

EFFECT OF NUTRIENT CONCENTRATION ON THE GROWTH AND LIPID CONTENT OF *CHLORELLA VULGARIS* CULTIVATION USING FROZEN SEAFOOD EFFLUENT

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Received: July 13, 2020; Revised: June 03, 2021; Accepted: June 14, 2021

Abstract

Microalgae can offer several benefits for wastewater treatment with their ability to produce amounts of lipid contents for biofuel production. The aim of this research was to evaluate the feasibility of microalgae cultivation using treated saline wastewater from frozen seafood factory. The effect of nutrient concentration on lipid content of *Chlorella vulgaris* was investigated. The frozen seafood effluent as a saline wastewater used for cultivating microalgae because it contains high nutrients for microalgae growth. Microalgae cultivation can provide several advantages consists of high photosynthesis, growth rate and lipid content. Furthermore, the microalgae are able to use carbon dioxide and produce the renewable energy such as fuels and chemicals. These productions can decrease the using of fossil fuel and greenhouse gas emission. The frozen seafood effluent was mixed with tap water in the ratio of effluent: tap water 10:0, 9:1, 8:2, 7:3, 6:4 and 5:5 by volume that cultivates in R1, R2, R3, R4, R5 and R6, respectively to decrease the concentrations of TKN and TP from the effluent. After microalgae cultivation, the results show that the maximum dry cell weight (DCW) of R1, R2, R3, R4, R5 and R6 was 1.265, 2.529, 1.859, 1.917, 2.729 and 2.533 g/L, respectively. The lipid content of *Chlorella vulgaris* in R1, R2, R3, R4, R5 and R6 was $3.82 \pm 0.06\%$, $4.58 \pm 0.01\%$, $5.09 \pm 0.04\%$, $4.30 \pm 0.02\%$, $3.70 \pm 0.08\%$ and $5.20 \pm 0.03\%$, respectively at the end of cultivation. This study found that nutrient concentration, especially TKN and TP, had more of an effect on the metabolisms of *Chlorella vulgaris* has been described. In this study, the lipid content of *Chlorella vulgaris* revealed good increasing trends in the low nutrient concentration from R1 to R3 and the highest lipid content of *Chlorella vulgaris* represented in R6, which was cultivated in the lowest nutrient concentration (58.1 ± 9.3 mg/L of TKN and 10.1 ± 0.9 mg/L of TP). The low nutrient condition can induce high lipid and biomass content of *Chlorella vulgaris*.

Keywords: Nutrient, *Chlorella vulgaris*, seafood effluent, lipid content

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Introduction

The current research has been interesting on renewable and biodegradable fuel and improving on the high requirement of energy due to fossil fuel decrement and environmental pollution (Yang *et al.*, 2018). The 80% of the world consumes petroleum production as energy for transportation and other energy requirement (Wu *et al.*, 2014; Al battab and Ghaly, 2015). Nevertheless, it cannot be replenished that becomes the crucial energy problem for people in the 21st century (Wu *et al.*, 2014). Furthermore, the fossil fuel burning produces the harmful gas such as sulfur dioxide, nitrogen oxide, and carbon monoxide, which is the cause of impacting on environment and well-being of human (Lv *et al.*, 2017). Therefore, the sustainable energy is the most essential resource for human nowadays (Al battab and Ghaly, 2015). Microalgae are recognized as the most suitable material for transforming to biodiesel (Chisti, 2007; Danquah *et al.*, 2009; Mercer and Armenta, 2011). The possible source of biological material for the biodiesel production is represented by microalgae, in particular by their lipid content (Chisti, 2008; Converti *et al.*, 2009). Biodiesel is a non-environmentally harmful alternative fuel that generated from renewable sources, it emits a smaller amount of carbon dioxide, sulfur dioxide, and other pollutants into the atmosphere than common diesel fuel (Miao and Wu, 2006; Al battab and Ghaly, 2015).

