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Effect of salinity concentrations on lipid content of *Chlorella vulgaris* in frozen seafood effluent

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Abstract

Microalgae have gained increased attention as an alternative source of bioenergy and eco-friendly. Wastewater is useful for microalgae cultivation because the nutrients in wastewater are consumed by microalgae. The salinity also can increase the lipid content of microalgae. The frozen seafood effluent is utilized for cultivating microalgae because it contains the nutrients and salinity for microalgae cultivation. The *Chlorella vulgaris* uses carbon dioxide to produce the renewable energy such as fuels and chemicals. These productions automatically reduce the consumption of fossil fuel and greenhouse gas emission. Therefore, this research aimed to study the effect of various salinity concentrations on lipid content of *Chlorella vulgaris*. The salinity concentrations of frozen seafood effluent before cultivation for R1, R2, R3, R4 and R5 was 1.34±0.01 ppt, 1.47±0.0 ppt, 2.96±0.04 ppt, 4.33±0.05 ppt and 6.11±0.05 ppt, respectively. After cultivation, the lipid content of *Chlorella vulgaris* was 2.82±0.5%, 2.20±0.8%, 1.31±0.7%, 1.83±0.8%, and 0.68±0.1% for R1, R2, R3, R4 and R5, respectively. According to the experiment, the various salinity concentrations had an effect on the lipid content of *Chlorella vulgaris*. The R1 was the original salinity concentration (1.34±0.01 ppt) of frozen seafood effluent which provided the maximum lipid content of *Chlorella vulgaris* after cultivation. Thus, the lipid content of *Chlorella vulgaris* was not representing the increasing trend under the difference of high salinity concentration. It can be concluded that the salinity concentration of the frozen seafood effluent from the factory was enough for *Chlorella vulgaris* cultivation and the nutrients in frozen seafood effluent were also removed after cultivation.

Keywords: Microalgae, Lipid content, *Chlorella vulgaris*, Seafood effluent

1. Introduction

Microalgae cultivation in wastewater is interesting because it does not cost much on cultivation [1]. The advantages of microalgae cultivation are high photosynthesis, growth rate and lipid content. Carbon source is also generated from organic carbon that is used in phototrophic and mixotrophic cultivation. However, the nutrients contain organic carbon that is able to produce carbon source which is the major nutrient for microalgae cultivation [2]. The organic carbon is consumed by the capacity of microalgae growth in wastewater under mixotropic cultivation [3]. However, the microalgae cultivation should be referred to low nutrients mix with the increased amount of carbon source makes microalgae growth cycle become shorter and enhances production capability [4].

Salinity has an effect on the microalgae growth because it is an essential stress component for microalgae. The salinity leads to change the metabolic in nutrient absorption and makes oxidative stress [5]. Microalgae are known that withstand in the rank of salinity condition, but if in this condition is changed by raining and evaporation, it will effect on microalgae cultivation in marine [6]. Moreover, different types of sodium salt such as sodium chloride (NaCl), sodium thiosulfate ($Na_2S_2O_3$), sodium bicarbonate ($NaHCO_3$), sodium acetate ($C_2H_3NaO_2$) are led to increase lipid on various microalgae. In addition, sodium chloride (NaCl) is shown to enhance the lipid content radically, but the microalgae biomass production is decreased slightly [4].

Normally, the seawater microalgae can tolerate in high salinity condition greater than freshwater microalgae. Several studies reported that the salinity is the choice for microalgae cultivation. The microalgae grow in less salinity is added some compound such as sodium chloride (NaCl) and sodium sulfate ($NaSO_4$) to support their growth [7]. However, too much of the light made evaporation in the water what is led to enhance salinity in the culture site that attacked photosynthesis and growth rate. There are three points which cause of salinity to attack microalgae such as leading to ion stress, absorption and deprivation of ion that causing ion stress, and ion ratio in the cell is changed [2, 8]. Additionally, high salinity can make the reaction in the algae cell to be stressed because of the higher osmotic pressure that is occurring in physiological and biochemical mechanisms [4]. Furthermore, high salinity activates help to balance export and absorb ions pass through the cell membrane and increment of osmo-protecting solute and stress proteins can induct high total lipid content in the cell [9].

According to the research of Bajwa and Bishnoi [10], the biomass and total lipid content of microalgae *Chlorella sp.* were increased under different level of salinity (5-25 mM). The salinity increment led to increase the lipid content from 12.57±0.56 to 43.84±0.40 %DCW [8]. Moreover, Church et al. [11] described sodium chloride (NaCl) and potassium chloride (KCl) concentrations which effect on *Chlorella vulgaris* that have also been shown that high concentration of both NaCl and KCl are cause to reduce microalgae growth

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rate, but low concentration of biomass was investigated with both NaCl and KCl are compared. In addition, the previous research reported that the microalgae growth and nutrient removal were affected by sodium concentration more than potassium chloride [11]. The *Chlorella vulgaris* cultivation is almost stopped under the concentration of NaCl at 34 g/L [12]. The *Chlorella protothecoides* is also showed that stopped growing under the high concentration of NaCl at 30 g/L due to toxic effecting [13]. Moreover, the growth rate of *Botryococcus braunii* is inhibited under the amount of salt from 0.3 to 0.7 M [14]. Therefore, different species of microalgae have different abilities to tolerate with salinity [4]. Additionally, the microalgae cultivate mixing with the stress of salinity and nitrogen condition in lab scale is able to increase lipid and cell biomass production for the unique life cycle. The main objective of this research was to investigate the lipid content of *Chlorella vulgaris* with the various salinity concentrations.

