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Enhancement of lipid production in marine microalga *Tetraselmis* sp. through salinity variation

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Abstract−The objective of this study was to enhance the lipid productivity in microalga *Tetraselmis* sp. through the salinity variation during cultivation. When marine alga *Tetraselmis* sp. was cultivated in a wide range of salinities, 0 through 70 practical salinity unit (PSU), enriched with F/2 medium, relatively low salinities below 35 PSU resulted in higher growth rates and lipid productivities under both N-deficient and -sufficient conditions, as compared to high salinities above 45 PSU. Nitrogen limitation did not stimulate lipid production in this species. Although high salinity increased lipid content, overall lipid productivities were lowered than those under low salinity conditions due to the decreased biomass production. When the salinity shifted from 35 to 22 PSU during cultivation, total lipid content increased from 20 to 26 (w/w) % within four days, and no significant change of fatty acids composition was observed.

Keywords: Microalgae, *Tetraselmis*, Lipid Production, Salinity Shift, Biodiesel

INTRODUCTION

Microalgae have been considered as a promising alternative feedstock for biodiesel production because of their higher photosynthetic efficiency, growth rate and oil yield in smaller area than that of conventional oil crops. They can contribute to fix carbon dioxide by photosynthesis for their growth and some microalgae have a good potential to produce bioethanol [1-3]. For achieving a high productivity of biodiesel from microalgae, it is important to increase both biomass production and cellular lipid content of microalgae cells. Microalgal species such as *Chlorella*, *Botryococcus*, *Dunaliella*, *Isochrysis*, *Nannochloris*, and *Scenedesmus* are widely used in lipid production, and they have oil content in the range of 15 and 50% [4]. It has been known that it is possible to enhance the cellular lipid content in microalgae under certain conditions of environmental stress, which enables us to achieve higher lipid productivity [5].

In some microalgae species, lipid accumulation can be induced through the starvation of nitrogen including nitrate, ammonium or urea [6]. It was also reported that phosphorous limitation could result in increasing lipid content in *Chlorella vulgaris* and *Nannochloropsis* sp. [7,8]. Limitations in certain mineral components can be used to accumulate lipid content [9]. Salinity changes also affect microalgal growth and cell composition, including lipids and carbohydrates, but the salinity tolerance and the responses to the salinity change are different from species to species [10-12].

Up to now, nitrogen starvation strategy has been applied successfully for enhancing lipid content in some microalgae species [13]. However, every microalga does not always accumulate lipids under nitrogen starvation condition, and the response to nitrogen deficiency is quite different depending on the microalgal species [8]. *Chlorella* and *Scenedesmus* accumulated lipid and carbohydrate simultaneously under nitrogen starvation condition [7,14], whereas *Dunaliella tertiolecta* accumulated carbohydrate, while lipid content decreased [15].

Our group has been interested in using marine alga *Tetraselmis* sp. for the purpose of marine culture with sea water on-site. *Tetraselmis* is a green microalga which has been used as an aquaculture feed, and it has been reported to have a wide salt tolerance, grow well in relatively cold sea water, and exhibit a high lipid content [16,17]. Some *Tetraselmis* strains are known to accumulate starch instead lipid under nitrate deprivation [18,19]. We also confirmed that several strains of *Tetraselmis* in our lab did not accumulate lipids under nitrogen starved condition through the preliminary experiments (data not shown).

Accordingly, we have searched other possible factors to stimulate lipid accumulation in *Tetraselmis* and found that they responded to the changes of salinity conditions. In this study, the influences of salinity changes on the cell growth and lipid production in *Tetraselmis* sp. were investigated. The salinity tolerance and lipid content were examined under a wide range of salinity values to select the most optimal salinity condition for the lipid production. Also, the change in the resulting profile of fatty acid methyl esters (FAME) accompanied by salinity shift was discussed.

MATERIALS AND METHODS

1. Microalgae Strain and Culture Medium

Marine microalga *Tetraselmis* sp. KCTC 12236BP (Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology) [17] was cultivated in Marine Biological Laboratory (MBL) artificial seawater enriched with F/2 medium which is composed of nutrients containing (g L^{-1}) $NaNO_3$, 0.075; $NaH_2PO_4 \cdot H_2O$, 0.005, trace metals and vitamins containing (mg L^{-1}) $FeCl_3 \cdot$

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$6H_2O$, 3.15; $Na_2EDTA{\cdot}2H_2O$, 4.36, $CuSO_4{\cdot}5H_2O$, 0.0098, $Na_2MoO_4{\cdot}2H_2O$, 0.0063; $ZnSO_4{\cdot}7H_2O$, 0.022; $CoCl_2{\cdot}6H_2O$, 0.01, $MnCl_2{\cdot}4H_2O$, 0.18, thiamine HCl (vitamin B_1), 0.2; biotin (vitamin H), 0.001 and cyanocobalamin (vitamin B_{12}), 0.001. MBL artificial seawater has the composition: 24.72 g/L of NaCl, 0.67 g/L of KCl, 1.36 g/L of $CaCl_2{\cdot}2H_2O$, 4.66 g/L of $MgCl_2{\cdot}6H_2O$, 6.29 g/L of $MgSO_4{\cdot}7H_2O$, 0.18 g/L of $NaHCO_3$ and 0.606 g/L of Tris (adjusted to pH 8.2, to make salinity 35 practical salinity unit (PSU)).

