

Advanced treatment of secondary effluent using filamentous algae

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ABSTRACT

In this study, we investigated the cultivation possibility using *Hydrodictyon reticulatum* in a continuous raceway pond as a tertiary sewage treatment plant. The cultivation possibility was evaluated by varying the light quantity, wavelength, and hydraulic retention time (HRT). Experimental results showed that the growth rates of algae and the removal efficiencies of nutrients increased as the light quantity increased, and the maximum photosynthetic rate was maintained at 100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ or higher. When wavelength was varied, nutrient removal efficiency and growth rate increased in the following order: green light, red light, white light, and blue light. The nutrient removal efficiencies and algae productivity in HRT 4 d were better than in HRT 8 d. We conclude that if *Hydrodictyon reticulatum* is cultivated in a raceway pond and used as a tertiary treatment facility in a sewage treatment plant, nutrients can be effectively removed, and production costs can be reduced.

1. INTRODUCTION

The incomplete removal of nutrients from sewage treatment plants causes eutrophication of rivers. However, since domestic sewage has very low organic matter content, the ability to effectively remove nitrogen and phosphorus through biological treatment process is limited. In Korea, phosphorus chemical treatment of effluent from sewage treatment plants has been introduced, and phosphorus concentration of effluent water has been reduced. There is a limit to the scalability of this method, and a large amount of sludge is generated, which can result in many problems (Kim 2017; Lee 2017a). Recently, several studies of algal pond technology, using microalgae to accumulate nitrogen and phosphorus as a tertiary sewage treatment, have been introduced (Park 2009).

Algae have been used extensively in the past for sewage treatment using ponds and lagoons (Park 2009). In the early 1980s, the possibility of nutrient recovery was investigated in the secondary treatment of sewage after establishing an artificial ecosystem including algae on a laboratory scale in the US (Kim 2013; Chan 2013). However, tertiary treatment by algae has not been widely used for a variety of reasons, but recently it has regained scientific attention in Norway and Sweden (Tepe 2006).

The wastewater treatment using algae can remove nutrients and produce useful biomass raw materials for organic fertilizer, feed and bio-energy (Wilkiea 2002; Park 2014; Lee 2017). Meanwhile, unicellular algae species that form colonies 50-200 μm in diameter have been used in wastewater treatment since they can be economically harvested (Caggs 2011; Park 2011). However, filamentous algae, such as *Hydrodictyon reticulatum* (family *Hydrodictyaceae*), can grow to more than several centimeters in size, which can reduce harvest costs (Tas 2011; Nguyen 2012; Lee 2017). The studies on filamentous algae have been actively carried out to improve economic efficiency (Hawes 1993; Hall 1997; Lee 2017a; Lee 2017b).

Algae is dependent on carbon dioxide and light quantity as an autotrophic organism (Wijffels 2010; Kim 2013; Teo 2014). Generally, when there is no nutrient limitation, it is very important to supply a large light quantity because the efficiency of photosynthesis increases in proportion to the amount of light quantity until reaching the saturation point of light (Richmond 2004). Research on the supply of large quantities of light has been conducted mainly in closed systems for recent years, but there are no cases in the open systems (Kim 2013; Teo 2014).

Large-scale cultivation systems for algae can be divided into two categories: open systems, which use raceway ponds, and closed systems, which use photobioreactors. Open systems have the advantages of low initial investment and easy maintenance. However, open systems require large areas for set up and are susceptible to contaminate with other algal species and bacteria. An additional disadvantage is that biomass productivity can be lower because the low solubility of carbon dioxide due to low water depth and top opening (Haag, 2007). In contrast, closed systems are convenient for operation because they have high cell growth rate and reduced probability of external pollution, are easy to scale-up, and can theoretically maximize the surface area available for incident light. However, when tested with actual sewage, and the experiment was scaled up, the contact area of light decreased, and light irradiation efficiency into the reactor was decreased. Consequently, nutrient removal efficiency and economic efficiency are very low because the growth of cells is greatly reduced (Hsieh 2009; Boonchai 2012; Choi 2013). Therefore, it is reasonable to apply an open system in order to improve economic efficiency. However, few studies have investigated the application of filamentous algae to a raceway pond for nutrient removal.

The purpose of this study was to investigate the cultivation possibility for the tertiary treatment process and the productivity of algae using *Hydrodictyon reticulatum* in an open system continuous raceway pond to treat sewage treatment plant effluent. For this purpose, quantitative evaluation of algae productivity, growth rate and nutrient removal efficiencies of sewage treatment plant effluent were carried out by varying light quantity, wavelength, and hydraulic retention time (HRT).

2. MATERIALS AND METHODS

2.1 *Hydrodictyon reticulatum* cultivation

In this study, *Hydrodictyon reticulatum* was obtained from Korea Research Institute of Chemical Technology. The *Hydrodictyon reticulatum* was cultured using MDM (Modified Diatom Medium). The cultured temperature was maintained $25 \pm 1^\circ\text{C}$

using a low temperature incubator (BI-81, HYSC Inc., Korea). The light quantity and light-dark cycle was maintained 100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ at 12 hour intervals using LED lamp (IOLUX 28W, IOLUX Lighting Inc., China). The influent used in the experiment is the secondary clarifier effluent of a sewage treatment plant located in Ansan city, Korea (Table 1). All distilled water used in the experiment was sterilized using an autoclave (JSAC-60T, JS Research Inc., Korea).

Table 1 Final effluent characteristic from the sewage treatment plant

Parameter	Concentration (Average \pm S.D.)
pH	6.81 \pm 0.11
COD (mg/L)	13.38 \pm 1.79
TN (mg/L)	7.19 \pm 0.68
NH ₄ ⁺ -N (mg/L)	0.18 \pm 0.08
NO ₃ ⁻ -N (mg/L)	5.84 \pm 0.62
NO ₂ ⁻ -N (mg/L)	0.04 \pm 0.01
TP (mg/L)	1.07 \pm 0.81
PO ₄ ³⁻ -P (mg/L)	0.97 \pm 0.84

2.2 Light quantity

Plants use light energy between 400 and 700 nm, known as photosynthetically active radiation (PAR). Light quantity for plant growth is measured by the instantaneous photosynthetic photon flux density in the PAR region. To determine the optimum light radiation, the distance between the white LED (IOLUX 28W, IOLUX Lighting Inc., China) and the water surface was controlled so that light quantity was 25, 50, 75, and 100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. And the case where light was not irradiated under the same conditions (0 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) was tested. A 410 (L) \times 300 (W) \times 200 (H_e) rectangular box made of open only to the top was used to prevent light entry from the outside. After the installation, *Hydrodictyon reticulatum* 0.059 \pm 0.002 g (dry weight) was placed in the box. The growth rate of *Hydrodictyon reticulatum* and the nutrient removal efficiencies were analyzed for 5 days by a light irradiation at intervals of 12 hours in water temperature of 25 \pm 2 °C. The culture medium was used after sterilization of the final effluent of the Ansan sewage treatment plant and all experiments were repeated 3 times.

2.3 Wavelength of light

A blue light (470 \pm 5 nm), a green light (525 \pm 5 nm), a red light (625 \pm 5 nm), and a white light (450-750 nm), respectively, were used to select the optimum wavelength of the light source. Initially, *Hydrodictyon reticulatum* (0.056 \pm 0.04 g, dry weight) was injected into a transparent acrylic rectangular box, and light was irradiated at intervals of 12 hours in a water temperature of 25 \pm 2 °C, and light of 100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ was irradiated. The growth rate of *Hydrodictyon reticulatum* and the nutrient removal efficiencies were analyzed by operating the reactor for 5 days. The culture medium was used after sterilization of the final effluent of the Ansan sewage treatment plant and all experiments were conducted in triplicate.