2. Materials and methods

2.1 Raw material characteristics

The effluent of frozen seafood processing was obtained from Chotiwat Manufacturing Co. Ltd in Songkhla province, Thailand (Figure 1). The sample was stored in the refrigerator before analyzing. The effluent characteristics such as total kjeldahl nitrogen (TKN), total phosphorus (TP), chemical oxygen demand (COD), total dissolved solid (TDS), turbidity, pH, salinity and temperature, were analyzed before cultivation.

Figure 1 The effluent of frozen seafood processing

2.2 Microalgae strain and pre-culture

The *Chlorella vulgaris* strain was obtained from the Coastal Aquaculture Research Institute in Songkhla province, Thailand (Figure 2). It was selected due to high lipid content. The *Chlorella vulgaris* was cultivated in urea fertilizer. The urea fertilizer consists of urea (0.2 g/L), di-ammonium phosphate (0.003 g/L), CaO (0.2 g/L) and glutamic mother liquid (0.8 mL/L). The sample was cultivated in 6 L of bottle at the ambient temperature with 6,000 lux of light intensity. The cell density, cell count and lipid content of *Chlorella vulgaris* were analyzed before transferring into the photobioreactor.

Figure 2 *Chlorella vulgaris*

2.3 Experimental design

The effluent of frozen seafood processing used as a controlled medium for microalgae cultivation. The frozen seafood effluent was used for *Chlorella vulgaris* cultivation by analyzing the salinity first to know the amount of salinity in the effluent. After that, NaCl was added for various salinity concentrations into the effluent to get the range of 0 M, 0.025 M, 0.05 M, 0.075 M and 0.10 M of NaCl for R1 (control), R2, R3, R4 and R5, respectively. Table 1 shows the controlled medium and NaCl addition in each biophotoreactor. These ranges applied for *Chlorella vulgaris* strain because they found that their growth rate was inhibited under the amount of NaCl at 34 g/L [15]. All the reactors carried out in duplicate (n=2).

Table 1 Controlled medium and NaCl addition in each photobioreactor

Reactor	*R1*	*R2*	*R3*	*R4*	*R5*
NaCl addition (M)	0 (Control)	0.025	0.05	0.075	0.10

The batch experiment used in the photobioreactor (length 60 cm, diameter 20 cm) and filled 10 L of effluent into each reactor (Figure 3). The cultures were continuously injected the air by the diffuser plate from the bottom of photobioreactors to make *Chlorella vulgaris* blend with the effluent but the carbon dioxide was not supplied in these photobioreactors because Chi et al. [16] expressed that the carbon dioxide in the air is enough for lipid production. All of the photobioreactor was cultured as an indoor scale up which the microalgae were grown under natural sunlight. The *Chlorella vulgaris* was cultivated until to reach the stationary phase. The cell density of *Chlorella vulgaris* was measured daily to know their growth rate. The cell count was counted before and after cultivation. At the end of cultivation, the cell count and the residual of effluent were analyzed again to compare with the initial value.

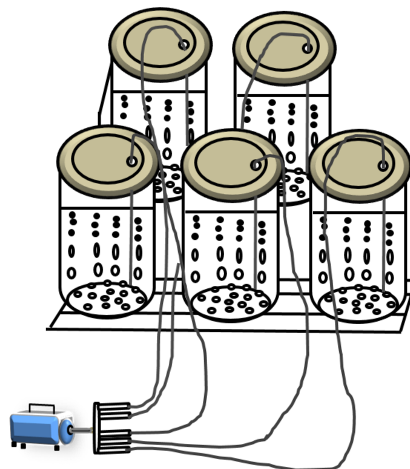

Figure 3 Photobioreactors

2.4 Microalgae characteristic analysis

The cell density of *Chlorella vulgaris* was measured daily to know the growth rate of microalgae by spectrophotometer at 680 nm. The cell count was counted by microscope with 40X of objective lens magnification and a Neubauer hemocytometer before and after cultivation.