2. Cultivation Conditions

Tetraselmis stock culture was inoculated in 250 mL Erlenmeyer flask containing 200 mL sterilized MBL artificial seawater with F/2 nutrients, and incubated in a shaking incubator at 160 rpm and 25 °C under continuous fluorescence illumination of 50 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ light intensity.

Cells were transferred from the stock culture to a conical-ended cylindrical photobioreactor (2 L working volume, 10 cm diameter) in an isolated cultivation room. For achieving aeration and mixing, sterilized ambient air was supplied to the reactor through a 0.2 μm PTFE membrane at a rate of 0.2 vvm. The volumetric gas supply was regulated by gas flow meter (RATE-MASTER, Dwyer, USA) and mass flow controller (MPR-2000, KITS, Korea). Temperature in the cultivation room was maintained at 20-25 °C and light intensity of 110-120 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ was continuously provided from both sides of the photobioreactor using white fluorescent lamps (FL20SPG/18, Kumho, Korea). The light intensity was checked by a light intensity meter (LI-COR, LI-250A, USA).

3. Experiments with Salinity Variation

3-1. Cultivation under a Wide Range of Salinity

Culture media with different salinities, 0 through 70 PSU, enriched with F/2 nutrients were prepared by adjusting MBL artificial seawater components. The inoculum cell density was 0.7 g/L as dry weight and the working volume was 800 mL. N-sufficient condition means that nitrate concentration was maintained above 75 mg/L $NaNO_3$, which is the original N concentration in F/2 medium, during the whole cultivation period by supplying $NaNO_3$ intermittently. N-deficient condition refers that no further nitrate was supplemented after the initial nitrate (75 mg/L $NaNO_3$) was all consumed within two days.

3-2. Stepwise Change in Salinity

Salinity was decreased stepwise from 35 to 9 PSU in a single photobioreactor. *Tetraselmis* cells were firstly inoculated to the medium with normal salinity of 35 PSU (inoculum cell density of 1.6 g/L in 1 L working volume). Once cell growth and lipid content were acclimated in the given salinity, salinity was lowered by replacing 200 mL of the spent medium to the fresh salt-deleted F/2 medium. Before changing salinity, a fraction of cells was harvested for the analysis of total lipid content and cell growth. Nitrate concentration was maintained above 75 mg/L $NaNO_3$.

3-3. Cultivation at Low Salinity Below 35 PSU

Exponentially-growing cells at 35 PSU were transferred equally to three reactors with different salinity conditions: (1) salinity 22 PSU, (2) salinity 28 PSU initially and then lowered to 22 PSU further after 1 day, and (3) salinity 35 PSU as a control. Inoculum cell density was 1.2 g/L for all three cases and nitrate was provided sufficiently and maintained above 75 mg/L $NaNO_3$. Experiment was performed in duplicate.

4. Measurement of Cell Growth

Microalgal growth rate was determined by measuring the optical density at a wavelength of 680 nm (OD_{680}) using a UV/VIS spectrophotometer (DR-4000U, Hach, USA). The dry cell weight (DCW) was measured by filtering 5 mL aliquots of culture broth through a glass microfiber filters (0.7 μm pore size, 47 mm diameter, Whatman, UK) which was pre-dried and pre-weighed. Before filtration, the cells were washed three times using 0.5 M ammonium bicarbonate solution to remove the interference by salts [20]. After washing and filtering, each loaded filter was dried at 80 °C for 12 h and kept in a desiccator until the weight was invariant. The relationship between OD_{680} absorbance and dry cell weight was established ($DCW=0.6045\times OD_{680}-0.0346$, $R^2=0.9991$).

5. Lipid Analysis

Total cellular lipids were extracted and the content was determined by gravimetric method [21]. From the culture broth, 50 mL sample was harvested by centrifugation at 3,000 rpm for 5 min and dried in a vacuum drying oven at 40 °C for 12 h. The dried cells were ground to be homogeneous and 9.5 mL of chloroform/methanol/water (1/2/0.8, v/v/v) was added to 50.0±0.1 mg of moisture-free biomass. The mixture was ultrasonicated for 1 min (100 W and 20 kHz, VCS 130, Sonics & Materials Inc., CT, USA) and mixed vigorously for 1 min by vortex mixer. Chloroform (2.5 mL) and distilled water (2.5 mL) were added and ultrasonicated by the same procedure above. After centrifugation at 3,000 rpm for 5 min, the upper layer of methanol with water was discarded, and the remaining cells in the middle layer were used for the second lipid extraction according to the same procedure as above. The bottom phase (chloroform layer) was transferred into a pre-weighed aluminum dish and evaporated in a drying oven at 80 °C. After the dish was cooled to room temperature in a desiccator, the weight of loaded dish was measured and the extracted amount of lipid was calculated by subtracting the weight of empty dish from loaded one. The total lipid content was expressed as the weight % of dried cell weight.

