1 to 6:1, the oil yield increased from 9% to 10.88%. Yet, there was not any remarkable change in the extraction yield above 6:1 solvent/biomass ratio [23]. Silitonga *et al*. also noticed that increasing solvent/biomass ratio increased the extraction yield up to an optimum condition. It was found that increasing the n-hexane to lipid molar ratio from 5:1 to 8:1 caused an increase in the oil yield in the range of 5%–8.36%. The tendency for the interaction of n-hexane to lipid molar ratio was continuing up to 6.7:1, but after that there was not any increase in the extraction yield [15].

In this study, it was observed that extraction yield increased with the increase in solvent/biomass ratio. The highest extraction yield was obtained as %75 under the conditions of at the temperature of 70°C, 30:1 solvent/biomass ratio and 8 h. Obtained results are in agreement with the literature studies.

3.5. The Effect of Extraction Time on the Extraction Yield

Extraction time is a significant parameter for oil extraction efficiency. Determining the optimum extraction time contributes in controlling the optimum interval time for the extraction process. In this study, it was found that the extraction time was determined as the least affecting parameter for oil extraction. The highest extraction yield was determined under the conditions of 8 h reaction time. However, after 8 h there was not any significant improvement in the extraction yield. Similar results were also obtained with the literature studies. According to Silitonga *et al*., the lipid yield increased and changed from 360 min to 496 min. On the other hand, after 496 min, a decrease was observed in the yield reversibly. It was reported that this parameter would influence the chemical reaction due to the rapid mass transfer between the solvent and the lipid into the oil phase [15].

Sayyar *et al*. also indicated that, after optimum reaction time, there was not any change in the extraction yield. It was found that the amount of extracted oil by hexane and petroleum ether did not change significantly after 6 h. Most of the oil is extracted after 6 h although maximum extracted oil is achieved after 8 h with 47.3% and 46.0% for hexane and petroleum ether, respectively [25].

Sulaiman *et al*. investigated extraction of oil from coconut waste using batch and soxhlet extractor and determined the optimum conditions and modelled the

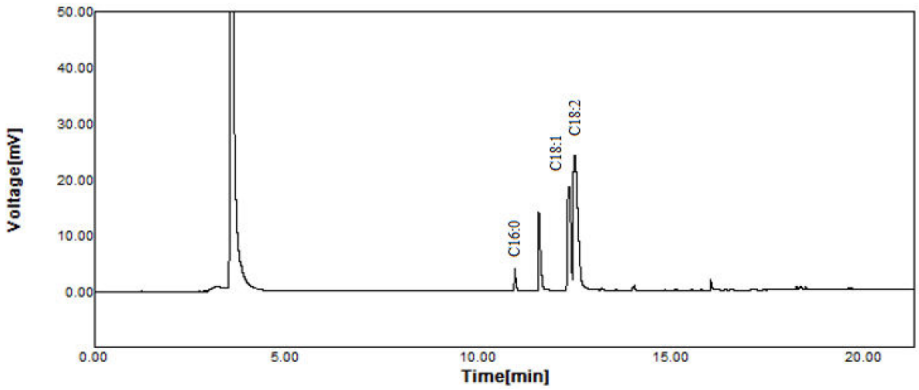

Figure 2: Gas chromatography (GC) chromatogram of fatty acid methyl ester (FAME) sample produced under the conditions of 9:1 methanol/oil molar ratio and 1.5 wt. % KOH.

extraction process. It was stated that the extraction rate was rapid at the beginning of the process and gradually slowed down because when the waste was exposed to the fresh solvent, the free oil on the surface of the waste was solubilized and oil was extracted quickly. This caused fast increase in extraction rate. At the initial extraction rate, the oil concentration was low in the solvent and mass transfer effect caused the oil to diffuse quickly from the waste to the solvent. When the maximum amount of extractable oil was reached, the oil yield remained the same even after extending the extraction time [28].

3.6. Biodiesel Production and Characterization

After the determination of optimum conditions for lipid extraction, algal oil was converted to biodiesel via transesterification reaction. Biodiesel quality depends on the composition of the fatty acid methyl ester profile of the product. A favorable fatty acid profile is necessary for high-quality biodiesel [29]. In order to meet the fuel properties for high quality biodiesel, it is required for the algal oil to have small amounts of polyunsaturated and saturated fatty acids. Song *et al*. [30] indicated that palmitic, stearic, oleic, linoleic, and linolenic acids are the most favorable for biodiesel production.