The linear relationship between the optical density (OD680) and the dry cell weight (DCW, g dry weight/L) was presented in equation 1:

$$DCW\ (g\ dry\ weight/L)=7.2471\times OD_{680};\ R2\ =\ 0.9996 \quad (1)$$

Biomass productivity ($P_{biomass}$, g/L/d) was determined by equation 2:

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

The growth rate per day was calculated using equation 3:

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

Where: μ= growth rate per day (μ, d^{-1})
DCW_1= dry cell weight at time t_1 (g/L)
DCW_2= dry cell weight at time t_2 (g/L)
t_1 = the period of time on d_1 (d)
t_2 = the period of time on d_2 (d)

At the end of cultivation, the cell of *Chlorella vulgaris* was harvested by centrifugation at 5,000 rpm for 10 min. After that, the *Chlorella vulgaris* were rinsed with distilled water for 2 times to get rid of the debris and medium. The cleaned cell was taken to dry in the oven at 105℃ until obtaining the constant weight. Dried cell was used for extracting the lipid content by Folch method [17]. The lipid was extracted by combining the dried cell with Chloroform: Methanol (2:1, v/v) in a fume hood. The samples were mixed using an ultrasonic cleaner for 30 min. The mixed samples were separated into two phases via centrifugation at 5,000 rpm for 15 min. The upper phase contained non-lipid substance which it was withdrawn and evaporated in the fume hood. The lower phase contained the solid which known as lipid. This phase continued to extract with adding Chloroform: Methanol (2:1, v/v) until obtaining the clear water.

Mass of lipid was calculated using equation 4:

$$\text{Mass of lipid}=(\text{mass of container}+\text{extracted lipid})-\text{mass of container} \quad (4)$$

Lipid content was calculated using equation 5:

$$\text{Lipid content (\%)}=\frac{\text{Total oil extracted (g/L)}}{\text{Biomass dry weight (g/L)}}\times 100\% \quad (5)$$

2.5 Effluent characteristic analysis

The effluent characteristics such as total kjeldahl nitrogen (TKN), total phosphorus (TP), chemical oxygen demand (COD) and total dissolved solid (TDS) were analyzed before and after cultivation. The monitoring parameters of effluent such as turbidity, pH, salinity, temperature, and dissolved oxygen (DO) were measured daily. Moreover, temperature in the atmosphere was also measured daily.

3. Results

3.1 Characterization of the effluent of frozen seafood processing

The initial characterization of frozen seafood effluent shows in Table 2. The initial characterization of frozen seafood effluent such as TKN, TP, COD, TDS, turbidity, salinity, pH, and temperature were analyzed. The previous study reported that *Chlorella vulgaris* growth was inhibited in the concentration of NaCl at 34 g/L (34 ppt) [12]. Whereas, the suitable TKN and TP for *Chlorella vulgaris* were known to range from 128-240 mg/L of TKN and 8.9-28.3 mg/L of TP [18]. Thus, it can be expressed that the frozen seafood effluent is able to use for microalgae cultivation.

The result of various NaCl concentrations in each photobioreactor after adjusting shows in the Table 3. As the inhibiting of NaCl on *Chlorella vulgaris* growth is mentioned above, the various concentrations of NaCl in each bioreactor were adjusted for obtaining the suitable concentration of NaCl on their growth. Moreover, the level of NaCl was organized to realize its effect on the growth rate and the lipid content of *Chlorella vulgaris*.

Table 2 Initial characterization of frozen seafood effluent

Parameter	Unit	Frozen seafood effluent
pH	-	7.8±0.06
Turbidity	NTU	10.5±4.7
Temperature	°C	29.9±2.3
COD	mg/L	102±8.3
TDS	mg/L	1,010±314
TKN	mg/L	140±4.8
TP	mg/L	18.5±8.5
Salinity	ppt	1.34±0.01

Table 3 Various of salinity concentrations in the photo bioreactor before cultivation

Reactor	R1	R2	R3	R4	R5
Salinity concentration (ppt)	1.34±0.01	1.47±0.0	2.96±0.04	4.33±0.05	6.11±0.05

3.2 The monitoring parameters

3.2.1 Salinity change

The amount of salinity was measured daily to observe the changing of it and how does it effect on microalgae. This point would explain how salinity changes in cultivation bioreactor. The changing of salinity in the frozen seafood effluent shows in Figure 4.