6. Transesterification and FAME Analysis

Acid-catalyzed direct-transesterification was performed according to Wychen and Laurens (2013) with some modifications [22]. Moisture-free biomass sample (50.0 mg) was prepared by drying in a vacuum oven at 40 °C overnight. 1.0 mL of chloroform:methanol (2 : 1, v/v) and 1.5 mL of 0.6 M HCl : methanol was added into the prepared biomass sample in a glass tube. The vials were sealed with the PTFE/silicone/PTFE crimp caps and mixed vigorously. The sealed vials were heated at 85 °C for 1 hour and cooled for 15 minutes at room temperature. After cooling, 5.0 mL HPLC grade *n*-hexane was added and mixed. The vials were left undisturbed at room temperature for 1 hour to allow a phase separation. After transferring the hexane layer into a new vial, 1 μL was taken by microsyringe, and the fatty acid methyl ester (FAME) composition was analyzed by GC-FID (YL6500 GC, Younglin Instrument, Korea) equipped with HP-INNOWAX capillary column (Agilent 19091N-213) under the conditions: 1 μL injection at 10 : 1 split ratio, inlet temperature of 260 °C, constant flow of 1 mL/min helium, oven temperature of 140 °C for 5 min, 4 °C/min up to 240 °C and hold for 10 min, FID temperature of 260 °C, flowrate of 300 mL/min zero air, 35 mL/min H_2, 20 mL/min helium. The FAME Mix (14 compound calibration mixture of C8:0-C24:0, Sigma Aldrich #18918)

was used as FAME standard. The GC analysis of FAME composition was conducted in triplicate for all samples.

7. Carbohydrate Analysis

Total carbohydrate content was analyzed by phenol-sulfuric acid method [23]. From the harvested culture broth, 0.1 mL was diluted to 0.5 mL, and 0.5 mL of phenol (5 w/w %) was added. 2.5 mL of concentrated sulfuric acid was added into the mixture, and immediately mixed on a vortex mixer vigorously. The tubes were left for 10 min and cooled to room temperature. The absorbance was measured at 490 nm against a reagent blank with a spectrophotometer.

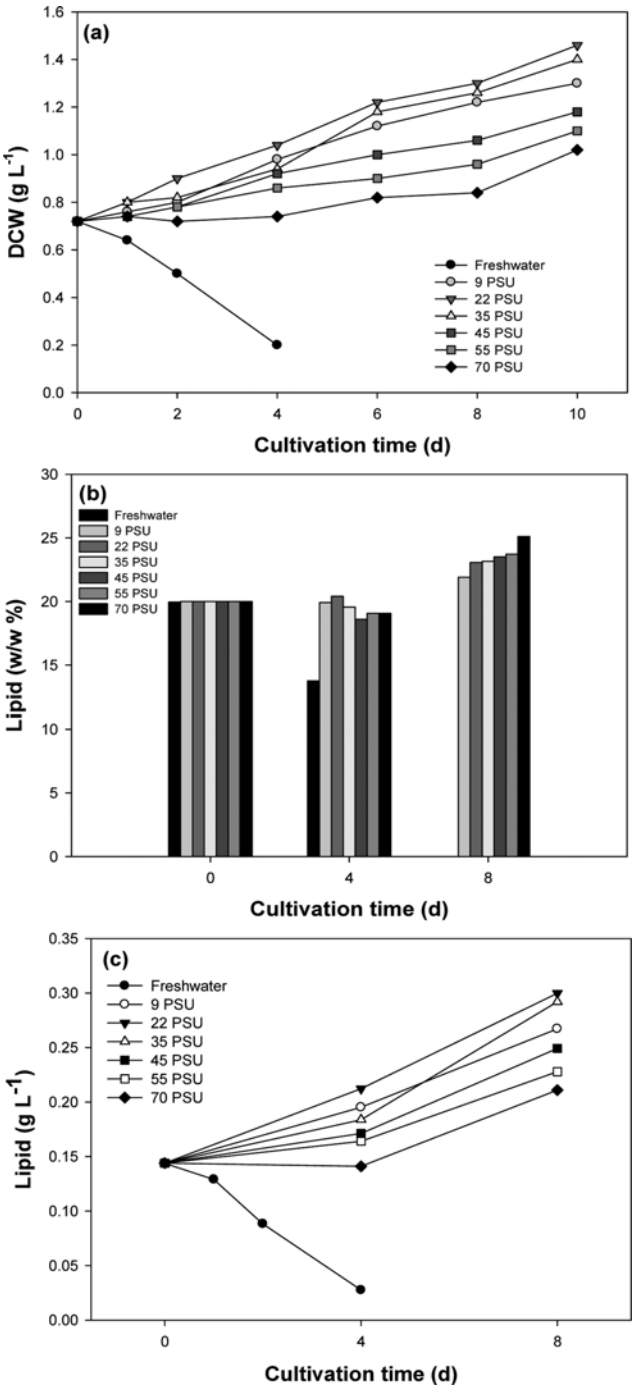

Fig. 1. (a) Cell concentration, (b) cellular lipid content and (c) lipid concentration per reactor volume under a wide range of salinity with nitrate repletion maintaining above 75 mg $NaNO_3$/L.