2.4 Hydraulic retention time

A lab-scale raceway pond reactor was prepared to conduct tertiary treatment sewage treatment plant effluent. The size of raceway pond made of transparent acrylic was 0.4 m (W) × 1.4 m (L) × 0.5 m (H_e), and the height of the outlet was adjusted to set the storage capacity to 50-200 L. In the same way as the general raceway pond operation, a variable-speed stainless steel paddle wheel (0.14 m (W) × 0.47 m (L) × 2 t) was installed in the pond. A fluorescent lamp (Dulux L 55W, OSRAM Opto Semiconductors GmbH, Italy) was installed on the top and two white LEDs were installed on both sides of the pond. The influent was injected using a metering pump (AD-P600, Daehan Science, Co. Ltd., Korea).

The final effluent from the Ansan sewage treatment plant was used as the influent and the feed rate was continuously controlled using a metering pump. The algae productivity, growth rate and nutrient removal efficiencies were analyzed at two hydraulic retention times (4 and 8 d). The pond was initially loaded with 1.05 g of *Hydrodictyon reticulatum* (dry weight), and was maintained at a surface velocity of 0.15 m/s using a paddle wheel (Park 2010) and a light quantity of $121 \pm 10 \mu\text{mol}/\text{m}^2\cdot\text{s}$ in a water temperature of $26.6 \pm 1.4 \text{ }^\circ\text{C}$. Five days after the start of the experiment, the algae were collected from 2 L of pond water daily, using a 3 mm filter net. The filtered water was injected again into the pond.

2.5 Analytical methods

All samples were filtered with a 0.45 μm syringe filter to remove suspended solids and analyzed according to HACH's DR-5000 manual (DR5000, HACH Inc., USA). The quantity of light was measured with a photosynthetic photon flux density (PPFD, $\mu\text{mol}/\text{m}^2\cdot\text{s}$) using a light quantity sensor (LI-190, LI-COR Inc., USA) and data logger (LI-1400, LI-COR Inc., USA). The dry weight of *Hydrodictyon reticulatum* was the weight dried in a dryer (DO-91, Hansol Science Inc., Korea) after dehydration of the algae biomass for 5 minutes using a centrifugal rotary electric dehydrator (WS-6600, HANIL Electric Co. Ltd., Korea). The pH was measured using an HQ-40d (HACH Inc., USA).

3. RESULTS AND DISCUSSION

3.1 Effects of light quantity

While evaluating the effects of light quantity on nutrient removal efficiency and growth rate of *Hydrodictyon reticulatum*, we found that pH tended to be proportional to the quantity of light (Fig. 1). The final pH values at the light quantities of 75, 100 and 200 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ were 8.70, 8.84, and 8.93, respectively. In general, algae grow during the day time through photosynthesis using sunlight and carbon dioxide present in the water. Algae use pH in carbonates and bicarbonates in the water during the photosynthesis process (Park 2010). Chan (2013) reported that pH increased during the incubation period when various microalgae were cultured using sewage treatment plant effluent. When pH value increase more than 11 in response to the growth of microalgae, the nutrients that algae can ingest are reduced due to precipitation of phosphorus and volatilization of ammonia (Park 2010). At a high pH, the growth of algae may be inhibited by the increase of free ammonia. In this experiment, the

observed highest pH value was 9, and the increase of pH was closely correlated with nutrient removal and algal growth.

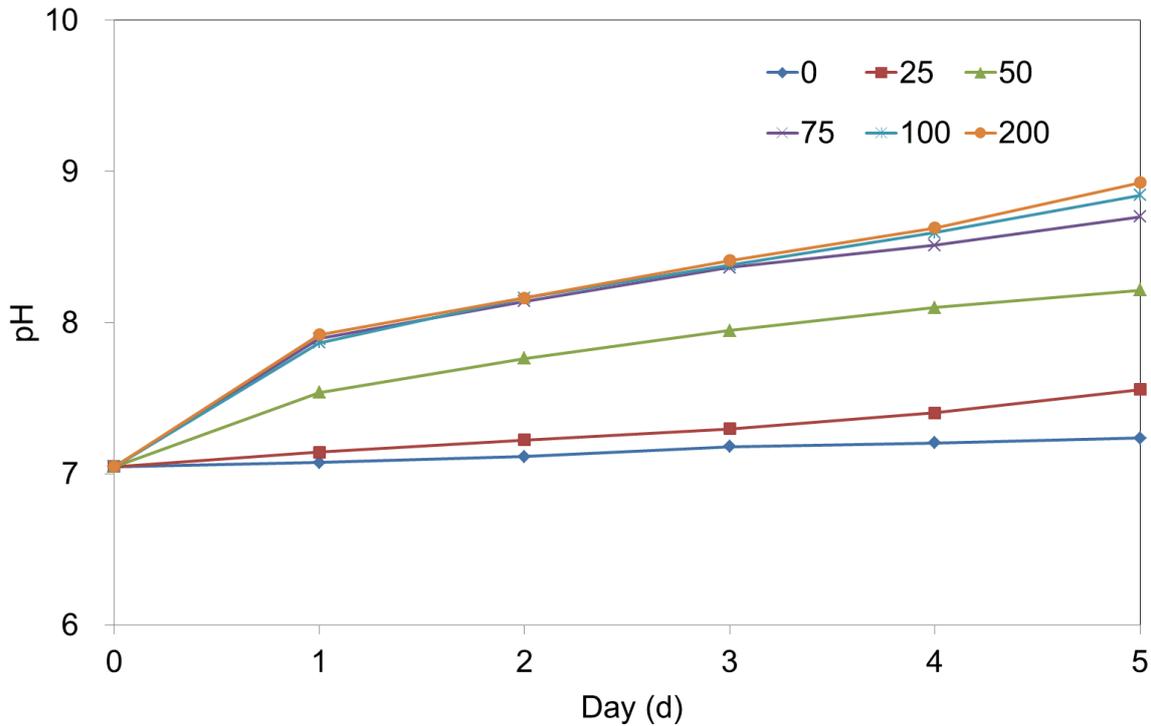


Fig. 1 pH changes according to light quantity

The concentration of NO_3^- -N in the influent was 6.17 mg/L. As the light quantity increased, the effluent concentration decreased to 5.70, 5.53, 2.79, 1.37, 1.33 and 1.20 mg/L (Fig. 2). In general, algae are known to prefer NH_4^+ -N among various nitrogen sources, since they lack intracellular nitrate reductase (Perez-Garcia 2011). Therefore, Hyenstrand (2000) reported that most microalgae begin to absorb NO_3^- -N only when NH_4^+ -N is depleted. Well growth of *Hydrodictyon reticulatum* with sewage treatment effluent NH_4^+ -N concentrations below 0.1 mg/L and NO_3^- -N concentrations over 6 mg/L was observed.