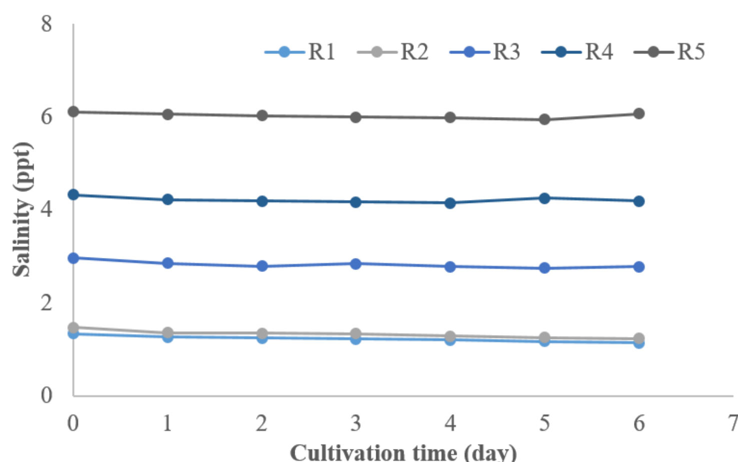

Figure 4 Changing of salinity in the effluent

The salinity in R1 and R2 was decreased gradually since Day-0 until the end of cultivation. The decreasing of salinity in R1 and R2 was from 1.34±0.01 ppt to 1.14±0.0 ppt and 1.47±0.0 ppt to 1.24±0.1 ppt, respectively. Nevertheless, the salinity in R3 was decreased from Day-0 to Day-5 which was from 2.96±0.04 ppt to 2.75±0.0 ppt, respectively, and increased on Day-6 which was 2.77±0.0 ppt. The salinity is one of an important condition for plant's life and able to inhibit their photosynthetic metabolism [19]. Thus, the salinity was decreased because the salinity is consumed by microalgae cell. While the evaporation can be a cause of increasing the salinity in the cultivation medium [6].

3.2.2 pH Change

The changing of pH in the effluent medium is shown in Figure 5. In this research, the pH was differently changed in each photobioreactor by natural. The pH in R1 and R2 was increased from 8.5±0.1 to 8.7±0.1 and 8.4±0.5 to 8.4±0.2, respectively, from Day-1 to Day-4. The pH in both R decreased from 8.3±0.7 to 6.5±0.0 in R1 and 8.1±0.6 to 6.2±0.0 in R2 from Day-5 to Day-6. Whereas pH in R3, R4, and R5 was increased from 8.4±0.3 to 8.8±0.2, 8.4±0.3 to 9±0.2, and 8.5±0.2 to 9±0.1, respectively, from Day-1 to Day-3. The pH decreased from 8.5±0.5 to 6.1±0.0 in R3, 8.8±0.2 to 7.6±0.0 in R4, and 8.9±0.1 to 8.9±0.0 in R5. It was observed that pH increased during 3 to 4 days and decreased after that day until the end of cultivation. The increasing of pH is impacted from the synthesis and carbon dioxide absorption of microalgae during daytime [4]. Moreover, the microalgae used bicarbonate (HCO_3^-) as carbon dioxide (CO_2) then they released hydroxyl ion into the water that cause to decrease bicarbonate (HCO_3^-) and then pH is increased [20]. Nevertheless, the decreasing pH is revealed that it is impacted from the respiration process of microalgae at night time [4]. The minimum and maximum pH of all bioreactors were 6.1 and 9.0 which were observed as the suitable condition for *Chlorella vulgaris* growth because it could grow well in this range of pH. Therefore, it was demonstrated that *Chlorella vulgaris* could grow in the range from 4 to 10 of pH conditions [21].

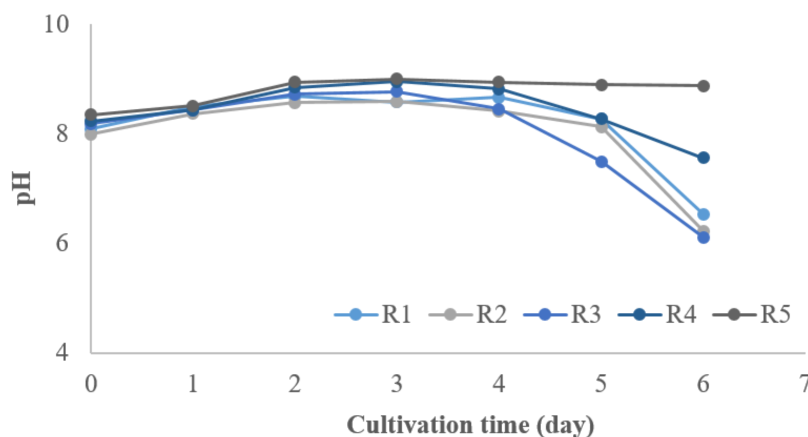

Figure 5 Changing of pH in the effluent

3.2.3 Temperature change

The temperature changed differently in each day. This research showed that the minimum and maximum of temperature, which were 25±0.2ºC and 29±0.0ºC, respectively. Moreover, it was observed that a range of temperature from 25ºC to 29ºC is the optimal temperature for *Chlorella vulgaris* growth. Similarly, it was reported that the optimum temperature for *Chlorella vulgaris* growth was from 25ºC to 30ºC [22]. The previous study revealed that the suitable temperature for *Chlorella vulgaris* was at 30ºC, but its growth rate decreased at 35ºC and died at 38ºC of temperature [21].

3.2.4 Microalgae growth

The *Chlorella vulgaris* was cultivated for 6 days. It was cultivated under various salinity conditions which operated in each photobioreactor. The *Chlorella vulgaris* growth was defined by direct cell count, optical density, dry cell weight, biomass productivity and specific growth rate.

