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Effect of Salinity on *Chlorella vulgaris* for Increasing Lipid Content

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Abstract

Microalgae growth with effluent from the frozen seafood wastewater treatment plant can provide some benefits such as produce high biomass and lipid for biodiesel production. *Chlorella vulgaris* was considered as the best feedstock of energy to produce biodiesel. The objective of this study was to increase lipid content under salinity stress. There were three reactors including R1, R2 and R3 for cultivating microalgae. The R1 used the effluent of seafood processing wastewater treatment plant without adding the NaCl. The R2 and R3 were added with the NaCl for increasing the salinity. The various NaCl concentrations of R1, R2 and R3 were 1.34 g/L (0.023 M of NaCl), 2.9 g/L (0.050 M of NaCl) and 4.4 g/L (0.075 M of NaCl), respectively. In this study, the *Chlorella vulgaris* growth in R1 and R2 was reached the maximum of dry cell weight (DCW) about 1.02 g/L and 1.16 g/L on Day-3, respectively. While the *Chlorella vulgaris* growth in R3 was reached the maximum of DCW about 1.47 g/L on Day-4. The total lipid content of *Chlorella vulgaris* was increased in different concentration of salinity. The total lipid content in R2 was lower than in R3, of which R2 and R3 contained 1.84% and 3.09% of lipid content, respectively. However, both reactors were lower than the lipid content of R1 which was 4.60% of lipid content. It could be concluded that the lipid content in *Chlorella vulgaris* strain was enhanced slightly between various concentrations of salinity. Therefore, the effluent from frozen seafood industry was suitable for growth *Chlorella vulgaris* without adding NaCl. The salinity content in the effluent from frozen seafood industry (0.023 M of NaCl) was enough for microalgae growth and the nutrient contained in the effluent, was also removed from microalgae cultivation.

Keywords : Microalgae growth; *Chlorella vulgaris*; Lipid; Salinity

Introduction

The high requirement of energy in the world is the crucial crisis faced nowadays, and fossil fuel is gradually decreased. Biomass is the sustainable energy source which can be utilized and substituted the carbon from fossil fuel source to produce the production of carbon-base. The production of carbon-base includes chemicals, raw materials and liquid fuels [1]. The consumption of fossil fuel causes many problems such as energy requirement, economic issue, rising of fuel utilization and fuel price, and the releasing of pollution gases [2].

Moreover, the fossil fuel burning induces to rise the greenhouse gas releasing and the environmental issue to the earth [3]. Therefore, the effect of fossil fuel burning has been seriously concerned nowadays [4]. Thus, the bioenergy has been interested for energy production. Microalgae are considered as the best feedstock for converting to biodiesel production [5]. There are many advantages of microalgae bioenergy which produce high lipid content, high growth rate, the precious of chemical production, great ability to absorb carbon dioxide and being able to combine with wastewater treatment for energy production [6]. Moreover, the microalgae cultivation does not need large land to grow compared to vegetable cultivation, which is also the renewable source for biodiesel production but requires large area [5, 7]. The microalgae can produce lipid content and biomass productivity which are the most important parameters. They require several limited conditions to increase lipid accumulation such as light, temperature, pH and nutrient [8]. Some researchers reported the lipid increment of microalgae cultivating in different conditions, such as the stress condition induced high lipid content in microalgae cell [2, 9]. Salinity is the one essential stress component for microalgae. It leads to change the metabolic in nutrient absorption, increase toxic ions, create osmose stress, and make oxidative stress [10]. The salinity stress induces to increase lipid productivity is suggested by previous researchers [11, 12]. High salinity helps to balance export and absorb ion pass through the cell membrane. The stress proteins can induct high total lipid content in the cell of microalgae [13]. The seafood processing wastewater contains high organic content which comes from blood, fish heads, intestine and meat residuals [14]. In addition, the microalgae are known that can use organic and inorganic nutrient in wastewater and produce biomass for biofuel production. Meanwhile, the microalgae have ability to uptake nutrient which are phosphorus and nitrogen contained in wastewater. Furthermore, there are few researches that have evaluated the potential of effluent reuse [15].