RESULTS AND DISCUSSION

1. Effect of Nitrogen Limitation and Salinity

To examine the effect of salinity on the cell growth and lipid production in *Tetraselmis* sp., cell concentration and lipid content were monitored under a wide range of salinity (0-70 PSU). Also, the influence of salinity variation was compared between N-sufficient and N-deficient conditions.

Fig. 1 shows the cultivation results at N-sufficient condition where the level of nitrogen was maintained above 75 mg/L of $NaNO_3$ by supplying it intermittently. *Tetraselmis* cells did not survive freshwater condition where their population decreased quickly. Although normal sea water salinity is 35 PSU, cell growth was highest at 22 PSU, which is 37% lower salinity compared to 35 PSU (Fig. 1(a)). Cell growth at 9 PSU was comparable to that at 35 PSU. Cell growth was inhibited at higher salinity conditions above 45 PSU. Results showed that cell growth rates tended to be higher at relatively low salinity range than those at high salinity range.

Fig. 2 shows the results at N-deficient condition where no further nitrate was supplemented during the cultivation, except the initial concentration of nitrate (75 mg/L as $NaNO_3$) in the culture medium. Nitrogen level decreased quickly and was completely consumed within two days in all cultures (data not shown). Similarly to N-sufficient condition (Fig. 1(a)), higher cell growth rates were obtained under relatively low salinity range including 35, 22 and 9 PSU, compared to those under high salinities above 45 PSU (Fig. 2(a)), although overall growth rates were smaller than those under N-sufficient condition (Fig. 1(a)).

Under both N-sufficient and -deficient conditions, high salinity conditions showed lowered cell growth and resulted in relatively low final biomass concentrations. The inhibition in cell growth under high salinity environment might be due to a high external ionic concentration and excessive ion flux into the cells [24,25]. Cellular water potential can be decreased by osmotic stress under high salinity, inducing excessive uptake of salt ions into the cells, and consequently cellular ion imbalance is caused [26]. Although some microalgae and cyanobacteria were reported to have salt tolerance up to 1.7 M of salt concentration, high salinity above 35 ppt may cause negative effects on both cell growth and efficiency of photosynthesis [27]. Takagi et al. [28] found that the increase of NaCl concentration above 1.0 M up to 2.0 M markedly inhibited the cell growth in marine microalga *Dunaliella tertiolecta* [28]. Freshwater green microalgae species such as *Scenedesmus obliquus* and *Chlorella vulgaris* were known to show inhibition of cell growth with an increase of salinity [12,29].

In the case of cultivation under fresh water condition (Fig. 1(a) and Fig. 2(a)), biomass concentrations were markedly reduced and the cells became dead within four days in both N-deficient and -sufficient conditions. Zhu et al. [30] reported that when salinity dereased to 0 (% w/v), cell growth rate of *Schizochytrium limacinum*, a marine fungus, was reduced significantly [30]. When some plants and algae encounter salinity variation, they are able to overcome external osmotic variations by osmolytes mechanism, controlling of water