Microalgae biomass production is generally more expensive than growing crops. Photosynthetic of microalgae growth required carbon dioxide, light, inorganic salts and water. The microalgae can offer several benefits from wastewater treatment with their ability to produce amount of lipid content for biofuel production and the economic value of harvested biomass for biogas (Church *et al.*, 2017). There are three main sources of wastewater, municipal, agricultural and industrial wastewater, which contain a variety of ingredients. The microalgae are acknowledged as extremely beneficial species because of high photosynthesis active, high growth rate, high lipid production, and capable of using wastewater (Carvalho *et al.*, 2006; Dalirynezhad *et al.*, 2017). Moreover, they can utilize carbon dioxide and generate the renewable energy like fuels and chemicals. The products from them help to reduce fossil fuel utilization and greenhouse gas releasing (Kube *et al.*, 2018). Some stressed conditions effect on enhancing the lipid accumulation such as nutrient, temperature, pH and light. For instance, cells of microalgae convert carbon source to lipid or starch in nutrient starvation condition (Shin *et al.*, 2018). The triacylglycerols

(TAG) are a type of lipid from microalgae, which might be transformed into biodiesel by the transesterification process (Wu *et al.*, 2014). *Chlorella vulgaris* is known ability to produce high-levels of lipid content for possible use as biofuel was evaluated for these abilities to thrive and produce in seawater and brackish water (Liang *et al.*, 2009; Teh *et al.*, 2021). It was found to thrive and produce lipid contents in full strength seawater (Luangpipat and Chisti, 2016).

In addition, the other global issue is the high cost of utilizing the large amount of water source and nutrient for microalgae growth (Xin *et al.*, 2010). Moreover, it is also high cost for biodiesel which produced from algae. Using wastewater for microalgae cultivation is beneficial in minimizing the use of fresh water, removing nitrogen and phosphorus from wastewater, production microalgae biomass for biofuel and reducing the cost of nutrient addition (Chiu *et al.*, 2015). However, it is low cost unless the microalgae grow with wastewater due to it can provides the essential nutrient for microalgae cultivation (Ahmad *et al.*, 2013). Even many researchers studied on microalgae cultivation with wastewater (Wu *et al.*, 2014; Chiu *et al.*, 2015), but many food industries have not practiced about the reusing of effluent because the information which is concerned about effluent reuse is limited (Guimarães *et al.*, 2018). Nitrogen has a prominent effect on microalgae lipid production (Liang *et al.*, 2009).

Hence, in this research selects *Chlorella vulgaris* strain for cultivating with the frozen seafood effluent. Because it contains high organic content which comes from blood, fish heads, intestine, and meat residuals (Jayashree *et al.*, 2016). The objective of this research is to increase the lipid content in microalgae with various nutrients deficiency in frozen seafood effluent.

Materials and Methods

Microalgae strain and pre-cultivation

The *Chlorella vulgaris* strain was taken from the Coastal Aquaculture Research Institute in Songkhla province, Thailand. The urea fertilizer was utilized for *Chlorella vulgaris* cultivation. The urea fertilizer contains 0.2 g/L of urea, 0.003 g/L of Diammonium phosphate, 0.2 g/L of CaO and 0.8 mL/L of Glutamic mother liquid (0.8 mL/L). The 6 L of reactor was used for pre-cultivation at ambient temperature with 6,000 lux of light intensity.

Microalgae growth determination

The cell counting was counted using microscope with a magnification of 40X and a Neubauer hemocytometer before and after cultivations. The optical density (OD) was measured using spectrophotometer at 680 nm. After cultivation, the microalgae cell was harvested using centrifugation at 5,000 rpm for 10 min and then, cleaned two folds with distilled water to remove the debris off. After that, the purified microalgae cell was dried at 105°C until getting constant weight. The linear relationship between OD₆₈₀ and dry cell weight (DCW, g dry weight/L) was shown as the Equation (1):

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

The growth rate per day (μ , d⁻¹) was determined using Equation (2):

$$\mu = \frac{(\ln DCW_2 - \ln DCW_1)}{t_2 - t_1} \quad (2)$$

Where: DCW₁ and DCW₂ were dry cell weight (g/L) at time t_1 and t_2 , respectively. The biomass productivity (P_{biomass} , g/L/d) was determined using Equation (3):

$$P_{\text{biomass}} = \frac{DCW_2 - DCW_1}{t_2 - t_1} \quad (3)$$

Characterization of the effluent of seafood processing

The treated frozen seafood effluent was collected from Chotiwat Manufacturing Co. Ltd in Songkhla province, Thailand. The properties of effluent were analyzed before and after cultivation that consist of total kjeldahl nitrogen (TKN), total phosphorus (TP), chemical oxygen demand (COD), total dissolved solid (TDS) and salinity. The monitoring parameters of effluent were measured daily during cultivation, such as turbidity, pH and temperature.