In this study, the effluent of frozen seafood industry due to high organic contained in effluent and microalgae as *Chlorella vulgaris* strain were utilized for cultivation. Thus, the objective was to study the effect of salinity stress on *Chlorella vulgaris* for increasing lipid content.

Methodology

Microalgae strain and pre-cultivation

The microalgae strain used in this study was *Chlorella vulgaris*. It was obtained from the Coastal Aquaculture Research Institute in Songkhla province, Thailand. In the first stage of cultivation, the *Chlorella vulgaris* strain was cultivated in urea fertilizer which contain urea (0.2 g/L), diammonium phosphate (0.003 g/L), CaO (0.2 g/L) and Glutamic mother liquid (0.8 mL/L). The sample was incubated in 5 L of bottle at the ambient temperature with light intensity at 6,000 Lux. The period of *Chlorella*

vulgaris culture was reached to the stationary phase in batch culture condition and then cell numbers were observed.

Microalgae growth determination

Cell density was determined by measuring optical density (OD) using spectrophotometer at 680 nm. The microalgae were harvested by centrifugation at 5,000 rpm for 10 min. After that, the microalgae were rinsed with distilled water two times to take medium off. Then the purified microalgae were dried in the oven at 105°C until obtaining constant weight and cooled in the desiccator. The linear relationship between OD_{680} and dry cell weight (DCW, g dry weight/L) is followed as equation (1):

$$\text{DCW (g dry weight/L)} = 7.2471 \times OD_{680} \quad R^2 = 0.9996 \qquad (1)$$

Where: DCW_1 and DCW_2 are dry cell weight (g/L) at time t_1 and t_2, respectively. The biomass productivity ($P_{biomass}$, g/L/d) is determined by equation (2):

$$P_{biomass} = \frac{DCW_2 - DCW_1}{t_2 - t_1} \qquad (2)$$

The specific growth rate is defined as the rate of increase of microalgae of a cell population per unit of microalgae concentration. The specific growth rate per day (μ, d^{-1}) is determined by equation (3):

$$\mu = \frac{(\text{Ln } DCW_2 - \text{Ln } DCW_1)}{t_2 - t_1} \qquad (3)$$

Experimental design

The effluent of frozen seafood processing factory was used for microalgae cultivation. Furthermore, the batch experiment was used in the photobioreactor (length 60 cm, diameter 20 cm) and filled with 10 L of effluent into each reactor. There were three reactors such as R1, R2 and R3 for cultivating microalgae. Sodium chloride (NaCl) is shown to increase the lipid content then the NaCl was used to enhance the lipid content. The R1 was used the effluent of seafood processing wastewater treatment plant without adding NaCl. The R2 and R3 were added NaCl for increasing salinity. The various NaCl concentrations of R1, R2 and R3 were 1.34 g/L (0.023 M of NaCl), 2.9 g/L (0.050 M of NaCl) and 4.4 g/L (0.075 M of NaCl), respectively. All reactors were performed in batch culture and conditions of cultivation were as follows: temperature was the ambient temperature; light intensity was 6,000 Lux in a whole day. The microalgae were cultivated until reach the stationary phase. The photobioreactor is used for microalgae cultivation as shown in Figure 1.

Lipid extraction

The lipid of microalgae was extracted from microalgae following the Folch method [16]. The biomass dry weight was homogenized with Chloroform: Methanol (2:1 by volume) in the fume hood. The mixture was put in ultrasonic cleaner for 30 min. The solvent mixture was separated into two phases by centrifugation at 5,000 rpm for 15 min. The upper phase contained all the non-lipid compounds and these compounds were evaporated in the fume hood. The lower phase contained the solid which was contained lipid and taken it by prolonged standing. Thereafter, the lipid was measured gravimetrically. The lipid content was calculated using equation (4).

$$\text{Lipid content (\%)} = \frac{\text{Total extracted lipid (g)}}{\text{Biomass dry weight (g)}} \times 100\% \qquad (4)$$