The microalgae cells were counted initial and final of cultivations to compare each other (Figure 6). The initial cell count of R1, R2, R3, R4 and R5 was 4.275×10^6 cells/mL, 3.625×10^6 cells/mL, 4.65×10^6 cells/mL, 2.025×10^6 cells/mL and 4.35×10^6 cells/mL, respectively. The final cell count was 6.2×10^6 cells/mL, 5.925×10^6 cells/mL, 5.9×10^6 cells/mL, 7.925×10^6 cells/mL and 8.075×10^6 cells/mL, respectively. As this result, it was observed that the cell number of R1 to R3 were similar and the cell number of the last other three reactors was also similar. In this research, the cell count of all reactors was increased under various salinity concentrations at the end of microalgae cultivation. It can be noticed that the cell count of *Chlorella vulgaris* was increased in high salinity concentration.

The optical density (OD) and dry cell weight (DCW) are the essential monitoring parameters to recognize microalgae growth daily (Figure 7). The maximum optical density (OD) of R1, R2, R3 and R4 was 0.16±0.0, 0.25±0.0, 0.22±0.0 and 0.24±0.0, respectively. The maximum dry cell weight (DCW) of R1, R2, R3, and R4 was 1.18±0.0 g/L, 1.79±0.0 g/L, 1.6±0.0 g/L and 1.7±0.0 g/L, respectively. The maximum optical density (OD) and dry cell weight (DCW) of R1 to R4 were reached on Day-6. Whereas, the maximum optical density (OD) and dry cell weight (DCW) of R5 were 0.18±0.04 g/L and 1.29±0.3 g/L which reached on Day-4, respectively. The previous research found that *Chlorella vulgaris* reached the maximum biomass concentration was 1.13 g/L, 1.01 g/L, and 0.74 g/L under 15 g/L, 30 g/L and 45 g/L of NaCl, respectively [11].

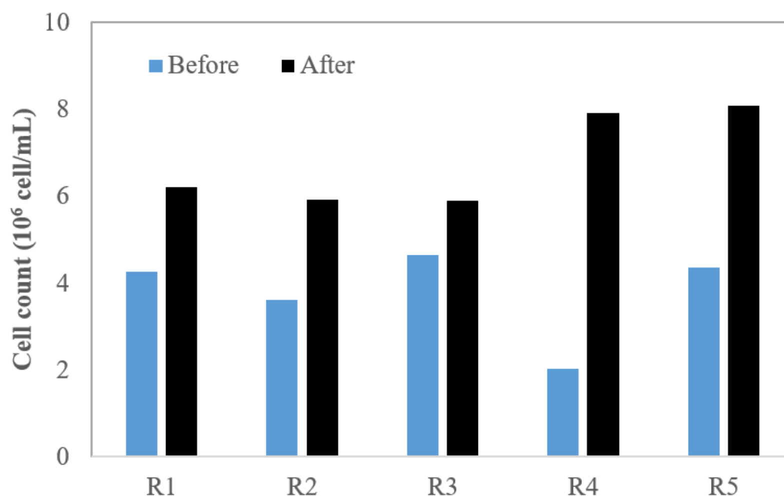

Figure 6 Initial and final cell count of *Chlorella vulgaris* strain

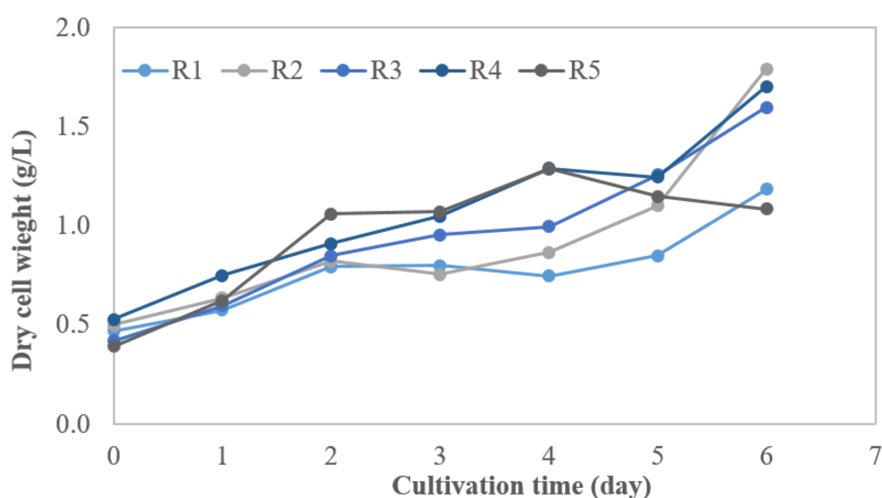

Figure 7 Effect of different salinity concentrations on Chlorella vulgaris growth as dry cell weight (DCW)