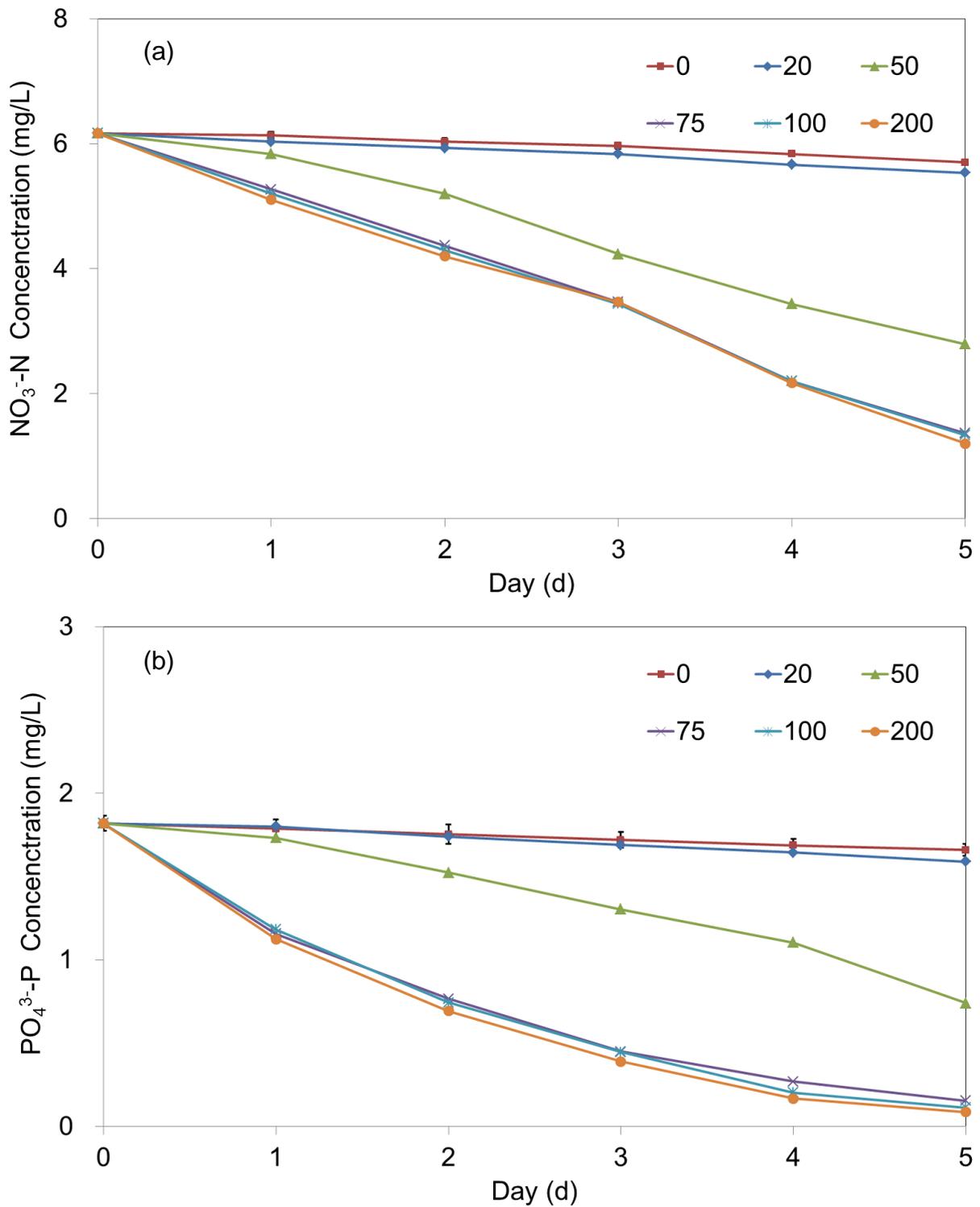


Fig. 2 Nutrient concentration changes according to light quantity (a) nitrate nitrogen and (b) phosphate phosphorus

The average influent concentration of PO₄³⁻-P was 1.82 mg/L, and as the quantity of light increased, the average effluent concentrations were 1.66, 1.59, 0.74, 0.15, 0.11 and 0.09 mg/L. The nitrogen concentrations in effluent tended to decrease as the light

quantity increased. However, phosphorous concentration in the reaction tank decreased rapidly to < 0.45 mg/L in the $75 \mu\text{mol}/\text{m}^2\cdot\text{s}$ during initial 3 days which showed a linear decreasing tendency. This is due to the fact that phosphorous can be stored intracellularly (Su 2012), whereas nitrogen cannot (Perez-Garcia 2011). In a variety of experiments using algae, many researchers have reported that non-biological precipitation of phosphorus can occur at pH 9 and above (Park 2010; Chan 2013). However, in this experiment, as the pH was only 8.93 at the highest light quantity ($200 \mu\text{mol}/\text{m}^2\cdot\text{s}$), it seems that the most of phosphorus was not removed by abiotic precipitation (Su 2011; Zhang 2011). Therefore, we conclude that most phosphorus removal was due to assimilation by *Hydrodictyon reticulatum*.

The removal efficiencies of TN and TP increased as the light quantity increased (Fig. 3). The average TN concentration of the influent was 7.40 mg/L and the TN effluent concentrations at the light quantities of 0, 25, 50, 75, 100 and $200 \mu\text{mol}/\text{m}^2\cdot\text{s}$ were 6.93, 6.70, 3.90, 2.20, 2.03 and 1.90 mg/L, respectively. The removal efficiencies of TN were calculated to be 6.3, 9.5, 47.2, 70.2, 72.5, and 74.3 %. The average effluent concentration of TP was 1.93 mg/L, and the effluent concentration was decreased to 1.78, 1.70, 0.85, 0.24, 0.20 and 0.19 mg/L as the light quantity increased. The TP removal efficiencies were 8.1, 11.9, 55.9, 87.6, 89.7, and 90.2 %, respectively. Therefore, the light saturation point is estimated to be $100 \mu\text{mol}/\text{m}^2\cdot\text{s}$, but when considering the cost, it is considered preferable to irradiate only $75 \mu\text{mol}/\text{m}^2\cdot\text{s}$.

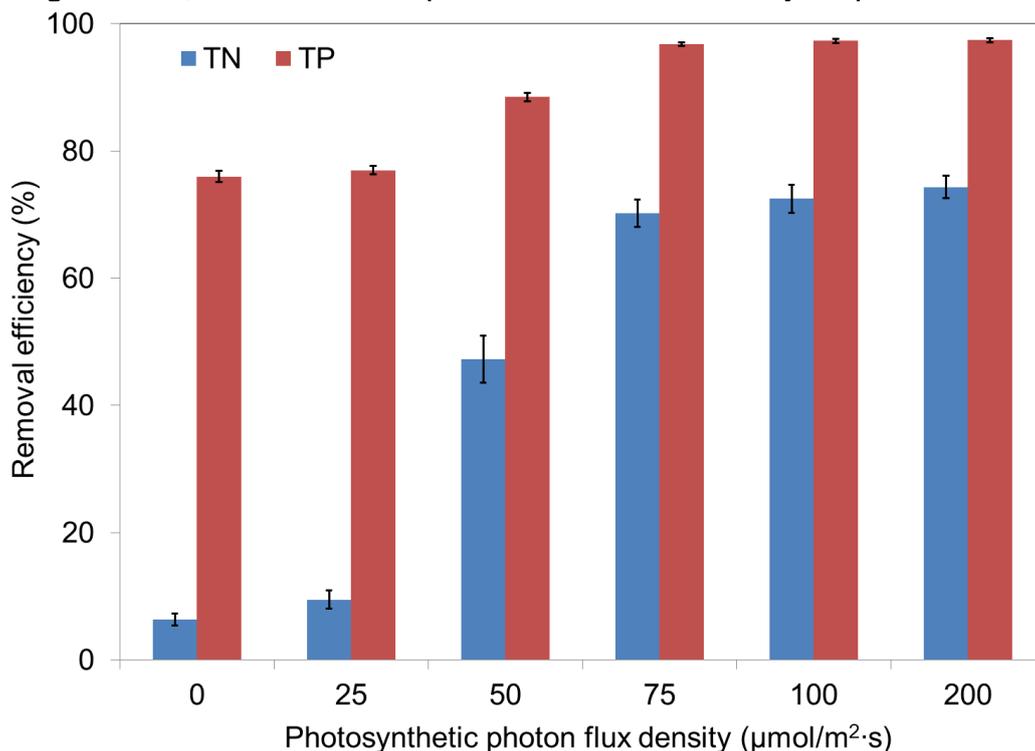


Fig. 3 TN and TP removal for each level of light quantity

Fig. 4 shows the productivity and growth rate (μ) of algae per unit area according to the quantity of light. The growth rate (μ) was calculated according to the following Eq. (1) using the biomass quantity at each light quantity (Hawes 1993; Hall 1997).