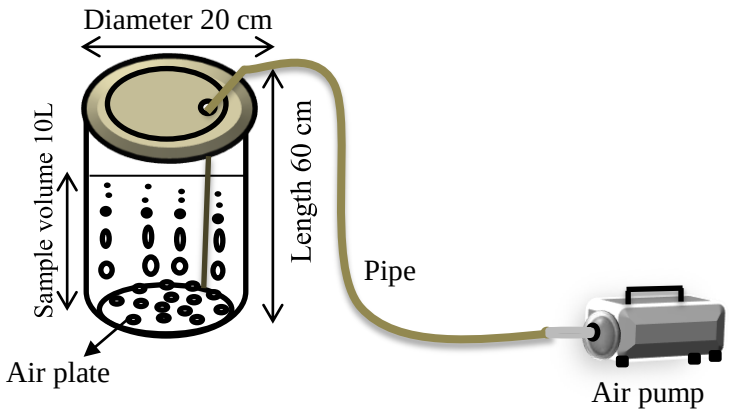

Figure 1 Photobioreactor Used for Microalgae Cultivation

Results and Discussions

Effluent properties before cultivation

In this study, the properties of frozen seafood effluent are shown as nutrients for microalgae growth such as TKN, TP and COD were measured. The turbidity, salinity, TDS and pH were also measured before growing microalgae (Table 1). These parameters were analyzed to recognize the ability of *Chlorella vulgaris* growth of using nutrient in effluent and to compare between the initial and final concentrations of effluent after microalgae cultivation.

Monitoring parameters

The salinity and pH were observed for the changing of their quantity during cultivation time. In this study, the content of salinity in R1 and R2 was decreased steadily until Day-3 and increased on Day-4. However, the R3 was decreased until Day-2 and increased from Day-3 to Day-4. The contents of salinity of all reactors were decreased again at last day of cultivation (Figure 2A). The decreasing of salinity indicated that the microalgae consumed the salinity for their growth. The salinity was the major parameter that provided the development of plant surviving and made the blocking on metabolic activity of photosynthesis [17]. The previous study reported that the salinity could be increased because of the evaporation [18]. The salinity could block the photosynthesis but *Chlorella vulgaris* was able to grow normally because it was cultivated in the suitable concentration of salinity [10].

Table 1 Initial concentration of effluent from frozen seafood factory

TKN (mg/L)	TP (mg/L)	COD (mg/L)	Turbidity (NTU)	Salinity (ppt)	TDS (mg/L)	pH
152.3	8.1	128	8.4	1.6	1,061	7.7

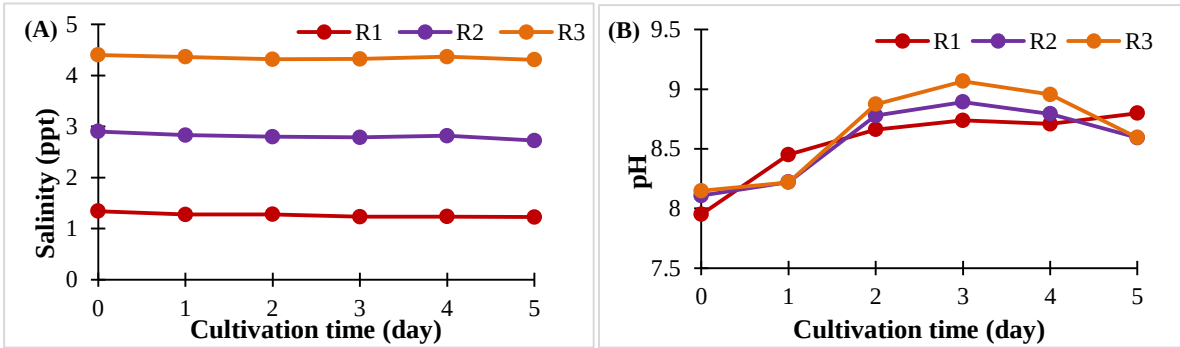

Figure 2 Results of Salinity (A) and pH (B)
(R1=0.023 M of NaCl, R2=0.050 M of NaCl, R3=0.075 M of NaCl)