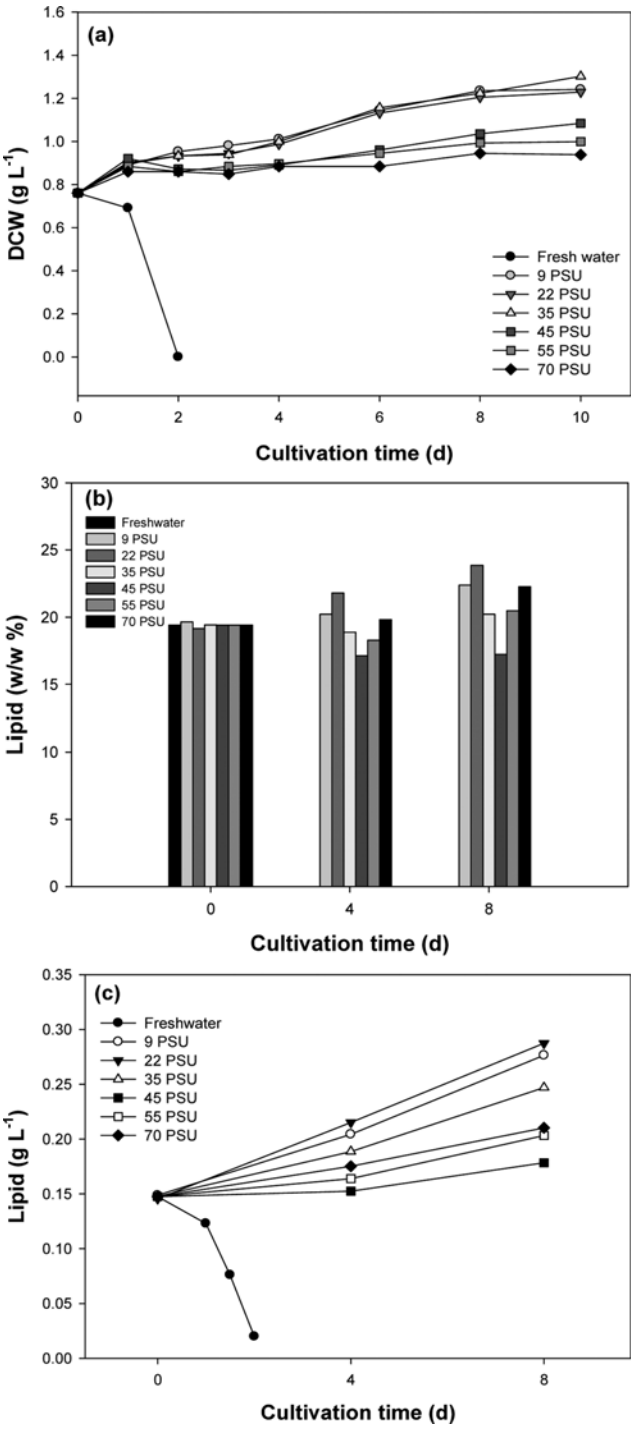

Fig. 2. (a) Cell concentration, (b) cellular lipid content and (c) lipid concentration per reactor volume under a wide range of salinity with nitrate limitation. Nitrate was added only one time at the beginning of the cultivation as 75 mg $NaNO_3$/L and consumed all within 2 days.

and ion fluxes [31]. When the salinity decreased, external water potential increased, leading to the uptake of water into the cell, consequently resulting in the expansion of cell volume and internal osmotic pressure against the cell membrane to adjust osmotic balance. However, the current *Tetraselmis* cells seemed not to tolerate the freshwater condition, and the osmotic adjustment was not achieved under the extremely low salinity condition, causing lethal damages in cell membranes or organelles [32]. Since *Tetraselmis* is a marine species, it is considered that certain optimal concentration of salts is required for retaining the osmotic balance [24]. The current *Tetraselmis* sp. which was used in this study was tolerable to a wide range of salinity (except fresh water) and, from the results in Figs. 1 and 2, it seemed that the standard sea water salinity value (35 PSU) is not optimal for the growth, and a lower salinity such as 22 PSU is preferred.

Figs. 1(b) and Fig. 2(b) show the change of lipid content under different salinity conditions. Under N-sufficient condition (Fig. 1(b)), lipid content which was about 20.0% before seeding (day 0, at 35 PSU) tended to decrease a little for the first four days, and then increase during next four days. The extent of increase in lipid content was highest at 70 PSU, increasing up to 25.1% on day eight. Under N-deficient conditions, lipid content was gradually increasing overall as time passed (Fig. 2(b)), the extent of increase was higher in low (9 and 22 PSU) and high salinity (55 and 70 PSU) conditions than those of middle range of salinity (35 and 45 PSU). Although a lower range of salinity is preferred to cell growth in this species, the low or high salinity is considered as an environmental stress factor because an adaptation including cell size change is required to adjust cells in new osmotic condition [28]. The induction of lipid synthesis is usually triggered by alleviation of photosynthesis under a variety of stress conditions, where excess electrons in NADPH or ATP are scavenged through the conversion of glyceraldehyde-3-phosphate to TAG [33].

From the comparison between Figs. 1(b) and 2(b), the extents of lipid content that increased under N-deficient conditions was not higher than that under N-sufficient conditions. This observation is unique because N-deficient or starvation condition has been widely used as a strategy for lipid synthesis induction condition in many microalgae species. It is well known that N-limitation converts the fixed carbon from protein synthesis to lipid or carbohydrate synthesis [13]. However, N-limitation did not show positive influence on lipid synthesis in the current *Tetraselmis* sp., and therefore there is no advantage in lipid production because N-deficient condition reduces cell growth rate, consequently lipid productivity.

Under both N-sufficient and deficient conditions, high lipid content was obtained at high salinity conditions. The reason for the increment of lipids under high salinity might be attributed to the accumulation of polar lipids as a response to the salt-stress condition. It was reported that marine diatom *Nitzschia laevis* accumulated polar lipids for tolerating high salinity stress by decreasing membrane permeability and fluidity [26]. However, although the highest lipid content was achieved at salinity 70 PSU in this study (Fig. 1(b)), the lipid concentration per reactor volume achieved on day eight was the lowest due to the lowered biomass production at 70 PSU (Fig. 1(c)). The results of Figs. 1 and 2 indicated that, for the current *Tetraselmis* sp., the low salinity stress below 35 PSU under N-sufficient condition increased both cell growth rate and lipid content, resulting in higher lipid productivities.