Experiment design

Microalgae were cultivated using frozen seafood effluent. The batch experiment was conducted in the photobioreactor (60 cm of length and 20 cm of diameter). The frozen seafood effluent was used as a controlled medium for microalgae cultivation. After analyzing the characteristic of frozen seafood effluent, the total kjeldahl nitrogen (TKN) and total phosphorus (TP) were found. The effluent was mixed with tap water to get the ratio of effluent: tap water into 10:0, 9:1, 8:2, 7:3, 6:4 and 5:5 by volume to decrease their

concentration from the frozen seafood effluent. The microalgae were cultivated until reach the stationary phase. The concentration of TKN and TP of each reactor (R) was analyzed after dilution (Table 1).

Table 1. The different concentrations in each reactor after dilution

Reactor	Effluent: Tap water (V/V)	TKN (mg/L)	TP (mg/L)
R1	10:0	122.2±20.8	18.5±1.5
R2	9:1	96.3±15.8	16.4±0.8
R3	8:2	77.8±0.8	15.6±1.0
R4	7:3	68.3±7.1	14.3±0.1
R5	6:4	62.7±4	11.7±0.2
R6	5:5	58.1±9.3	10.1±0.9

Lipid extraction

The lipid content was extracted from microalgae using a modified method of Folch (Folch *et al.*, 1957). The lipid was extracted with chloroform: methanol of 2:1 (v/v) in fume hood. The ultrasonic cleaner was used for mixing the sample with solvent for 30 min. The solvent mixture was divided into two phases by centrifugation at 5,000 rpm for 15 min. The upper phase consists of non-lipid substance and substance, was removed and evaporated in a fume hood. The lower phase is a solid that consists of lipid. The solid was repeated by adding chloroform: methanol of 2:1 (v/v) until getting clear water. The lipid content was calculated using Equation (4).

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

Statistical analysis

The results are shown as mean value±standard deviation using Microsoft excel to evaluate the different level of growth rate and lipid content in different concentration of nutrient.

Results and Discussion

Characterization of frozen seafood effluent

Frozen seafood effluent contained 140.1±4.8 mg/L of TKN, 18.5±8.5 mg/L of TP, 101.9±8.3 mg/L of COD, 1,010±31.4 mg/L of TDS, 10.5±4.7 NTU of turbidity, 1.3±0.0 ppt of salinity, 7.8±0.1 of pH and 29.9±2.3°C of temperature. The previous study of Keo and Kaosol (2020) shows that the frozen seafood effluent was suitable for microalgae cultivation because it contained the suitable salinity content. The salinity in wastewater can help to increase the lipid content. The suitable TKN and TP for *Chlorella vulgaris* was known from 128-240 mg/L of TKN and 8.9-28.3 mg/L of TP

(Sreesai and Pakpain, 2017). Therefore, the frozen seafood effluent was able to use for microalgae cultivation.

Monitoring parameters

1) Correlation between turbidity and optical density (OD)

Turbidity is a photosensitive measurement of clearness of liquid that the light scatter on the sample. It is necessary to determine the presence of dense of algae cell as well (Abdel-Raouf *et al.*, 2012). The turbidity is one of monitoring parameters which was measured daily to find the correlation with OD on R1, R2, R3, R4, R5 and R6 that shown in Figure 1. It was shown high correlation between turbidity and OD which R1, R2, R3, R4, R5 and R6 were displayed the correlation coefficient $R^2 = 0.821$, $R^2 = 0.9171$, $R^2 = 0.9045$, $R^2 = 0.9043$, $R^2 = 0.9756$ and $R^2 = 0.9726$, respectively. Similarly, the previous research that cultivated *Chlorella sp.* was found the correlation coefficient R^2 for vials 1, 2, A and B was $R^2 = 0.8323$, $R^2 = 0.8621$, $R^2 = 0.8675$ and $R^2 = 0.8459$, respectively (Abdel-Raouf *et al.*, 2012). These high correlation coefficients (R^2) have been indicated that turbidity correlated with OD.