Moreover, the pH was also changed as its natural in the cultivation reactor. In this study, the pH in all reactors was increased from the first day until Day-3. For three days, the pH of R1, R2, and R3 was increased from 7.95 to 8.74, 8.11 to 8.89 and 8.15 to 9.07, respectively. The previous study reported that pH increased gradually when the microalgae synthesized and consumed CO_2 at daytime [19]. Additionally, the other research described the cell of microalgae excreted hydroxyl ion to the cultivation medium during it used HCO_3 as CO_2. The reducing of HCO_3 led to increase the pH [20]. However, it was demonstrated that the respiration process at night time induces pH decreasing [19].

Microalgae growth

1. Dry cell weight of *Chlorella vulgaris* strain

The *Chlorella vulgaris* strain was cultivated for 5 days in this study. The dry cell weight (DCW) was an important parameter for microalgae growth. The microalgae growth in R1 and R2 was reached the maximum DCW about 1.02 and 1.16 g/L on Day-3, respectively. While the microalgae growth in R3 was reached the maximum DCW about 1.47 g/L on Day-4 (Figure 3). The previous researches reported that *Chlorella vulgaris* growth was decreased in various high concentrations of salinity which the salinity concentrations were 0, 0.26, 0.51 and 0.77 M [21]. However, this study observed that *Chlorella vulgaris* could increase their growth in increasing of salinity at 0.050 and 0.075 M of NaCl. The previous study demonstrated that microalgae were able adapt in the range of salinity at 0.05, 0.15 and 0.20 M [22]. According to the previous study, the range of salinity in this study was grown in the suitable condition of salinity.

2. Biomass productivity of *Chlorella vulgaris* strain

The results showed that the highest biomass productivity of *Chlorella vulgaris* for R1 and R2 was reached on Day-2, whereas R3 was reached on Day-3. The highest biomass productivity of R1, R2 and R3 was 0.395, 0.424 and 0.322 g/L/d, respectively (Figure 4). The R2 (0.050 M of NaCl) provided the highest biomass productivity. The high concentration of NaCl was caused to reduce the microalgae productivity [21].

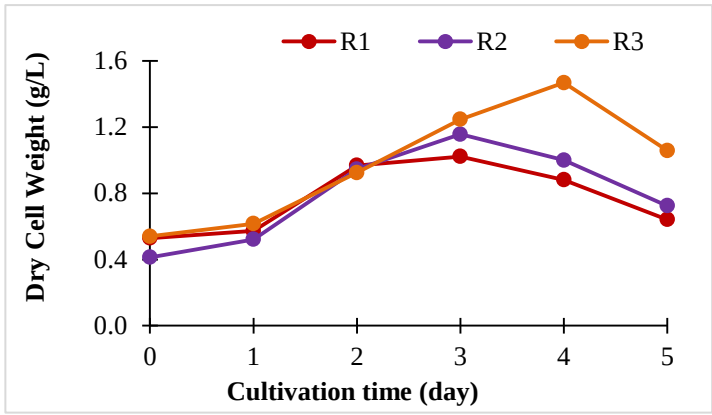

Figure 3 *Chlorella Vulgaris* Growth as Dry Cell Weight
(R1=0.023 M of NaCl, R2=0.050 M of NaCl, R3=0.075 M of NaCl)