In this study, GC analysis (Figure **2**) showed that there are three main fatty acids: Palmitic, oleic, and linoleic which are shown in Figure **2**. Other fatty acids, such as C16:1, C18:0, C20:3, and C20:5, were found as trace amounts and could not be determined by the results. The highest amount of fatty acid methyl ester determined in algal biodiesel was linoleic acid (C18 = 2). According to the results, it can be said that, *C. minutissima* oil can be evaluated for biodiesel production.

4. CONCLUSION

In this study, the extraction of *C. minutissima* microalgae was performed and modelled by applying Box-Behnken experimental design. The parameters that affect the extraction yield, such as temperature, solvent/biomass ratio and reaction time were also studied. The optimum parameters were as follows: solvent/biomass ratio of 30:1, reaction temperature of 70°C, and reaction time of 8 h using BBD. The results showed that the maximum yield obtained from *C. minutissima* was 75%. After determining the extraction conditions, biodiesel production was carried out from microalgal oil. According to the FAME profile, it was found that high quality biodiesel can be obtained from *C. minutissima* microalgae. Although algal biofuel production is quite expensive, significant progress has been made with studies of optimizing the production steps to reduce the operational cost. Extraction is one of the most important steps for algal biodiesel production. Even tough there are novel methods applied in laboratory for extraction process, it is significant to optimize the parameters for conventional methods that are used in large scale operations. Moreover, effect of other parameters on microalgal oil extraction yield such as particle size and solvent type should be also explored.

REFERENCES

[1] Özçimen D, İnan B, Koçer AT, Vehapi M. Bioeconomic Assessment of Microalgal Production. Microalgal Biotechnol 2018. https://doi.org/10.5772/intechopen.73702

[2] Koçer AT, Özçimen D. Investigation of the biogas production potential from algal wastes. Waste Manag Res 2018; 36: 1100-5. https://doi.org/10.1177/0734242X18798447

[3] Markou G, Nerantzis E. Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnol Adv 2013; 31: 1532-42. https://doi.org/10.1016/j.biotechadv.2013.07.011

[4] Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae: A review. Biotechnol Adv 2012; 30: 709-32. https://doi.org/10.1016/j.biotechadv.2012.01.001

[5] Doucha J, Lívanský K. Outdoor open thin-layer microalgal photobioreactor: Potential productivity. J Appl Phycol 2009; 21: 111-7. https://doi.org/10.1007/s10811-008-9336-2

[6] Hurtado DX, Garzón-Castro CL, Cortés-Romero J, Camacho ET. Comparison of lipid extraction methods for the microalgae Acutodesmus obliquus. Int J Agric Biol Eng 2018; 11: 211-7. https://doi.org/10.25165/j.ijabe.20181105.3748

[7] Kumar RR, Rao PH, Arumugam M. Lipid extraction methods from microalgae: A comprehensive review. Front Energy Res 2015; 3. https://doi.org/10.3389/fenrg.2014.00061

[8] Lee AK, Lewis DM, Ashman PJ. Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. Biomass and Bioenergy 2012; 46: 89-101. https://doi.org/10.1016/j.biombioe.2012.06.034

[9] Minyak P, Daripada A, Air M, Chlorella T. Algae oil extraction from freshwater microalgae Chlorella vulgaris. Malaysian J Anal Sci 2017; 21: 735-44. https://doi.org/10.17576/mjas-2017-2103-23

[10] Sati H, Mitra M, Mishra S, Baredar P. Microalgal lipid extraction strategies for biodiesel production: A review. Algal Res 2019; 38. https://doi.org/10.1016/j.algal.2019.101413

[11] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energy Rev 2010; 14: 217-32. https://doi.org/10.1016/j.rser.2009.07.020

[12] Koçer AT, Mutlu B, Özçimen D. Investigation of biochar production potential and pyrolysis kinetics characteristics of microalgal biomass. Biomass Convers Biorefinery 2019. https://doi.org/10.1007/s13399-019-00411-7

[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951. https://doi.org/10.1016/0304-3894(92)87011-4

[14] Dubois M, Gilles K, Hamilton JK, Rerbers P, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 28: 350-6. https://doi.org/10.1021/ac60111a017