$$\mu = \frac{\ln\left(\frac{B_2}{B_1}\right)}{(t \times \ln 2)} \quad (1)$$

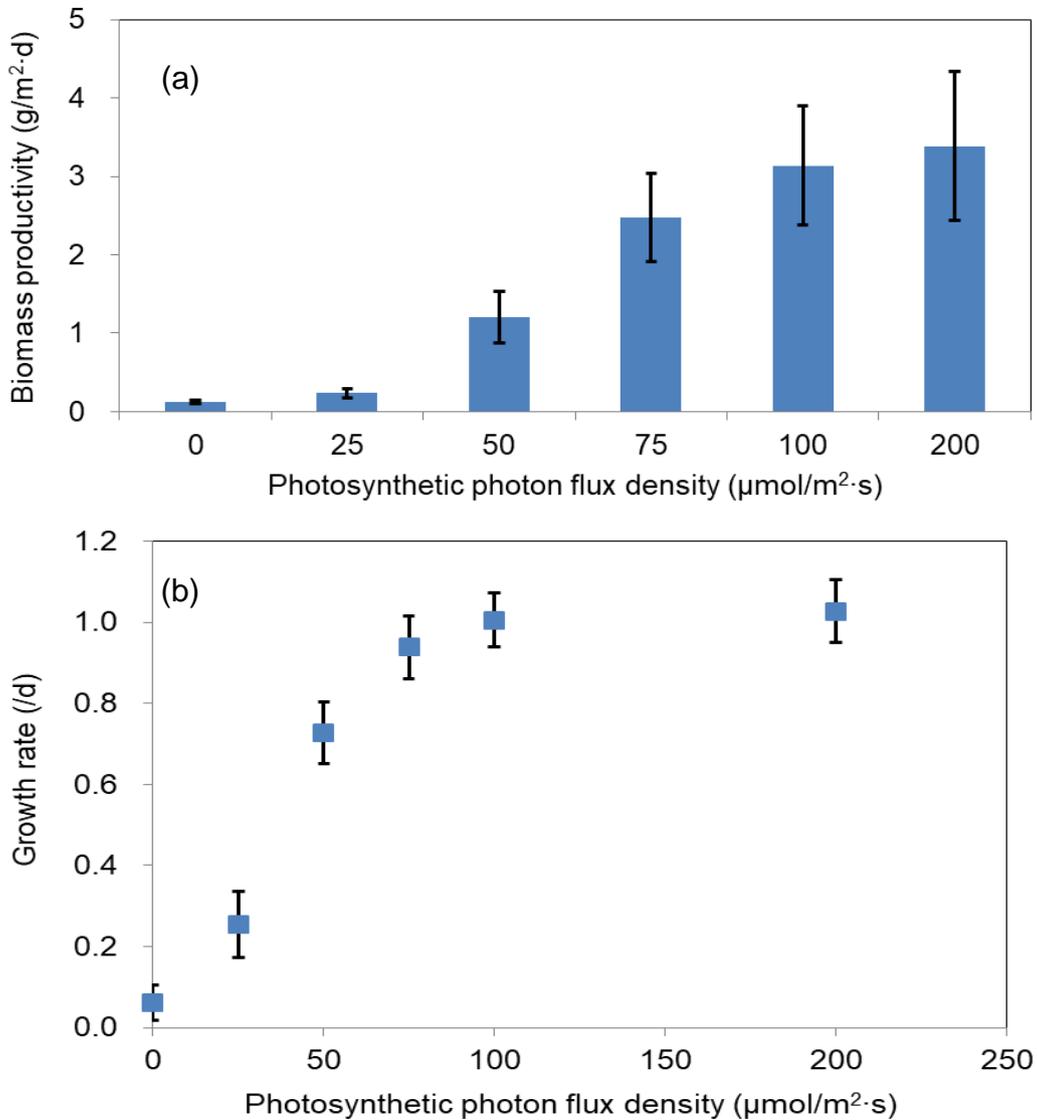


Fig. 4 Productivity and growth of biomass according to light quantity (a) biomass productivity and (b) growth rates

Where B1 and B2 are biomass (dry weight, g) and t is the incubating period (d). The productivity of algae per unit area was estimated to be 0.12, 0.23, 1.20, 2.47, 3.14, and 3.38 g/m²·d (dry weight) as the light quantity increased, so that as the removal efficiency of nutrients increased, the productivity of algae increased. Although there are many differences in the experimental conditions and productivity, comparable results were observed in previous researches. Nishikawa (2001) reported that the algal growth rate was maximized at 200 μmol/m²·s

The growth rate (μ) of algae also increased to 0.06, 0.25, 0.73, 0.94, 1.01, and 1.03 /d as the light quantity increased. Hall (1997) reported that the growth rate of

Hydrodictyon reticulatum in New Zealand showed an average of 0.14-0.21 /d and a maximum of 0.33 /d. The results of this experiment demonstrate that the growth rate of *Hydrodictyon reticulatum* in sewage effluent is three times higher than that of the natural state. Li (2010) reported that biomass concentrations were significantly reduced when *Hydrodictyon reticulatum* used $\text{NH}_4^+\text{-N}$ compared to $\text{NO}_3^-\text{-N}$ as a nitrogen source. It seems that $\text{NO}_3^-\text{-N}$ was used as a nitrogen source while the water temperature was maintained above 23 °C, and the maximum amount of photosynthesis was maintained.

Hawes (1993) found that the compensation point of *Hydrodictyon reticulatum* was lower than $35 \mu\text{mol/m}^2\cdot\text{s}$ and that the light saturation point is $160 \mu\text{mol/m}^2\cdot\text{s}$ at 20 °C. In this experiment, we found that the light saturation point was $100 \mu\text{mol/m}^2\cdot\text{s}$ at the water temperature of 23 °C and that the compensation point was $>25 \mu\text{mol/m}^2\cdot\text{s}$. This low optimum light quantity is consistent with the view of Raven (1979) that algae with large cells tend to adapt to the shade, and suggests that *Hydrodictyon reticulatum* has high light availability. Therefore, *Hydrodictyon reticulatum* can be cultivated in areas and climates where light quantity is insufficient for other algae, and that the water depth of the raceway pond can be increased.