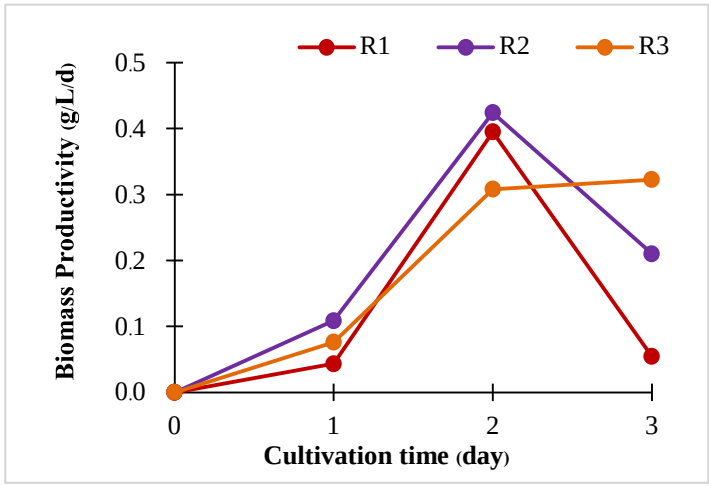

Figure 4 *Chlorella Vulgaris* Growth as Biomass Productivity
(R1=0.023 M of NaCl, R2=0.050 M of NaCl, R3=0.075 M of NaCl)

3. Specific growth rate of *Chlorella vulgaris*

The results showed that the specific growth rate was determined by calculating with dry cell weight curve. Figure 5 shows the specific growth rate in the different salinity during the cultivation time. The maximum specific growth rate was observed on Day-2 for R1, R2 and R3 which was 0.525, 0.595 and 0.405 d^{-1}, respectively. These microalgae growth rates were decreased gradually until last day of cultivation.

Effect of salinity on lipid content

The survival ability of *Chlorella vulgaris* in the diverse and extreme conditions is reflected in the changes of lipid content of these cells. Total lipid content of *Chlorella vulgaris* was increased in different concentrations of salinity. In this study, the total lipid content in R2 (0.050 M of NaCl) was lower than that in R3 (0.075 M of NaCl), of which the R2 and R3 contained 1.84% and 3.09% of lipid content, respectively. However,

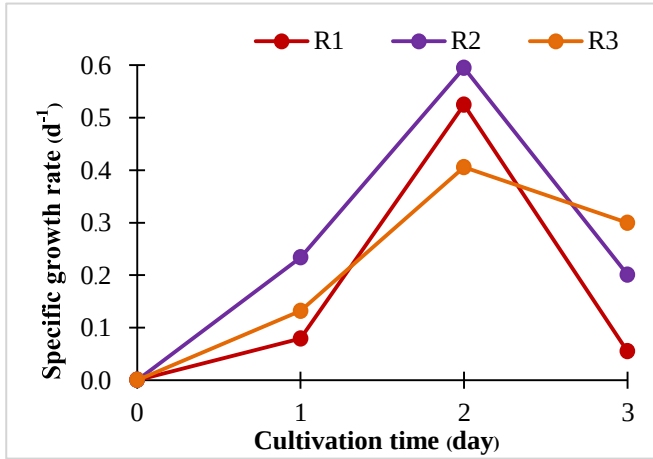

Figure 5 *Chlorella Vulgaris* Growth as Specific Growth Rate (R1=0.023 M of NaCl, R2=0.050 M of NaCl, R3=0.075 M of NaCl)

the lipid content of both reactors was lower than that the lipid content of R1 (0.023 M of NaCl) which was 4.60% of lipid content (Figure 6). Similarly, the previous study showed that the lipid content was increased depending on NaCl concentration, but it was lower than effluent without adding NaCl (R1 – 0.023 M of NaCl) [23]. In addition, the other studies reported that the total lipid content was increased slightly in high content of salinity. The *Chlorella vulgaris* cultivation is almost stopped when the NaCl concentration is 34.0 g/L [7]. Hu et al. [24] discovered that under various kinds of stresses, the photosynthetic membrane of the microalgae was rapidly degraded and TAG-enriched lipids were accumulated in cytosol because of microalgae mechanism to store energy. Moreover, some microalgae can modify their lipid metabolism pathway effectively according to the change in environmental conditions [25]. However, the previous study showed that the increasing salinity concentration was able to accumulate lipid content for *Chorella sp.* which was increased from 1.92 ± 0.012% to 3.49 ± 0.016% [26].