2) The changing of pH

The pH was measured daily in the purpose of observing how does it change in the microalgae growth medium and how is it effected. This study found the similar changing of pH increasing from Day-1 to Day-3 in R1, R5 and R6 were 7.9 ± 0.1 to 8.4 ± 0.1 , 7.7 ± 0.04 to 8.1 ± 0.04 and 7.6 ± 0.06 to 8.1 ± 0.1 , respectively. After that, the pH of these reactors was decreased from Day-4 until Day-6 which R1 was from 7.5 ± 0.1 to 6.2 ± 0.00 , R5 was from 7.5 ± 0.7 to 6.0 ± 0.05 and R6 was from 7.5 ± 0.7 to 5.8 ± 0.04 . Whereas the similarly of R2, R3 and R4 was also detected the pH rose from Day-1 to Day-2. The pH rising of R2, R3 and R4 were from 7.9 ± 0.05 to 5.6 ± 0.00 , 8.0 ± 0.03 to 6.1 ± 0.5 and 7.5 ± 0.7 to 6.0 ± 0.05 between both days, respectively. The result of pH in the cultivation medium each day shows in Figure 2. The rising of pH was explained that it increased during microalgae utilized the carbon dioxide (CO_2) and photosynthesize under the sunlight (Sajjadi *et al.*, 2018). It was described that the decreasing microalgae growth can be caused from the free ammonia that produce high pH in wastewater (Tan *et al.*, 2016).

Moreover, another study reported that the microalgae cell release hydroxyl ion into the medium while it utilizes bicarbonate (HCO_3) instead of CO_2 . Furthermore, the HCO_3 is reduced that induce pH increasing (Mandalam and Palsso, 1995; Abdel-Raouf *et al.*, 2012). Nevertheless, the respiration process is the cause of pH reducing

(Sajjadi *et al.*, 2018). Similarly, other research observed that microalgae growth was decreased due to low of photosynthesis activity which can be induced pH decreasing (Abdel-Raouf *et al.*, 2012). In this study, the pH in all the reactors was decreased gradually until the last of cultivation. The pH at 7 is suitable for microalgae growth and increased lipid production. The pH at 9 is low kinetic model for microalgae growth (Bélanger-Lépine *et al.*, 2018). Therefore, it can be expressed that the microalgae were growing up while they synthesized and led to pH increasing. Subsequently, the microalgae were starting lower which caused pH decreasing at the end of cultivation.

Effect of various nutrient on microalgae growth and lipid content

1) Microalgae growth

TKN and TP used as food for microalgae growth (Cai *et al.*, 2013; Markou *et al.*, 2014; Salama *et al.*, 2017; Razzak *et al.*, 2017). In this study, *Chlorella vulgaris* was cultivated for 6 days.

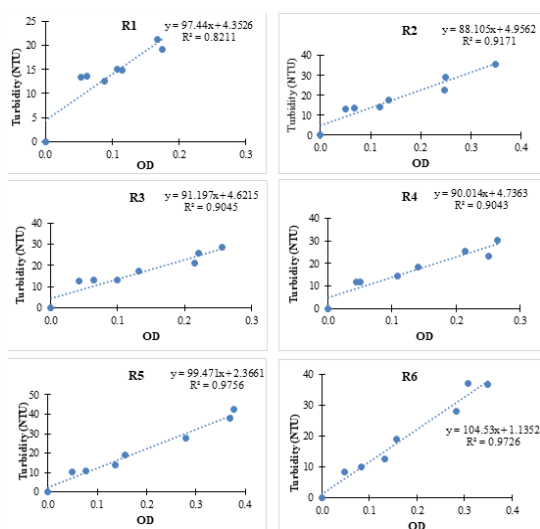


Figure 1. The correlation between turbidity with OD of R1, R2, R3, R4, R5 and R6

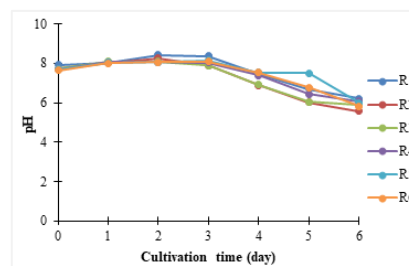


Figure 2. The result of pH in the cultivation medium each day

It was cultivated under the blending between frozen seafood effluent and tap water to reduce the concentration of nutrient (TKN and TP). After reducing the concentration of nutrient, TKN and TP were decreased from 122.2 to 58.1 mg/L and 18.5 to 10.1 mg/L, respectively (Table 1). The microalgae growth was determined by direct cell counting, OD, DCW, biomass productivity and specific growth rate. The cells were counted before and after cultivation to compare each other. The initial cell counting of R1, R2, R3, R4, R5 and R6 was 2.225×10^6 , 2.500×10^6 , 2.325×10^6 , 1.500×10^6 , 2.075×10^6 and 2.025×10^6 cell/mL, respectively. It was observed that cell counting of all reactors after cultivation was higher than that before cultivation. Moreover, the cell counting of R5 was higher than other reactors which was 21.375×10^6 cell/mL that shows in Figure 3. The final cell counting of R5 was