3.2 Effects of wavelength

Hydrodictyon reticulatum was evaluated for its nutrient removal performance and growth potential at diverse light wavelengths using LED. As a result, the pH value was 8.07 (green light, $525 \pm 5 \text{ nm}$), 8.46 (red light, $625 \pm 5 \text{ nm}$), 8.92 (white light, 450-750 nm) and 9.04 (blue light, $470 \pm 5 \text{ nm}$) (Fig. 5). In general, photoautotrophic microalgae absorb light energy (i.e., photons) using carotenoids and chlorophyll pigments that make up photochemical systems. The absorbed light energy is sent to the reaction center and converted to chemical energy such as ATP and NADP for photosynthesis. Microalgae are used for photosynthesis by using light energy of 400-700 nm wavelengths. It is known that there is variability in the preferred wavelength for different species of microalgae (Richmond, 2004; Kim 2013). Energy uptake in photosynthetic organisms depends on the chemical nature of the constituent pigments (Carvalho 2011). Chlorophyll-a is the core of the reactive pigment and the light absorption range is extended by the auxiliary pigments chlorophyll-b, -c and -d, all green pigments absorb light energy from 450-475 nm and 630-675 nm (Richmond 2004). Therefore, the growth of algae can be improved by using red light or blue light. *Hydrodictyon reticulatum* creates the highest pH value under blue light followed by white light and red light. These results suggest that *Hydrodictyon reticulatum* is highly dependent on light energy of 450-475 nm.

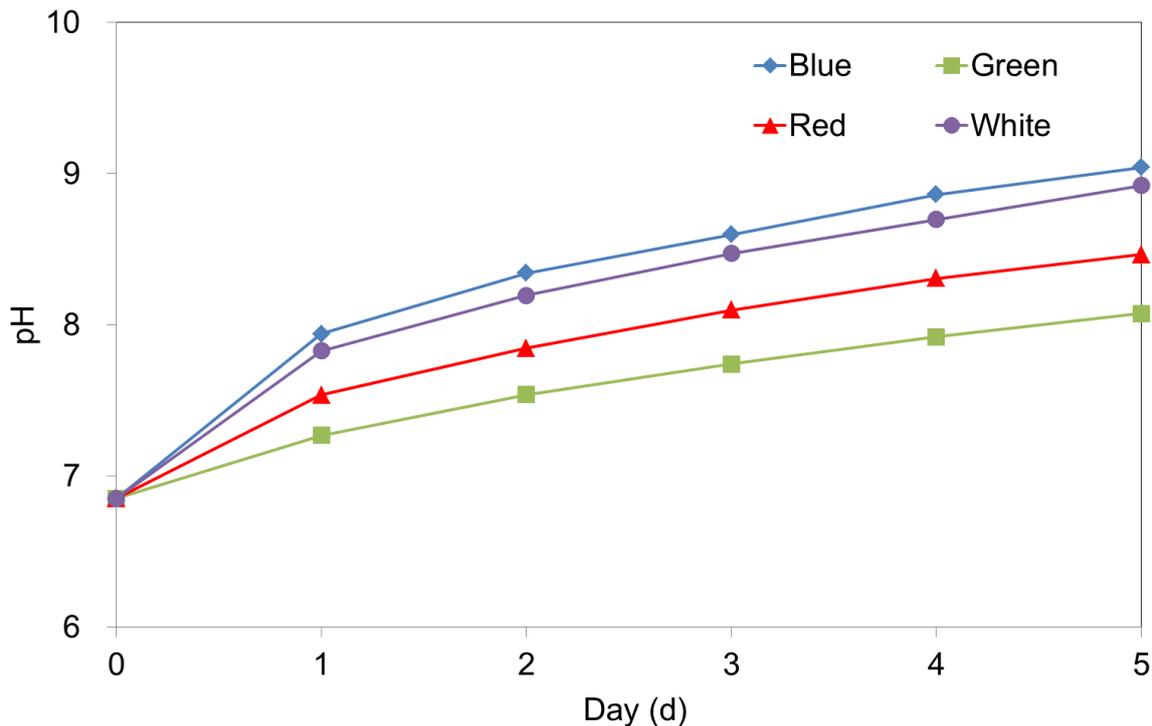


Fig. 5 pH changes according to light wavelength

The changes in nitrogen concentration and phosphate phosphorus concentration during the experiment at each light wavelength are shown in Fig. 6. The $\text{NH}_4^+\text{-N}$ concentrations of effluent water were lower than 0.1 mg/L in all experimental conditions because the average $\text{NH}_4^+\text{-N}$ concentration of influent was low at 0.24 mg/L. The concentration of $\text{NO}_3^-\text{-N}$ in effluent was 5.33, 3.77, 1.60 and 1.33 mg/L for each the light wavelength, and showed a linear decrease with increasing of pH. Kim (2013) evaluated the nitrogen removal of white, red, blue, and mixed (red and blue) light using *Scenedesmus sp.* cultured in Bold's Basal Medium (BBM). Their study reported that nitrogen removal was best under blue light. The same results were obtained in our experiment using *Hydrodictyon reticulatum*.

The trend in phosphorous concentration mirrored that of nitrogen concentration; the $\text{PO}_4^{3-}\text{-P}$ concentration of the effluent tended to decrease as pH increased. The $\text{PO}_4^{3-}\text{-P}$ concentrations with green, red, white, and blue light were 0.65, 0.24, 0.15, and 0.13 mg/L. The results of these experiments differ from those obtained using *Scenedesmus sp.* (Kim 2013). In the case of *Scenedesmus sp.*, the amount of nitrogen removal was similar under red light and white light, but the amount of phosphorus removal was larger under red light than white light. However, in our study using *Hydrodictyon reticulatum*, the removal efficiency difference of $\text{NO}_3^-\text{-N}$ under white and red light was 30.1 %, but the removal efficiency difference of $\text{PO}_4^{3-}\text{-P}$ was only 4.7 %. This is a significant increase compared to phosphorous removal in previous studies. The removal efficiency of $\text{PO}_4^{3-}\text{-P}$ was particularly higher in the green light treatment, where the removal efficiency of $\text{NO}_3^-\text{-N}$ was the lowest. Therefore, *Hydrodictyon reticulatum* can be considered to have relatively high phosphorus removal capacity.