The maximum biomass productivity of R1, R2, R3, R4 and R5 was 0.22±0.2 g/L/d, 0.38±0.0 g/L/d, 0.26±0.8 g/L/d, 0.27±0.0 g/L/d and 0.44±0.3 g/L/d, respectively. The maximum growth rate of R1, R2, R3, R4 and R5 was 0.299±0.3 d^{-1}, 0.467±0.3 d^{-1}, 0.356±0.3 d^{-1}, 0.333±0.3 d^{-1} and 0.518±0.3 d^{-1}, respectively. The maximum biomass productivity and growth rate of R2 to R5 under the sodium chloride (NaCl) adding were higher than that R1. The highest biomass productivity and growth rate were observed in R5. The previous study revealed that *Chlorella vulgaris* growth was reduced under different salinity increment that were 0 M, 0.26 M, 0.51 M and 0.77 M [11]. Nevertheless, it was able to enhance the growth under the 0.025 M, 0.05 M, 0.075 M, 0.10 M, and 0.125 M of salinity concentration in this research. The previous research discovered that algae could grow under the 0.05 M, 0.15 M and 0.20 M of salinity concentration [5]. Thus, it can be assumed that the 0.025 M, 0.05 M, 0.075 M, 0.10 M, and 0.125 M of salinity concentration were the suitable condition for *Chlorella vulgaris* cultivation.

3.2.5 Lipid content

Lipid content was extracted before and after cultivations to compare each other. The lipid content of various concentrations of sodium chloride (NaCl) is shown in Figure 8. The lipid content of *Chlorella vulgaris* was increased in different concentrations of salinity. The lipid content of R1, R2 and R4 was found 2.82±0.5%, 2.2±0.8% and 1.83±0.8%, respectively, while the initial value of lipid content was 1.65±0.8%. It showed that the R1, R2, and R4 were slightly increased, compared to the initial value of lipid content. However, it was not shown the increasing lipid content trend in salinity increment between these photobioreactors.

These results were similar to the previous study, which reported that the lipid content of some microalgae such as *M. subterraneus*, *Dunaliella spp.*, *Nannochloropsis sp.*, *Chlamydomonas nivalis* and *Chlorella vulgaris* was enhanced slightly in the increasing of salinity concentration [11]. However, the lipid content of R3 and R5 was known 1.31±0.7% and 0.68±0.1%, respectively. The lipid content of R3 and R5 was lower than the initial value. The previous research showed the opposite result that the lipid content of *Chlorella vulgaris* increased about 12%, 13.5%, and 16% with 15 g/L, 30 g/L, and 45 g/L of salt (NaCl), respectively [11]. The lipid content of *Chlorella sp.* was reported that increased from 12.57±0.56% to 43.84±0.40% under salinity enhancement from 0.005 M to 0.025 M, respectively [10].

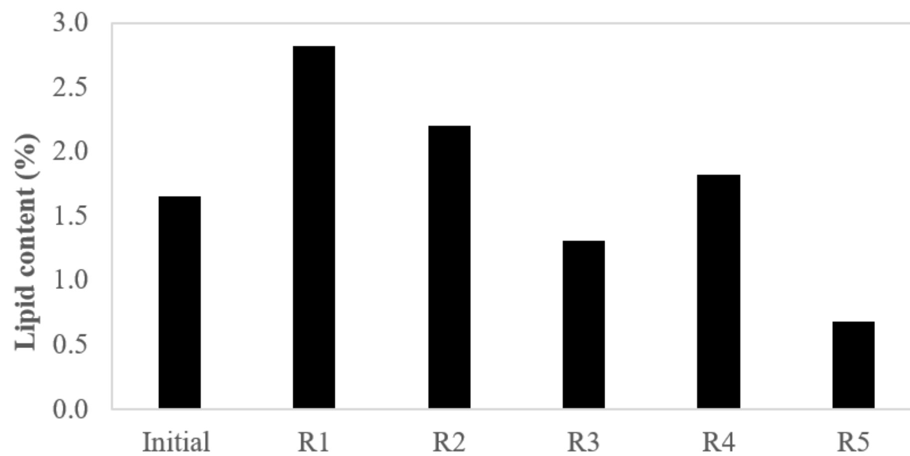

Figure 8 Lipid content of various concentration of salinity

3.2.6 Efficiency of nutrient uptake

The properties of frozen effluent after *Chlorella vulgaris* cultivation are shown in Table 4. In this research, the frozen seafood effluent used as the nutrient which the nutrients were TKN and TP for *Chlorella vulgaris* cultivation. These parameters were analyzed at the end of cultivation to determine the efficiency of nutrient uptake. The initial concentration of TKN and TP was 143±12.5 mg/L and 12.5±6.15 mg/L, respectively. The final concentration of TKN and TP was 48.44±35.6 mg/L and 9.28±4.5 mg/L of R1, 41.86±32.3 mg/L and 10.04±5 mg/L of R2, 38.71±28.2 mg/L and 10.08±5.8 mg/L of R3, 53.06±35.4 mg/L and 9.57±4.5 mg/L of R4, 61.39 ± 5.8 mg/L and 9.75 ± 5.3 mg/L of R5, respectively. In addition, the efficiency of TKN and TP uptake was 66.13% and 46.97% of R1, 70.73% and 42.63% of R2, 72.93% and 42.40% of R3, 62.9% and 45.31% of R4 and 57.07% and 44.29% of R5, respectively.