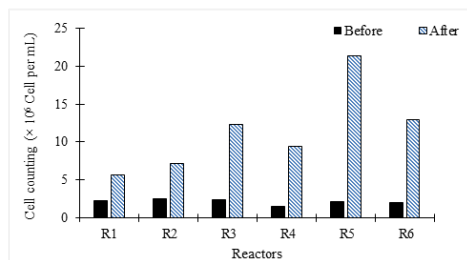


Figure 3. Cell counting of *Chlorella vulgaris* before and after cultivation

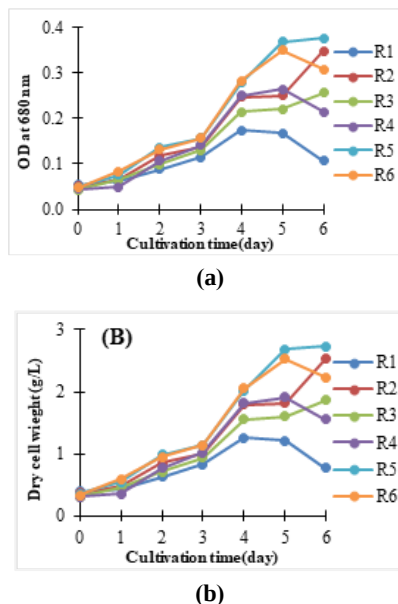


Figure 4. Effect of different nutrient concentrations on *Chlorella vulgaris* growth showed as (a): OD at 680 nm and (b): DCW

higher than the initial cell counting for 10 folds. Higher TKN and TP concentration produced higher biomass productivity in 6 days. The previous study revealed that biomass production is decreased under the nutrient deficiency condition since microalgae's structure cell and physical process are not improved (Scarsella *et al.*, 2010).

Furthermore, the OD and DCW were the important monitoring parameters to identify the microalgae growth day by day. Both parameters were correlated with each other which the correlation coefficient is $R^2 = 0.9996$. The microalgae growth in R1 was 13 reached the maximum OD and DCW were 0.18 and 1.27 g/L on Day-4. Whereas R2, R3 and R5 were reached the maximum of OD and DCW on Day-6. The maximum OD and DCW of R2 were 0.35 and 2.53 g/L, respectively. The maximum OD and DCW of R3 were 0.26 and 1.86 g/L, respectively. The maximum OD and DCW of R5 were 0.38 and 2.73 g/L, respectively. While R4 and R6 were reached the maximum OD and DCW on Day-5. The maximum OD and DCW of R4 were 0.27 and 1.92 g/L, respectively. The maximum OD and DCW of R6 were 0.35 and 2.53 g/L, respectively. In among of all reactors, the highest OD and DCW were also found on R5. The maximum OD and DCW of each reactor are shown in Figure 4(a) and Figure 4(b), respectively. In this study found that the optimum TKN and TP for *Chlorella vulgaris* growth were from 58.1 to 122.2 mg/L and 10.1 to 18.5 mg/L, respectively. These concentrations were in the rank of optimum TKN and TP of previous research which were from 128 to 240 mg/L and 8.9 to 28.3 mg/L, respectively (Sreesai and Pakpain, 2007).

The maximum biomass productivity for all reactors was reached on Day-4. It was observed that the maximum of biomass productivity was increased in low nutrient concentrations which shows in Figure 5(a). However, the maximum biomass productivity of R2 contained high nutrient concentration and it was higher than that R3 which contained low nutrient concentration. The previous study described that toxicity of free ammonia can induce pH increment and can be a major reason of reducing algae growth in the wastewater (Tan *et al.*, 2016). In contrast, some study reported nutrient starvation induced low biomass production because of no ability to improve the structure of cell and physical process (Scarsella *et al.*, 2010). The highest specific growth rate was discovered on R4 on Day-2 which was higher than other cultures. The maximum specific growth rate of R4 was 0.65 d^{-1} . The result of specific growth rate in all cultures is shown in Figure 5(b).