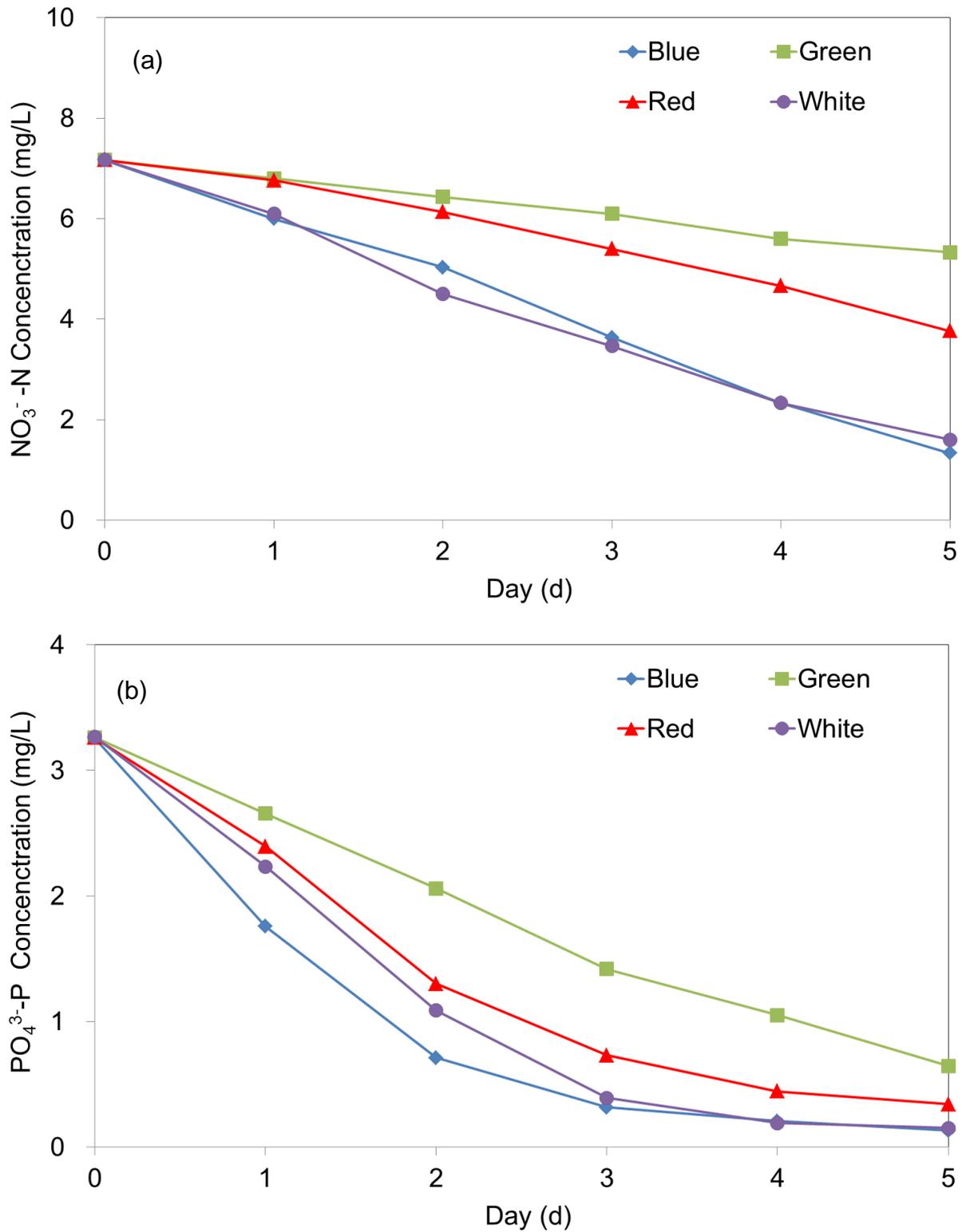


Fig. 6 Nutrient concentration changes according to light wavelength (a) nitrate nitrogen and (b) phosphate phosphorus

The removal efficiencies of TN and TP at each the light wavelengths are shown in Table 2. The removal efficiencies of TN were 24.5, 41.5, 71.3, and 74.4 % for green, red, white, and blue light, respectively, and 79.4, 88.0, 94.8, and 95.3 % for TP. The results of these experiments are consistent with the productivity and growth rates of algae.

Table 2 The removal of TN and TP according to light wavelength

		Blue Light	White Light	Red Light	Green Light
TN	Influent (mg/L) (Average ± S.D.)	8.60 ± 0.02			
	Effluent(mg/L) (Average ± S.D.)	2.20 ± 0.06	2.47 ± 0.02	5.03 ± 0.05	6.50 ± 0.05
	Removal Efficiency (%) (Average ± S.D.)	74.4 ± 2.2	71.3 ± 2.6	41.5 ± 6.8	24.5 ± 1.7
TP	Influent (mg/L) (Average ± S.D.)	3.54 ± 0.06			
	Effluent(mg/L) (Average ± S.D.)	0.17 ± 0.05	0.18 ± 0.02	0.43 ± 0.02	0.73 ± 0.23
	Removal Efficiency (%) (Average ± S.D.)	95.3 ± 0.6	94.8 ± 0.2	88.0 ± 0.2	79.4 ± 2.9

Table 3 shows the results of the productivity and growth rate according to the light wavelength. In the case of productivity, green, red, white, and blue light showed 2.16, 2.69, 3.42 and 3.54 g/m²-d, respectively. The growth rate (μ) was also 0.85, 0.92, 0.99, and 1.00 /d in light wavelength order. For productivity, white light and blue light are 1.27 and 1.32 times higher, respectively, than productivity under red light. In general, blue light tends to increase the chlorophyll and growth rate of some microalgae, but it shows the opposite trend in red algae (Mercado 2002). Growth of green algae, Phaeophyceae and red flora, diatoms *Thalassiosira gravida*, *Chaetoceros sp.*, *Phaeodactylum tricornutum* and *Cyclotella caspia* has been reported to increase under blue light.

Table 3 Productivity and growth rate of *Hydrodictyon reticulatum* according to the light wavelength

	Initial weight (g)	Final weight (g)	Biomass productivity (g/m ² -d)	Growth rate (/d)
Blue Light	0.056 ± 0.007	1.77 ± 0.14	3.54 ± 0.27	1.00 ± 0.06
White Light		1.71 ± 0.14	3.42 ± 0.27	0.99 ± 0.06
Red Light		1.35 ± 0.04	2.69 ± 0.09	0.92 ± 0.03
Green Light		1.08 ± 0.12	2.16 ± 0.23	0.85 ± 0.06

However, in the case of some benthic diatoms (Gabriel 2001), *Heterocapsa pygmaea* (Nelson 1990) and diatoms *Skeletonema costatum* (Tremblin 2000), there was no difference in growth rate under multiple light wavelengths, even under blue light.

Teo (2014) reported that the growth of algae in blue light was superior to the growth in red light, and that growth in white light was superior to that in red light, as tested by *Teraselmis sp.*, marine microalgae. Atta (2013) reported that the *Chlorella vulgaris* growth rate was best under blue light as a result of evaluation the growth rate according to multiple light wavelengths. In this experiment, *Hydrodictyon reticulatum* showed that the best growth potential occurred under blue light, but there was no significant difference from white light.

3.3 Effects of hydraulic retention time

In order to investigate the possibility of tertiary sewage treatment using *Hydrodictyon reticulatum* in raceway pond, the nutrient removal efficiency and productivity in HRTs (4 and 8 d) were evaluated. The results are summarized in Table 4. In HRT 4 d, the pH value remained at 9 or higher after 2 days of the experiment, and in HRT 8 d the pH value remained above that threshold for after 3 days of the experiment. Martinez (2000) reported that when $\text{NH}_4^+\text{-N}$ is used as a nitrogen source, H^+ is released in the synthesis process, decreasing pH. However, when $\text{NO}_3^-\text{-N}$ is used, H^+ ions are consumed, and emit OH^- , increasing pH. Therefore, we concluded that the increasing of pH was probably due to the use of CO_2 in the influent and the use of $\text{NO}_3^-\text{-N}$ as a nitrogen source. Additionally, there was no significant effect on the nutrient removal efficiency and growth rate of *Hydrodictyon reticulatum* even at a high pH (11+).