Table 4 Properties of frozen seafood effluent after cultivation

Parameter	R1	R2	R3	R4	R5
pH	7.5±2.0	7.3±1.9	7.3±1.6	8.0±1.5	8.9±0.1
TKN (mg/L)	48.4±35.6	41.9±32.3	38.7±28.2	53.1±35.4	61.4±5.8
TP (mg/L)	9.3±4.5	10.0±5.0	10.1±5.8	9.6±4.5	9.8±5.3
COD (mg/L)	128±45.2	63.2±4.1	51.9±3.35	30.9±4.25	20.6±3.4
TDS (mg/L)	1,030±42.2	1,156±24.0	2,596±36.6	4,400±48.6	5,536±77.2
Turbidity (NTU)	4.6±1.2	4.1±1.4	3.4±0.0	4.3±0.1	3.4±0.1
Salinity (ppt)	1.2±0.1	1.3±0.1	2.8±0.0	4.2±0.2	5.8±0.2

4. Discussion

The pH in all photobioreactors was increased during *Chlorella vulgaris* cultivation. The previous research reported that the pH increased when the microalgae synthesized and consumed carbon dioxide at the daytime [4]. The salinity increment leads to increase the lipid content is known that high salinity may impact on the osmotic concentration in microalgae cell [23]. Additionally, the salinity is the important stress factor for microalgae, which induces to alter the metabolism for their nutrient uptake, toxic ion accumulation, osmotic stress, and oxidative stress [24]. In a plant cell, the reactive oxygen species (ROS) are generated under stress condition for example high salinity. Moreover, the oxidative stress induces to produce the ROS accumulation in microalgae. The ROS causes to change the function of cell in the structure of lipid peroxidation, protein oxidation and DNA destruction [25, 26]. Furthermore, the superoxide dismutase (SOD) and catalase (CAT) are the anti-oxidation enzymes. The enzymes are related to the detoxification of ROS and preventing of cell damage under stress condition [25]. Nevertheless, the increasing of salt condition led to reduced polyunsaturated lipid production [11]. The previous research reported that the salinity could be increased due to the evaporation of the daytime [6]. The salinity could block the photosynthesis but *Chlorella vulgaris* was able to grow normally because it was cultivated in the suitable salinity concentration [5]. Compared to other studies, the microalgae produced the lowest lipid content at 0.88% in freshwater treatment which was lower than the results above in this research [23].

TKN and TP could be uptake from 55.9% to 72.9% and 42.4% to 46.9%, respectively. The previous study showed that TKN and TP could be uptake from 72% to 85% and 57% to 77%, respectively, which were higher than the result of this research [18]. However, the other study demonstrated that total nitrogen (TN) and TP could be uptake from 11.9% to 74.3% and 22.5% to 94.8%, respectively [27]. Thus, the previous study result showed the similarity of TKN uptake efficiency range, and it was a higher range of TP uptake efficiency which are compared to this research. It can be assumed that *Chlorella vulgaris* has efficiently to uptake the nutrient from frozen seafood effluent. Under saline conditions, *Chlorella vulgaris* could adjust their biochemical identity, change biomass yield, and the efficiency of pollutant removal.

5. Conclusions

The effect of salinity concentrations on the lipid content of *Chlorella vulgaris* cultivation was observed in this research. The salinity concentrations of frozen seafood effluent before cultivation for R1, R2, R3, R4 and R5 was 1.34±0.01 ppt, 1.47±0.0 ppt, 2.96±0.04 ppt, 4.33±0.05 ppt and 6.11±0.05 ppt, respectively. The lipid content of *Chlorella vulgaris* after cultivation was 2.82±0.5%, 2.20±0.8%,

1.31±0.7%, 1.83±0.8%, and 0.68±0.1% for R1, R2, R3, R4 and R5, respectively. The salinity in effluent for cultivation could increase the lipid content of *Chlorella vulgaris*. But too high salinity concentration led to the slow growth of microalgae. The R1 (the original frozen seafood effluent) provided the maximum lipid content of *Chlorella vulgaris* after cultivation. Therefore, it can be concluded that the salinity concentration in the original frozen seafood effluent as a saline wastewater from the factory was enough for the *Chlorella vulgaris* cultivation, and the nutrients in frozen seafood effluent were also removed.