2. Effect of Stepwise Salinity Change on the Cell Growth and Lipid Content

Previous results verified that the low salinity below 35 PSU was

advantageous to achieve increased biomass and lipid production than high salinity condition. Therefore, to figure out the acclimation behavior of cells to the salinity shift from 35 PSU to lower values, changes in growth recovery and cellular lipid content were monitored through stepwise lowering of salinity value under N-sufficient cultivation. Culture at the standard 35 PSU was diluted after 0.5 d by adding non-salt medium to create lowered salinity condition, 28 PSU. The recovery of the cell density to the initial level through acclimation was checked, and it took two days at 28 PSU. Then the dilution-recovery cycles were repeated stepwise to create 22, 18, 14 PSU, and so on, and the difference in the required period of acclimation was observed.

As shown in Fig. 3(a), right after lowering salinity at all changing points, cell density decreased temporarily due to the dilution. But, cells soon acclimated to the new lowered salinity environment and cell density increased again gradually. The required time for acclimated recovery at 28 and 22 PSU was within two days. However, under further low salinities below 18 PSU, longer periods of time were required for recovering cell density, implying that the acclimation to achieve normal cell growth became difficult when salinity was too low. Fig. 3(b) shows the lipid content in the cells at the end of culture under the specified salinity value (right before next dilution). Lipid content was 20.0% at the beginning of the cultivation (35 PSU), and increased to 26.4% when the salinity decreased to 22 PSU. The enhancement of lipid content was main-

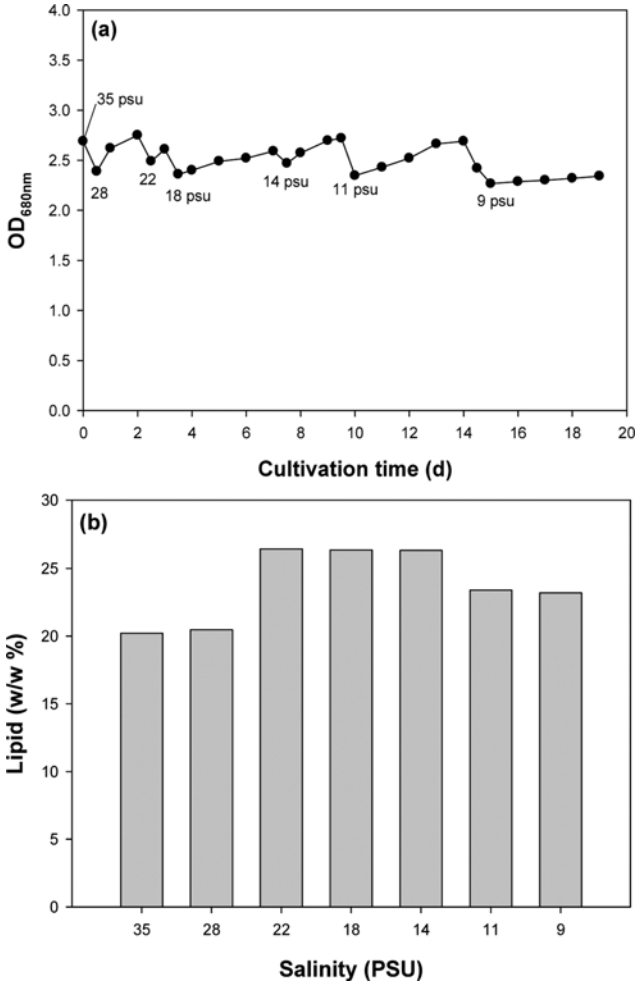

Fig. 3. Changes in (a) cell concentration and (b) total lipid content during stepwise salinity shift. Total lipid contents were analyzed right before shifting salinity.