Table 4 Productivity and growth rate of *Hydrodictyon reticulatum* according to the light wavelength

		Influent (mg/L) (Average \pm S.D.)	Effluent (mg/L) (Average \pm S.D.)	Removal Efficiency (%) (Average \pm S.D.)
pH	HRT 4 d	6.76 \pm 0.08	10.87 \pm 0.91	-
	HRT 8 d	6.80 \pm 0.08	10.73 \pm 1.05	-
COD	HRT 4 d	13.44 \pm 1.79	11.94 \pm 1.65	11.11 \pm 4.10
	HRT 8 d	13.31 \pm 1.85	11.63 \pm 0.09	12.39 \pm 4.95
TN	HRT 4 d	6.84 \pm 0.51	1.94 \pm 0.41	71.46 \pm 6.27
	HRT 8 d	7.23 \pm 0.57	2.33 \pm 0.41	67.50 \pm 6.82
$\text{NH}_4^+\text{-N}$	HRT 4 d	0.15 \pm 0.04	0.10 \pm 0.03	60.00 \pm 6.67
	HRT 8 d	0.23 \pm 0.07	0.11 \pm 0.03	77.42 \pm 16.67
$\text{NO}_3^-\text{-N}$	HRT 4 d	5.59 \pm 0.49	0.93 \pm 0.37	83.24 \pm 6.66
	HRT 8 d	5.78 \pm 0.49	1.16 \pm 0.45	79.50 \pm 9.01
$\text{NO}_2^-\text{-N}$	HRT 4 d	0.04 \pm 0.01	0.03 \pm 0.01	-
	HRT 8 d	0.03 \pm 0.01	0.05 \pm 0.02	-
TP	HRT 4 d	0.76 \pm 0.09	0.12 \pm 0.10	84.61 \pm 12.25
	HRT 8 d	0.76 \pm 0.11	0.17 \pm 0.11	77.86 \pm 13.28
$\text{PO}_4^{3-}\text{-P}$	HRT 4 d	0.67 \pm 0.09	0.09 \pm 0.09	86.53 \pm 12.20
	HRT 8 d	0.67 \pm 0.11	0.13 \pm 0.09	80.19 \pm 13.02

In general, *Hydrodictyon reticulatum* is known as an autotrophic organism, and no studies have been reported its ability to remove organic matter. In the HRT 4 d, the

chemical oxygen demand (COD) had an average removal efficiency of 11.1 % as the influent COD averaged 13.4 mg/L and the effluent averaged 11.9 mg/L. In the HRT 8 d, the influent was 13.3 mg/L, the effluent was 11.6 mg/L, and the average removal efficiency was 12.4 %. The removal of organics in this experiment was likely due to bacterial seeding in the pond during the cultivation of *Hydrodictyon reticulatum*. However, even if the bacteria were propagated, no tissue necrosis of *Hydrodictyon reticulatum* was observed during the experiment, suggesting that co-culture with bacteria may be feasible.

The average removal efficiency of TN in HRTs (4 and 8 d) were 71.5 and 67.5 %, respectively. In the case of $\text{NH}_4^+\text{-N}$, the average influent concentration of 0.15 mg/L in HRT 4 d was discharged 0.10 mg/L, and the average concentration of influent and effluent in HRT 8 d were 0.23 and 0.11 mg/L, respectively. During the experiment, most of the pH was maintained above 11, but $\text{NH}_4^+\text{-N}$ was very low at 0.35 mg/L or less under all experimental conditions and it was estimated that there was no loss due to ammonia stripping and no effect of free ammonia. Therefore, most of $\text{NH}_4^+\text{-N}$ is considered to be removed by assimilation of *Hydrodictyon reticulatum*. In the case of $\text{NO}_3^-\text{-N}$, removal efficiency was 83.2 % in HRT 4 d with 5.59 mg/L of influent and 0.93 mg/L of effluent. The influent concentration in HRT 8 d was 5.78 mg/L and the effluent concentration was 1.16 mg/L. The average $\text{NO}_3^-\text{-N}$ removal efficiency was 79.5 %. These results are attributed to the high light availability of *Hydrodictyon reticulatum*, maintenance of proper biomass concentration according to nutrient, and low "self-shading" due to minimization of shadows in the pond (Park 2011a). From this experiment, we concluded that the tertiary treatment of the sewage treatment plant using *Hydrodictyon reticulatum* in a raceway pond could effectively remove $\text{NO}_3^-\text{-N}$ and reduce the TN concentration of effluent.

In the case of TP, the influent concentration in HRTs (4 and HRT 8d) was 0.76 mg/L on average, and the effluent concentrations were 0.12 and 0.17 mg/L, respectively. The TP removal efficiencies were 84.6 and 77.9 %, respectively, and the removal efficiency of HRT 4 d was better than that of HRT 8 d, as in TN. Also, in the case of $\text{PO}_4^{3-}\text{-P}$, the average influent concentration was 0.67 mg/L, the average effluent concentration was 0.07 and 0.13 mg/L in HRT 4 and 8 d, respectively, and the average removal efficiencies were 86.5 and 80.2 %. We note that the removal efficiency in HRT 4 d was particularly high.

The productivity and growth rate of *Hydrodictyon reticulatum* according to the hydraulic retention time are shown in Table 5. The total productivity of 87.8 g (dry weight) was obtained in HRT 4 d, and the productivity was 10.44 g/m²-d and the growth rate (μ) was 0.40 /d. In HRT 8 d, a total of 69.5 g was harvested demonstrating that there was a productivity of 8.26 g/m²-d and a growth rate (μ) of 0.38 /d. Productivity and growth rate of *Hydrodictyon reticulatum* in HRT 4 d were evaluated to be better than HRT 8 d. Our experimental results showing that the efficiency of nutrient removal is proportional to the productivity of algae are consistent with previous results according to the above light quantity and wavelength. In the case of productivity and growth rate (μ), compared with the results at the light quantity of 100 $\mu\text{mol/m}^2\text{-s}$, the productivity of HRT 4 d increased 4.2 times, but the growth rate was only 25 %. We suggest that this low growth rate is due to abiotic precipitation occurring since the pH value was maintained above 9, which resulted in a decrease in available phosphorus.

Table 5 Productivity and growth rate of *Hydrodictyon reticulatum* according to HRT

	Initial weight (g)	Final weight (g)	Biomass productivity (g/m ² ·d)	Growth rate (/d)
HRT 4d	1.05	87.78	10.44	0.40
HRT 8d	1.07	69.49	8.26	0.38

Hydrodictyon reticulatum is an alga with large, multinucleate cells that are continually divided until colonies are not formed or mature unless they are individual cells. Therefore, conventional chemostat-type cultivation techniques are not suitable for *Hydrodictyon reticulatum*, so existing studies have investigated and reported the nutrient removal efficiency on growth using batch culture techniques. However, since these experiments have been applied in relatively small amounts of other nutrients, and with N or P in excessively saturated environmental conditions, high nutrient removal efficiencies and growth rates have been reported due to the formation of colonies and segmentation growth (Hawes 1993). Therefore, the growth rate in the batch experiment tested at relatively high concentrations was higher than the growth rate in this continuous experiment. However, the productivity in the continuous experiment was higher than that in the batch experiment, which is considered to be due to the characteristics of the filamentous algae. Unlike unicellular algae, filamentous algae generally form large cell aggregates that are entangled with each other. Filamentous algae are further characterized by the ability to easily absorb oxygen into the cell matrix.

Therefore, when the *Hydrodictyon reticulatum* was cultured in a batch manner, it was floated on the surface during the culturing process, and light was not transmitted below the water surface, limiting the cultivation area. On the other hand, when cultured in a continuous raceway pond, turbulence is generated by the flow of the culture medium, so oxygen is not attached to the cell matrix. As a result, *Hydrodictyon reticulatum* is scattered in the water without floating on the surface of the underwater, so the light permeability to the water is increased and the cultivation area is relatively increased. Also, this suggests that productivity per unit area has increased because *Hydrodictyon reticulatum* has high light availability and is maintained at the proper concentration to produce of a low self-shading effect. Therefore, when *Hydrodictyon reticulatum* is cultured in a raceway pond using sewage treatment effluent, effective removal of nutrients and cost-effective mass production is possible.

4. CONCLUSIONS

The nutrient removal efficiency, productivity, and growth rate of *Hydrodictyon reticulatum* were increased up to 100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. Due to the high light availability, it will be applicable to the region or the climate where the light quantity is insufficient. We found that the efficiency of nutrient removal and growth rate of *Hydrodictyon reticulatum* at hydraulic retention time 4 d were excellent. In particular, productivity in the continuous raceway pond was higher than that in batch culture, suggesting that the

cultivation of *Hydrodictyon reticulatum* in a continuous-type reactor is a valid method in terms of productivity.