2) Lipid content of *Chlorella vulgaris*

The lipid content was extracted for initial and final microalgae cultivation. It was observed under different concentrations of nutrient deficiency that presents in Figure 6. The lipid content under various reducing of nutrient concentration at the end of cultivation was higher than the initial lipid content which was $3.13 \pm 0.09\%$. In this study observed that the lipid content was increased

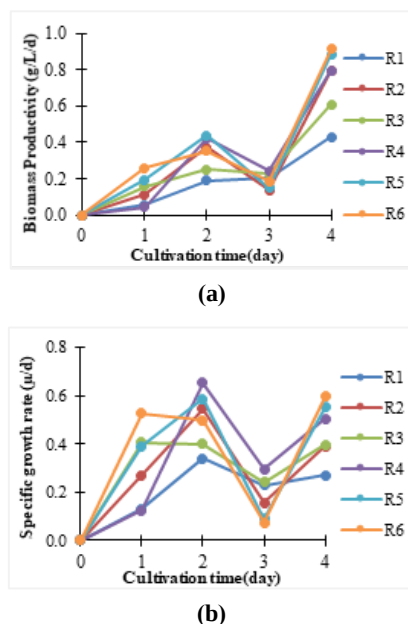


Figure 5. (a) Biomass productivity and (b) Specific growth rate of *Chlorella vulgaris*

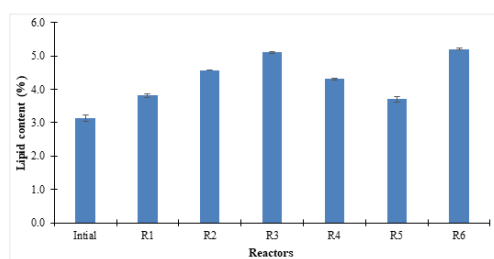


Figure 6. Lipid content of *Chlorella vulgaris* before and after cultivation

in order between R1, R2 and R3 which was $3.82 \pm 0.06\%$, $4.58 \pm 0.01\%$ and $5.09 \pm 0.04\%$, respectively. Whereas the lipid content of R4 ($4.30 \pm 0.02\%$) and R5 ($3.70 \pm 0.08\%$) was higher than the initial lipid content. But the lipid content of both reactors was lower than the lipid content of R2, R3 and R6. Moreover, the lipid content of R6 was $5.20 \pm 0.03\%$, which was extremely higher than all the reactors. The lipid content of *Chlorella vulgaris* displayed good increasing trends in the nutrient deficiency from R1 to R3 and the highest lipid content of *Chlorella vulgaris* represented in R6 which was cultivated in the lowest nutrient concentration. The lipid content of *Chlorella vulgaris* was reported that it increased from 18% to 40% in low nitrogen condition (Illman *et al.*, 2000). In nutrient deficiency condition cultivation, the metabolism of microalgae cell with carbon alter the flow pathway to produce two types of energy such as carbohydrates and lipids that both energies contain the carbon source (Wu *et al.*, 2014). The other study reported that the changing of carbon fixation pathway since the supplement of essential nutrient like nitrogen, phosphorus and sulfur are not enough for microalgae cell (Rai *et al.*, 2017). Furthermore, starch synthesis is inhibited because of ADP-glucose pyrophosphorylase inactivation, which can enhance TAG content and lipid droplet generation (Gong and Jiang, 2011).

The properties of seafood effluent after cultivating microalgae

The properties of frozen seafood effluent at the end of *Chlorella vulgaris* cultivation are presented in Table 2. Normally, COD decreases after microalgae cultivation. In this study, COD increased from initial to final concentration in the original effluent reactor (R1) which was 107.7 ± 43.1 mg/L to 145.3 ± 37.9 mg/L. It was explained that there were two processes which were the reasons of COD increasing. These processes, the bacteria may break down the old microalgae, and microalgae may excrete the organic compound during its absorbing the carbon dioxide (Lee *et al.*, 2019). Moreover, the initial TKN and TP were 136.8 ± 38.21 and 24.5 ± 0.3 mg/L, respectively. These parameters were depleted