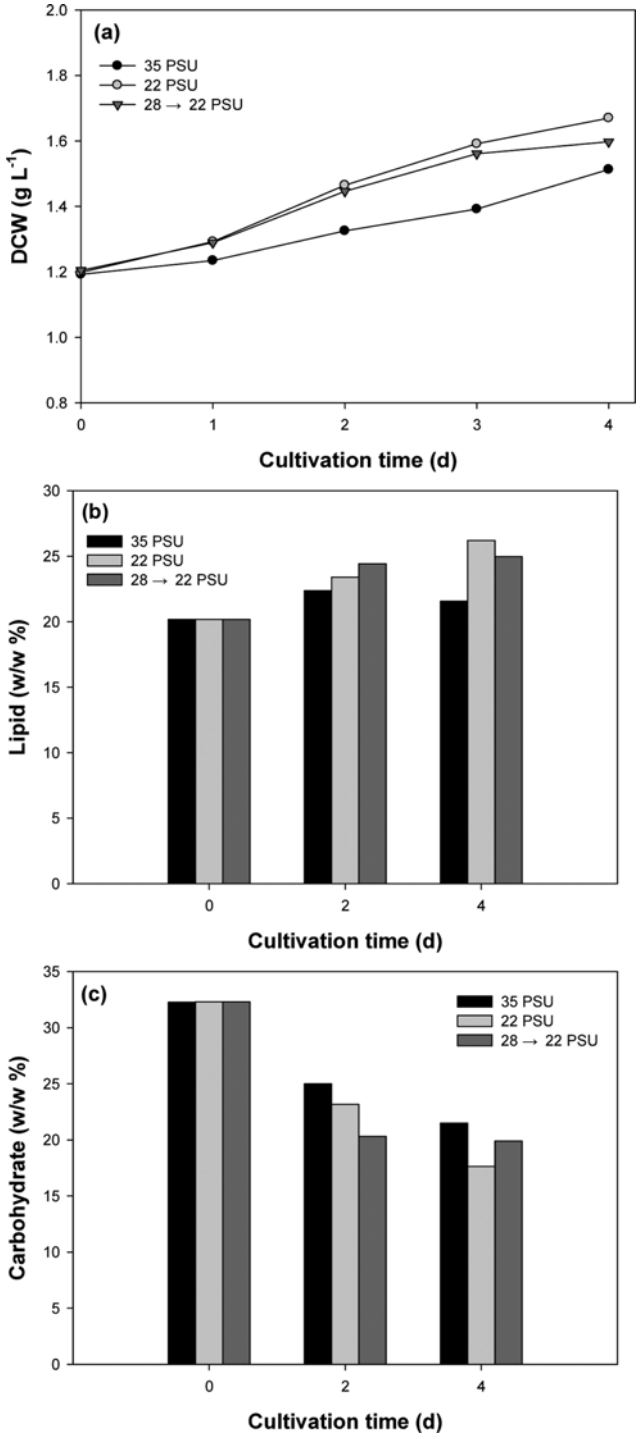

Fig. 4. Changes in (a) cell concentration, (b) total lipid content and (c) carbohydrate content after shifting salinity from 35 PSU to 22 PSU directly; to 28 PSU first and then 28→22 after 1 day; and to 35 PSU as a control.

Table 1. Comparison in lipid content and productivity of *Tetraselmis* strains

Microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/d)	Reference
Tetraselmis suecica	8.5-23.0	27.0-36.4	[8]
Tetraselmis sp.	-	18.6-22.7	[16]
Tetraselmis sp.	12.6-14.7	43.4	[8]
Tetraselmis sp.	20.7-25.2	21.4-48.9	This study

tained during further decrease in salinity to 18 and 14 PSU. However, lipid content was lowered to 22-23% under salinity 11 PSU and 9 PSU. Since the 18 and 14 PSU required longer time for the acclimation than 22 PSU, salinity value of 22 PSU was considered optimal for achieving high lipid productivity.

3. Cultivation at Low Salinity below 35 PSU

Since 22 PSU salinity condition proved to be the best for lipid production, different approaches to reach 22 PSU from normal 35 PSU were compared under N-sufficient condition: (i) direct change from 35 to 22 PSU and (ii) two step change from 35 to 28 (for 1 day) and then to 22 PSU. As shown in Fig. 4(a), the direct change to 22 PSU and two step change were similarly better than 35 PSU culture. Salinity 22 PSU culture resulted in the highest biomass concentration of 1.67 g/L on day four after salinity change. The biomass production by stepwise decreasing salinity was slightly lower than direct decreasing to 22 PSU. Biomass productivity of 0.118 g/L/d in salinity 22 PSU was 1.5-fold higher than that under salinity 35 PSU (0.08 g/L/d), indicating that salinity 22 PSU with sufficient nitrate is a favorable condition for biomass production in *Tetraselmis* sp. in accordance with the previous results in Fig. 2(a).

The optimal salinity for microalgal cell growth depends on species to species, and their response to salinity variation is also species specific [34]. Bartley et al. [11] reported that the optimal salinity for growth of marine microalga *Nannochloropsis salina* was also below standard sea water salinity [11]. Marine microalgae *Isochrysis* sp., *Nannochloropsis oculata* and diatom *Nitzschia* were reported to show a higher growth rate under salinity 20 ppt when they were tested under salinity from 10 to 35 ppt [35].

When salinity shifted from 35 to 22 PSU, lipid content increased from 20.2 to 26.2% on day 4 (Fig. 4(b)), corresponding to 49 mg/L/d of productivity, which is more than 2-fold higher than that in control culture at 35 PSU (21.4 mg/L/d). The enhancement of lipid production under 22 PSU with N-sufficient condition is attributed to both higher biomass concentration and increased lipid content, compared to those under 35 PSU. On the other hand, carbohydrate content decreased at salinity 22 PSU, as seen in Fig. 4(c). The opposite trend between lipid and carbohydrate implies that the carbon flux changed from carbohydrate synthesis to lipid synthesis as a response to the lowered salinity. It is known that lipid accumulation is usually induced by environmental stressful conditions such as nutrient limitation, which is unfavorable to microalgal growth, due to the decrease in photosynthetic activity [6,13]. However, *Tetraselmis* sp., which was used in the present study, exhibited an increase in lipid content as well as in biomass production at low salinity environment. The salinity shift from 35 to 22 PSU resulted in a higher lipid productivity than constant salinity 35 PSU, inducing both faster cell growth and increase in lipid accumulation. Pal et al. [36] reported similar phenomenon in *Nannochloropsis* sp. that both biomass and total fatty acids were increased under 13 g/L NaCl with nitrogen-depleted condition, among three different salinity ranges of 13, 27 and 40 g/L NaCl [36]. On the contrary, Bartley et al. [11] reported that when the salinity changed from 22 to 34 PSU, the lipid productivity increased due to both enhanced lipid content and biomass production in *Nannochloropsis salina* [11].