The efficiency of nutrient removal from effluent

The previous studies showed that some types of wastewater were suitable for microalgae to growth. The different microalgae can adapt with different type of wastewater. It is also depended on the characteristic of wastewater conditions. The chlorophyte of microalgae can utilize the various kinds of wastewater and have the capacity to remove nutrient waste from wastewater as well [3]. In this study, *Chlorella vulgaris* was a species of Chlorophyte which was used for growing in the frozen seafood effluent. As mentioned above, the microalgae used nutrient from frozen seafood effluent as their food such as TKN, TP, and COD. These parameters were analyzed after cultivating to know how many percent that microalgae could uptake. The final concentration of each reactor are shown in Table 2, presenting that the microalgae could remove the TKN, TP, and COD in R1 was 51.7%, 24.5% and 25.0%, respectively. While the removal efficiency of TKN, TP, and COD in R2 was 61.5%, 26.0% and 43.8%, respectively. Whereas the removal efficiency of

TKN, TP, and COD in R3 was 48.9%, 23.6% and 31.8%. The removal efficiency of nutrient in each reactor is shown in Table 3. The removal efficiency of nutrient was lower than the previous research which the TKN and TP could be removed in the range of 72% to 85% and 57% to 77%, respectively [27]. The previous research reported that TN and TP could be removed from 11.9% to 74.3% and 22.5% to 94.8%, respectively [28]. Thus, it could be assumed that *Chlorella vulgaris* had highly efficient to remove the nutrient from the effluent of frozen seafood industry. The phytoremediation was the common wastewater treatment by microalgae for pollutant removal such as organic and inorganic [29, 30].

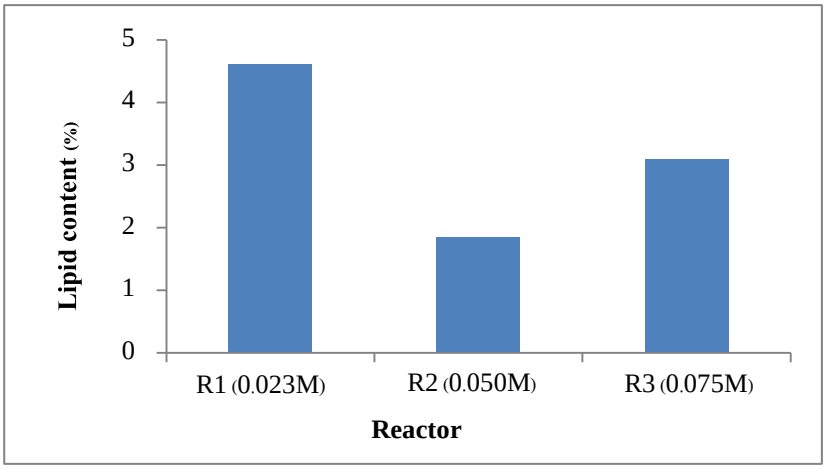

Figure 6 Effect of Salinity on Lipid Content of *Chlorella Vulgaris* Growth

Table 2 Characteristics of effluent after growing microalgae

Reactor	TKN (mg/L)	TP (mg/L)	COD (mg/L)	Turbidity (NTU)	Salinity (ppt)	TDS (mg/L)	pH
R1 (0.023M of NaCl)	73.6	6.1	96.0	3.7	1.2	970	8.97
R2 (0.050M of NaCl)	58.7	6.0	72.0	3.4	2.8	2,079	8.46
R3 (0.075M of NaCl)	78.1	6.2	87.2	4.2	4.4	3,803	8.94

Table 3 Nutrient removal efficiency of effluent after growing microalgae

Nutrient removal efficiency (%)	Reactor		
	R1 (0.023M of NaCl)	R2 (0.050M of NaCl)	R3 (0.075M of NaCl)
TKN	51.7	61.5	48.9
TP	24.5	26.0	23.6
COD	25.0	43.8	31.8

Conclusion

Salinity condition had the effect on lipid content of microalgae cultivation. The effect of salinity concentration on lipid content of *Chlorella vulgaris* was observed that the salinity concentration of 0.023, 0.05 and 0.075 M of NaCl induced to increase lipid content about 4.60%, 1.84% and 3.09%, respectively. The effluent from frozen seafood industry contained 0.023 M of NaCl, the growth of *Chlorella vulgaris* with this effluent provided 4.60% of lipid content increasing that provided the best condition of Salinity for *Chlorella vulgaris* cultivation. Then, the effluent from frozen seafood industry was suitable for microalgae growth especially the *Chlorella vulgaris* due to this effluent contained enough salinity. It could be concluded that the increased salinity led to enhance lipid content in *Chlorella vulgaris* strain. But it was slightly enhanced among various concentrations of salinity.