Table 2. The properties of effluent after cultivation microalgae

Reactor	TKN (mg/L)	TP (mg/L)	COD (mg/L)	Turbidity (NTU)	pH	Temp. (°C)	TDS (mg/L)
R1	41.3 ± 10.8	18.3 ± 0.9	145.3 ± 37.9	4.7 ± 0.6	6.2 ± 0.4	31.0 ± 0.0	598 ± 30.6
R2	37.4 ± 5.3	16.9 ± 0.8	117.2 ± 14.9	4.3 ± 0.4	5.6 ± 0.3	31.4 ± 0.6	790 ± 11.0
R3	30.8 ± 7.9	15.1 ± 0.6	116.8 ± 31.1	4.2 ± 0.3	5.9 ± 0.2	31.3 ± 0.4	786 ± 27.9
R4	21.0 ± 0.4	10.2 ± 2.9	98.8 ± 64.4	3.5 ± 0.7	6.1 ± 0.3	30.8 ± 0.4	463 ± 15.9
R5	22.5 ± 2.3	10.8 ± 0.4	72.9 ± 69.6	3.4 ± 0.1	6.0 ± 0.2	30.8 ± 0.4	658 ± 5.9
R6	18.1 ± 3.7	5.0 ± 5.4	82.0 ± 48.9	2.7 ± 0.4	5.8 ± 0.3	30.8 ± 0.4	506 ± 5.6

for original effluent reactor after cultivation, which TKN and TP were 41.3 ± 10.8 and 18.3 ± 0.9 mg/L, respectively. According to this result, the capacity of TKN and TP consumption by *Chlorella vulgaris* was found up to 69.81% and 25.47%, respectively. Wang *et al.* (2020) reported that TKN was removed by *Chlorella vulgaris* from 70.84% to 73.99%, which similar with removal efficiency of this study. However, the capacity of TP removal was higher than this study, which removed from 52.16% to 65.22%. The capacity of TKN consumption of the other previous study was lower than this study, which TKN was removed about 62%. But TP consumption was higher than this study, which TP was removed by *Chlorella vulgaris* about 55% (Sreesai and Pakpain, 2007). Furthermore, the results of TKN and TP consumption efficiency of this study were in the range from 11.9-74.3% and 22.5-94.8% for TKN and TP removal efficiencies of previous research (Khoo *et al.*, 2017). Thus, it can be attributed that this study found the high efficiency of TKN consumption, but low efficiency of TP consumption by *Chlorella vulgaris*.

TDS of frozen seafood effluent before cultivation was $1,010 \pm 31.4$ mg/L. The frozen seafood effluent provided the high TDS content because it contained sodium chloride. The salinity, such as NaCl of frozen seafood effluent was 1.3 ± 0.0 ppt, which was suitable for microalgae cultivation. After microalgae cultivation, TDS of R1, R2, R3, R4, R5 and R6 was 598 ± 30.6 , 790 ± 11.0 , 786 ± 11.0 , 463 ± 15.9 , 658 ± 5.9 and 506 ± 0.5 mg/L, respectively. TDS of all reactors decreased after microalgae cultivation because the microalgae consumed salinity in effluent for growth.

Conclusion

Biomass and lipid productivities of *Chlorella vulgaris* under different nutrient conditions were investigated. The frozen seafood effluent is suitable for *Chlorella vulgaris*. The *Chlorella vulgaris* strain has produced high biomass with the frozen seafood effluent using cultivating in the nutrient deficiency condition which can be considered as the best feedstock for accumulating the lipid content. The lipid content represented the good trend in R1 to R3 and the highest lipid content in R6. Growing on nutrient with 5:5 of effluent: tap water ratio (58.1 ± 9.3 mg/L of TKN and 10.1 ± 0.9 mg/L of TP) produced the highest lipid productivity compared with other growth modes. Both TKN and TP had inhibitory effects at high nutrient concentrations. The 5:5 of effluent: tap water ratio (R6) provided the fastest growth rate. It can be concluded that the low

nutrient condition can induce high lipid and biomass content of *Chlorella vulgaris* strain.

Acknowledgments

The authors would like to thank the Royal Scholarship under Her Royal Highness Princess Maha Chakri Sirindhorn Education Project to Kingdom of Cambodia, Faculty of Engineering (PSU) and PSU graduate school for providing financial support. Thanks to the Department of Civil and Environmental Engineering, PSU for providing the equipment to run this research.

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