[15] Silitonga AS, Masjuki HH, Ong HC, Mahlia TMI, Kusumo F. Optimization of extraction of lipid from Isochrysis galbana microalgae species for biodiesel synthesis. Energy Sources, Part A Recover Util Environ Eff 2017; 39: 1167-75. https://doi.org/10.1080/15567036.2017.1310957

[16] Karakaş CY, Şahin HT, İnan B, Özçimen D, Erginer YÖ. In-vitro cytotoxic activity of microalgal extracts loaded nano-micro particles produced via electrospraying and microemulsion methods. Biotechnol Prog 2019: e2876. https://doi.org/10.1002/btpr.2876

[17] Sert BS, Inan B, Özçimen D. Effect of chemical pre-treatments on bioethanol production from Chlorella minutissima. Acta Chim Slov 2018; 65: 160-5. https://doi.org/10.17344/acsi.2017.3728

[18] Margarites ACF, Costa JAV. Increment of carbohydrate concentration of Chlorella minutissima microalgae for bioethanol production. J Eng Res Appl WwwIjeraCom ISSN 2014; 4: 2248-962280.

[19] Illman AM, Scragg AH, Shales SW. Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme Microb Technol 2000; 27: 631-5. https://doi.org/10.1016/S0141-0229(00)00266-0

[20] Feng X, Walker TH, Bridges WC, Thornton C, Gopalakrishnan K. Biomass and lipid production of Chlorella protothecoides under heterotrophic cultivation on a mixed waste substrate of brewer fermentation and crude glycerol. Bioresour Technol 2014; 166: 17-23. https://doi.org/10.1016/j.biortech.2014.03.120

[21] Mani S, Jaya S, Vadivambal R. Optimization of solvent extraction of moringa (Moringa Oleifera) seed kernel oil using response surface methodology. Food Bioprod Process 2007; 85: 328-35. https://doi.org/10.1205/fbp07075

[22] Bhutada PR, Jadhav AJ, Pinjari D V., Nemade PR, Jain RD. Solvent assisted extraction of oil from Moringa oleifera Lam. seeds. Ind Crops Prod 2016; 82: 74-80. https://doi.org/10.1016/j.indcrop.2015.12.004

[23] Suganya T, Renganathan S. Optimization and kinetic studies on algal oil extraction from marine macroalgae Ulva lactuca. Bioresour Technol 2012; 107: 319-26. https://doi.org/10.1016/j.biortech.2011.12.045

[24] Jeong GT, Park DH. Optimization of lipid extraction from marine green macro-algae as biofuel resources. Korean J Chem Eng 2015; 32: 2463-7. https://doi.org/10.1007/s11814-015-0083-1

[25] Sayyar S, Abidin ZZ, Yunus R, Muhammad A. Extraction of oil from Jatropha seeds-optimization and kinetics. Am J Appl Sci 2009; 6: 1390-5. https://doi.org/10.3844/ajassp.2009.1390.1395

[26] Baboli ZM, Safe Kordi AA. Characteristics and composition of watermelon seed oil and solvent extraction parameters effects. JAOCS, J Am Oil Chem Soc 2010; 87: 667-71. https://doi.org/10.1007/s11746-010-1546-5

[27] Meziane S, Kadi H. Kinetics and thermodynamics of oil extraction from olive cake. JAOCS, J Am Oil Chem Soc 2008; 85: 391-6. https://doi.org/10.1007/s11746-008-1205-2

[28] Sulaiman S, Abdul Aziz AR, Aroua MK. Optimization and modeling of extraction of solid coconut waste oil. J Food Eng 2013; 114: 228-34. https://doi.org/10.1016/j.jfoodeng.2012.08.025

[29] Talebi AF, Mohtashami SK, Tabatabaei M, Tohidfar M, Bagheri A, Zeinalabedini M, *et al*. Fatty acids profiling: A selective criterion for screening microalgae strains for biodiesel production. Algal Res 2013; 2: 258-67. https://doi.org/10.1016/j.algal.2013.04.003

[30] Song M, Pei H, Hu W, Ma G. Evaluation of the potential of 10 microalgal strains for biodiesel production. Bioresour Technol 2013; 141: 245-51. https://doi.org/10.1016/j.biortech.2013.02.024

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