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References

[1] Veronesi, D., Idà, A., D'Imporzano, G. and Adani, F. 2015. Microalgae cultivation: nutrient recovery from digestate for producing algae biomass. Chemical Engineering Transactions, 43: 1201-1206.

[2] Chi, N.T.L., Duc, P.A., Mathimani, T. and Pugazhendhi, A. 2019. Evaluating the potential of green alga *Chlorella* sp. for high biomass and lipid production in biodiesel viewpoint. Biocatalysis and Agricultural Biotechnology, 17: 184-188.

[3] Chui, S.Y., Kao, C.Y., Chen, T.Y., Chang, Y.B., Kuo, C.M. and Lin, C.S. 2015. Cultivation of microalgal Chlorella for biomass and lipid production using wastewater as nutrient resource. Bioresource Technology, 184: 179-189.

[4] Gonçalves, A.L., Pire, J.C. and Simões, M. 2013. Lipid production of *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*. International Journal of Energy and Environmental Engineering, 4(1): 1-6.

[5] Danquah, M.K., Gladman, B., Moheimani, N., and Forde, G.M. 2009. Microalgal growth characteristics and subsequent influence on dewatering efficiency. Chemical Engineering Journal, 151: 73-78.

[6] Wu, Y.H., Hu, H.Y., Yu, Y., Zhang, T.Y., Zhu, S.F., Zhuang, L.L., Zhang, X., and Lu, Y. 2014. Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review. Renewable and Sustainable Energy Reviews, 33, 675-688.

[7] Daneshvar, E., Santhosh, C., Antikainen, E. and Bhatnagar, A. 2018. Microalgal growth and nitrate removal efficiency in different cultivation conditions: Effect of macro and micronutrients and salinity. Journal of Environmental Chemical Engineering, 6(2): 1848-1854.

[8] Shin, Y.S., Choi, H.I., Choi, J.W., Lee, J.S., Sung, Y.J. and Sim, S.J. 2018. Multilateral approach on enhancing economic viability of lipid production from microalgae: A review. Bioresource Technology, 258: 335-344.

[9] Yoo, C., Jun, S.Y., Lee, J.Y., Ahn, C.Y. and Oh, H.M. 2009. Selection of microalgae for lipid production under high levels carbon dioxide. Bioresource Technology, 101: S71-S74.

[10] Kebeish, R., El-Ayouty, Y. and Hussein, A. 2014. Effect of salinity on biochemical traits and photosynthesis-related gene transcription in *Chlorella vulgaris*. Egyptian Journal of Botany, 54(2): 281-294.

[11] Ben Moussa-Dahmen, I., Chtourou, H., Rezgui, F., Sayadi, S. and Dhouib, A. 2016. Salinity stress increases lipid, secondary metabolites and enzyme activity in *Amphora subtropica* and *Dunaliella* sp. for biodiesel production. Bioresource Technology, 218: 816-825.

[12] Mohan, S.V. and Devi, M.P. 2014. Salinity stress induced lipid synthesis to harness biodiesel during dual mode cultivation of mixotrophic microalgae. Bioresource Technology, 165: 288-294.

[13] Ishika, T., Moheimani, N.R., and Bahri, P.A. 2017. Sustainable saline microalgae co-cultivation for biofuel production: A critical review. Renewable and Sustainable Energy Reviews, 78: 356-368.