Utilization of salinity shift could be one way to achieve simultaneous enhancement of biomass and lipid production without halt or loss of biomass productivity. Biomass productivity is usually sacrificed when the strategy of limitation or starvation of major nutrients (nitrogen or phosphate) is used for the enhancement of lipid production [7]. Lipid productivity in this study was improved a little, when compared to the previous studies with *Tetraselmis* strains (Table 1).

4. FAME Profiles

Fatty acid composition of biodiesel is an important factor influencing fuel quality because the ratio of saturation/unsaturation governs low temperature fluidity and oxidative stability [26]. Some important fuel properties, such as cetane number, viscosity, cold flow property, oxidative stability and lubricity, are determined by the fatty ester composition of the biodiesel fuel. The cold flow property and oxidative stability are especially closely related to the major

Table 2. Fatty acid composition of FAME biodiesel at different salinity conditions

Fatty acids	Fatty acids (%)	
	Salinity 35 PSU	Salinity 22 PSU
C10:0	0.43±0.38	3.93±0.4
C14:0	1.20±0.10	1.33±0.06
C16:0	33.07±0.46	33.03±0.42
C16:1	3.10±0.17	3.10±0.10
C17:0	2.00±0.36	2.83±0.12
C16:4	5.73±0.06	4.87±0.06
C18:0	1.00±0.10	1.20±0.00
C18:1	27.10±0.40	24.33±0.15
C18:2	6.33±0.12	7.10±0.17
C18:3	13.23±0.55	11.60±0.17
C18:4	3.27±0.21	3.27±0.21
C22:0	3.53±0.21	3.40±0.44
SFA	41.23	45.73
UFA	58.77	52.23
MUFA	30.20	27.43
PUFA	28.57	24.80

Data expressed as mean±SD (n=3)

fatty acid components. The biodiesels produced from the microalgae containing a high fraction of saturated fatty acids (SFAs) will have poor cold-flow properties. However, if the fraction of unsaturated fatty acids (UFAs) is too high, they will have poor oxidative stability. Microalgal FAMEs usually possess higher UFA fraction than those of conventional energy crops [37].

To investigate the influence of different salinity on the fatty acid compositions, fatty acids of the FAME biodiesel obtained from 35 and 22 PSU cultures were compared (Table 2). The most abundant fatty acids in *Tetraselmis* sp. were palmitic acid (C16:0) and oleic acid (C18:1). Palmitic acid, oleic acid, linoleic acid (C18:2) and linolenic acid (C18:3) accounted for more than 70% of the fatty acids in *Tetraselmis* sp. under both salinity conditions. Bondioli et al. (2012) and Huerlimann et al. (2010) also reported similar FAME compositions that palmitic, oleic and linolenic acids were the major component of fatty acid in *Tetraselmis* sp. [16,19].

The FAME composition of microalgae is known to be different from species to species, and it can be changed due to the different nutrition and cultivation conditions. Some other marine microalgae showed quite different results in fatty acid composition against salinity shifting. Renaud and Parry [35] reported that the most abundant fatty acid in marine microalga *Isochrysis* sp. was myristic acid (C14:0), but UFAs increased by 14% under salinity 20 PSU compared to salinity 35 PSU [34]. Whereas, *Nannochloropsis aculata* had high contents of palmitic acid (C16:0), palmitoleic acid (C16:1) and eicosapentaenoic acid (C20:5), and no significant change was observed in the SFA/UFA ratio under salinity change between 20 and 35 PSU.

In this study, the fatty acid compositions did not change significantly by salinity shift. As the culture shifted from salinity 35 to 22 PSU, the fraction of SFAs slightly increased from 41.2 to 45.7%, while unsaturated fatty acids (UFAs) decreased from 58. 8 to 52.2%. The reduction of UFA portion would be desirable in that high content of UFAs such as linolenic acid (C18:3) causes oxidative instability [38].

CONCLUSIONS

Marine microalga *Tetraselmis* sp. showed a high salinity tolerance under a wide range of salinity, 9 through 70 PSU. A high salinity of 70 PSU exhibited an increase in lipid content; however, the overall lipid productivity was the lowest due to the lowered cell growth rate. Relatively low salinities below 35 PSU showed higher biomass concentrations, resulting in higher lipid productivities, than those under high salinities above 45 PSU. When the salinity shifted from 35 to 22 PSU, the lipid production was enhanced, without significant change in fatty acids composition. Lipid productivity was enhanced under salinity 22 PSU with N-sufficient condition, compared to 35 PSU which is the standard sea water condition. Salinity shift can be an option to enhance lipid production without limiting any major nutrients, which can sacrifice active cell growth.

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