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7. References

[1] Chiu SY, Kao CY, Chen TY, Chang YB, Kuo CM, Lin CS. Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource. Bioresource Tech. 2015;184:179-89.
[2] Darvehei P, Bahri PA, Moheimani NR. Model development for the growth of microalgae: a review. Renew Sustain Energ Rev. 2018;97:233-58.
[3] Razzak SA, Ali SAM, Hossain MM, deLasa H. Biological CO_2 fixation with production of microalgae in wastewater-a review. Renew Sustain Energ Rev. 2017;76:379-90.
[4] Sajjadi B, Chen WY, Raman AAA, Ibrahim S. Microalgae lipid and biomass for biofuel production: a comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. Renew Sustain Energ Rev. 2018;97:200-32.
[5] Kebeish R, El-Ayouty Y, Hussein A. Effect of salinity on biochemical traits and photosynthesis-related gene transcription in Chlorella vulgaris. Egypt J Bot. 2014;54(2):281-94.
[6] Ahmad A, Buang A, Bhat AH. Renewable and sustainable bioenergy production from microalgal co-cultivation with palm oil mill effluent (POME): a review. Renew Sustain Energ Rev. 2016;65:214-34.
[7] Enamala MK, Enamala S, Chavali M, Donepudi J, Yadavalli R, Kolapalli B, et al. Production of biofuels from microalgae-a review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renew Sustain Energ Rev. 2018;94:49-68.
[8] Ishika T, Moheimani NR, Bahri PA. Sustainable saline microalgae co-cultivation for biofuel production: a critical review. Renew Sustain Energ Rev. 2017;78:356-68.
[9] Mohan SV, Rohit MV, Chiranjeevi P, Chandra R, Navaneeth B. Heterotrophic microalgae cultivation to synergize biodiesel production with waste remediation: progress and perspectives. Bioresource Tech. 2015;184:169-78.
[10] Bajwa K, Bishnoi NR. Osmotic stress induced by salinity for lipid overproduction in batch culture of *Chlorella pyrenoidosa* and effect on others physiological as well as physicochemical attributes. J Algal Biomass Utilization. 2015;6(4):26-34.
[11] Church J, Hwang JH, Kim KT, McLean R, Oh YK, Nam B, et al. Effect of salt type and concentration on the growth and lipid content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production. Bioresource Tech. 2017;243:147-53.
[12] Daneshvar E, Santhosh C, Antikainen E, Bhatnagar A. Microalgal growth and nitrate removal efficiency in different cultivation conditions: effect of macro and micronutrients and salinity. J Environ Chem Eng. 2018;6(2):1848-54.
[13] Wang T, Ge H, Liu T, Tian X, Wang Z, Guo M, et al. Salt stress induced lipid accumulation in heterotrophic culture cells of *Chlorella protothecoides*: mechanisms based on the multi-level analysis of oxidative response, key enzyme activity and biochemical alteration. J Biotechnol. 2016;228:18-27.
[14] Tasic MB, Pinto LFR, Klein BC, Veljkovic VB, Filho RM. *Botryococcus braunii* for biodiesel production. Renew Sustain Energ Rev. 2016;64:260-70.
[15] Ramaraj R, Unpaprom Y, Dussadee N. Cultivation of green microalga, *Chlorella vulgaris* for biogas purification. Int J Sci Tech Res. 2016;2(3):117-22.
[16] Chi NTL, Duc PA, Mathimani T, Pugazhendhi A. Evaluating the potential of green alga *Chlorella* sp. for high biomass and lipid production in biodiesel viewpoint. Biocatalysis Agr Biotechnol. 2019;17:184-8.
[17] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J biol chem. 1957;226(1):497-509.
[18] Sreesai S, Pakpain P. Nutrient recycling by *Chlorella vulgaris* from septage effluent of the Bangkok city, Thailand. Sci Asia. 2007;33(3):293-9.
[19] Adenan NS, Yusoff FM, Shariff M. Effect of salinity and temperature on the growth of diatoms and green algae. J Fish Aquat Sci. 2013;8(2);397-404.
[20] Mandalam RK, Palsso BO. *Chlorella vulgaris* (Chlorellaceae) does not screte autoinhibitors at high cell densities. Am J Bot. 1995;82(8):955-96.
[21] Daliry S, Hallajisani A, Roshandeh JM, Nouri H, Golzary A. Investigation of optimal condition for *Chlorella vulgaris* microalgae growth. Global J Environ Sci Manag. 2017;3(2):217-30.
[22] Cassidy KO. Evaluating algal growth at different temperatures. Kentucky: University of Kentucky; 2011.
[23] Suyono EA, Haryadi W, Zusron M, Nuhamunad M, Rahayu S, Nugroho AP. The effect of salinity on growth, dry weight and lipid content of the mixed microalgae culture isolated from Glagah as biodiesel substrate. J Life Sci. 2015;9:229-33.
[24] Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. 2006;45;523-39.
[25] Zhang Y, Xu Q, Xi B. Effect of NaCl salinity on the growth, metabolites, and antioxidant system of *Microcystis aeruginosa*. J Freshwat Ecol. 2013;28(4):477-87.
[26] Sun XM, Ren LJ, Zhao QY, Ji XJ, Huang H. Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. Biotechnol Biofuels. 2018;11(272):1-16.
[27] Khoo CG, Woo MH, Yury N, Lam MK, Lee KT. Dual role of *Chlorella vulgaris* wastewater treatment for biodiesel production: growth optimization and nutrient removal study. J Jpn Inst Energ. 2017;96(8):290-9.