[14] Jayashree, C., Tamilarasan, K., Rajkumar, M., Arulazhagan, P., Yogalakshmi, K.N., Srikanth, M. and Banu, J.R. 2016. Treatment of seafood processing wastewater using upflow microbial fuel cell for power generation and identification of bacterial community in anodic biofilm. Journal of Environmental Management, 180: 351-358.

[15] Guimarães, J.T., Souza, A.L.M., Brígida, A.I.S., Furtado, A.A.L., Chicrala, P.C.M.S., Santos, V.R.V., Rosiana, R.A., Luiz, D.B. and Mesquita, E.F.M. 2018. Quantification and characterization of effluents from the seafood processing industry aiming at water reuse: A pilot study. Journal of Water Process Engineering, 26: 138-145.

[16] Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry, 226(1): 497-509.

[17] Adenan, N. S., Yusoff, F. M. and Shariff, M. 2013. Effect of salinity and temperature on the growth of diatoms and green algae. Journal of Fisheries and Aquatic Science, 8(2): 397-404.

[18] Ahmad, A., Buang, A. and Bhat, A.H. 2016. Renewable and sustainable bioenergy production from microalgal co-cultivation with palm oil mill effluent (POME): A review. Renewable and Sustainable Energy Reviews, 65: 214-234.

[19] Sajjadi, B., Chen, W.Y., Raman, A.A.A. and Ibrahim, S. 2018. Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. Renewable and Sustainable Energy Reviews, 97: 200-232.

[20] Mandalam, R. K. and Palsso, B. 1995. *Chlorella vulgaris* (Chlorellaceae) does not secrete autoinhibitors at high cell densities. American Journal of Botany, 82(8): 955-963.

[21] Church, J., Hwang, J.H., Kim, K.T., McLean, R., Oh, Y.K., Nam, B., Joo, J.C. and Lee, W.H. 2017. Effect of salt type and concentration on the growth and lipid content of Chlorella vulgaris in synthetic saline wastewater for biofuel production. Bioresource Technology, 243: 147-153.

[22] Kebeish, R., El-Aouty, Y. and Hussein, A. 2014. Effect of salinity on biochemical traits and photosynthesis-related gene transcription in *Chlorella vugaris*. Egyptian Journal of Botany, 54(2): 281-294.

[23] Yun, C.J., Hwang, K.O., Han, S.S. and Ri, H.G. 2019. Effect of salinity stress on the biofuel production potential of freshwater microalgae *Chlorella vulgaris* YH703. Biomass and Bioenergy, 127: 1-7.

[24] Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. and Darzins, A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The Plant Journal, 54: 621-639.

[25] Thompson, G.A. 1996. Lipids and membrane function in green algae. Biochemica et Biophysica Acta, 1302: 17-45.

[26] Bajwa, K. and Bishnoi, N.R. 2015. Osmotic stress induced by salinity for lipid overproduction in batch culture of *Chlorella pyrenoidosa* and effect on others physiological as well as physicochemical attributes. Journal of Algal Biomass Utilization, 6(4): 26-34.

[27] Sreesai, S. and Pakpain, P. 2007. Nutrient recycling by *Chlorella vulgaris* from septage effluent of the Bangkok city, Thailand. Science Asia, 33: 293-299.

[28] Khoo, C.G., Woo, M.H., Yury, N., Lam, M.K. and Lee, K.T. 2017. Dual role of *Chlorella vulgaris* in wastewater treatment for biodiesel production: growth optimization and nutrients removal study. Journal of the Japan Institute of Energy, 96(8): 290-299.

[29] Cuellar-Bermudez, S.P., Aleman-Nava, G.S., Chandra, R., Garcia-Perez, J.S., Contreras-Angulo, J.R., Markou, G., Muylaert, K., Rittamann, B.E. and Parra-Saldivar, R. 2017. Nutrients utilization and contaminants removal: A review of two approaches of algae and cyanobacteria in wastewater. Algal Research, 24: 438-449.

[30] Pal, D., Khozin-Goldberg, I., Cohen, Z. and Boussiba, S. 2011. The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp. Applied Microbiology Biotechnology, 90(4): 1429-1441.