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REFERENCES

- Atta, M., Idris, A., Bukhari, A. and Wahidin, S. (2013), "Intensity of blue LED light: a potential stimulus for biomass and lipid content in fresh water microalgae *Chlorella vulgaris*", *Bioresour. Technol.*, **148**, 373-378.
- Boonchai, R., Seo, G.T., Park, D.R. and Seong, C.Y. (2012), "Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant", *Int. J. Biosci. Biochem. Bioinforma.*, **2**(6), 407-410.
- Carvalho, A.P., Silva, S.O., Baptista, J.M. and Malcata, F.X. (2011), "Light requirements in microalgal photobioreactors: an overview of biophotonic aspects", *Appl. Microbiol. Biotechnol.*, **89**(5), 1275-1288.
- Chan, A., Salsali, H. and McBean, E. (2013), "Nutrient removal (nitrogen and phosphorous) in secondary effluent from a wastewater treatment plant by microalgae", *Can. J. Civ. Eng.*, **41**(2), 118-124.
- Choi, H.J., Lee, J.M. and Lee, S.M. (2013), "A Novel Optical Panel Photobioreactor for Cultivation of Microalgae", *Water Sci. Technol.*, **67**(11), 2543-2548.
- Craggs, R.J., Heubeck, S., Lundquist, T.J. and Benemann, J.R. (2011), "Algal biofuels from wastewater treatment high rate algal ponds", *Water Sci. Technol.* **63**(4), 660-665.
- Gabriel, J. and Sánchez-Saavedra, M.D.P. (2001), "Isolation and growth of eight strains of benthic diatoms, cultured under two light conditions", *J. Shellfish Res.*, **20**(2), 603-610.
- Haag, A.L. (2007), "Algae Bloom Again", *Nature*, **447**(7144), 520-521.
- Hall, J. and Payne, G. (1997), "Factors controlling the growth of field populations of *Hydrodictyon reticulatum* in New Zealand", *J. Appl. Phycol.*, **9**(3), 229-236.
- Hawes, I. and Smith, R., (1993), "Influence of environmental factors on the growth in culture of a New Zealand strain of the fast-spreading alga *Hydrodictyon reticulatum* (water-net)", *J. Appl. Phycol.*, **5**(4), 437-445.
- Hsieh, C.H. and Wu, W.T. (2009), "A Novel Photobioreactor with Transparent Rectangular Chambers for Cultivation of Microalgae", *Biochem. Eng. J.*, **46**(3), 300-305.
- Hyenstrand, P., Rydin, E. and Gunnerhed, M. (2000), "Response of pelagic cyanobacteria to iron additions-enclosure experiments from Lake Erken", *J. Plankton Res.*, **22**, 1113-1126
- Kim, D., Min, K.J., Lee, K., Yu, M.S. and Park, K.Y. (2017), "Effects of pH, molar ratios and pre-treatment on phosphorus recovery through struvite crystallization from effluent of anaerobically digested swine wastewater", *Environ. Eng. Res.*, **22**(1), 12-18.

- Kim, T.H., Lee, Y., Han, S.H. and Hwang, S.J. (2013), "The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus sp.* for wastewater treatment", *Bioresour. Technol.*, **130**, 75-80.
- Lee, J., Lee, K., Jang, H.M., Shin, J., Park, K.Y., Cho, J. and Kim Y.M. (2017a), "Biomethanation and anaerobic co-digestion via microbial communities of microalgal *Hydrodictyon reticulatum* biomass residues with sewage sludge", *Desalination Water Treat.*, **77**, 185–193.
- Lee, K., Chantrasakdakul, P, Kim, D., Kong, M. and Park, K.Y. (2014), "Ultrasound pretreatment of filamentous algal biomass for enhanced biogas production", *Waste Manag.*, **34**(6), 1035–1040.
- Lee, K., Chantrasakdakul, P., Kim, D., Kim, J.S. and Park. K.Y. (2017b), "Biogas productivity of algal residues from bioethanol production", *J. Mater. Cycles Waste Manag.*, **19**(1), 235–240.
- Li, X., Hu, H.-y., Gan, K. and Yang, J. (2010), "Growth and nutrient removal properties of a freshwater microalga *Scenedesmus sp.* LX1 under different kinds of nitrogen sources", *Ecol. Eng.*, **36**, 379–381.
- Martinez, M.E., Sánchez, S., Jimenez, J.M., El Yousfi, F. and Munoz, L. (2000) "Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*", *Bioresour. Technol.*, **73**(3), 263-272.
- Mercado, J.M., Sánchez, P., Carmona, R. and Niell, F.X. (2002), "Limited acclimation of photosynthesis to blue light in the seaweed *Gracilaria tenuistipitata*", *Physiol. Plant.*, **114**(3), 491-498.
- Nelson, N.B. and Prezelin, B.B. (1990), "Chromatic light effects and physiological modeling of absorption properties of *Heterocapsa pygmaea* (= *Glenodinium sp.*)", *Mar. Ecol. Prog. Ser.*, 37-46.
- Nguyen, C.M., Kim, J.S., Hwang, H.J., Park, M.S., Choi, G.J., Choi, Y.H., Jang, K.S. and Kim, J.C. (2012), "Production of L-lactic acid from a green microalga, *Hydrodictyon reticulum*, by *Lactobacillus paracasei* LA104 isolated from the traditional Korean food, makgeolli", *Bioresour. Technol.*, **110**, 552-559.
- Nishikawa, T. (2001), "Measuring the growth rate of the harmful diatom *Eucampia zodiacus* by in vivo chlorophyll fluorescence", *Bull. Hygo. Prefect. Fish. Exp. Stn.*, **36**, 21-23.
- Park, J.B.K. and Craggs, R.J. (2010), "Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition", *Water Sci. Technol.*, **61**(3), 633-639.
- Park, J.B.K. and Craggs, R.J. (2011a), "Nutrient removal in wastewater treatment high rate algal ponds with carbon dioxide addition". *Water Sci. Technol.*, **63**(8), 1758-1764.
- Park, J.B.K., Craggs, R.J. and Shilton, A.N. (2011b), "Wastewater treatment high rate algal ponds for biofuel production", *Bioresour. Technol.* **102**(1), 35-42.
- Park, K.Y., Kweon, J., Chantrasakdakul, P., Lee, K. and Cha, H.Y. (2013), "Anaerobic digestion of microalgal biomass with ultrasonic disintegration". *Int. Biodeterior. Biodegradation*, **85**, 598-602.
- Park, K.Y., Lim, B.R. and Lee, K. (2009), "Growth of microalgae in diluted process water of the animal wastewater treatment plant", *Water Sci. Technol.*, **59**(11), 2111-2116.

- Perez-Garcia, O., Escalante, F.M.E., De-Bashan, L.E. and Bashan, Y. (2011), "Heterotrophic culture of microalgae: Metabolism and potential products", *Water Res.*, **45**(1), 11-36.
- Raven, J.A., Smith, F.A. and Glidewell, S.M. (1979), "Photosynthetic capabilities and biological strategies of giant-celled and small-celled macro-algae", *New Phytol.*, **83**, 299-309.
- Richmond, A. (2004), "Principles for attaining maximal microalgal productivity in photobioreactors: an overview", *Hydrobiologia*, **512**, 33–37.
- Singh, A., Mehta, S.K. and Gaur, J.P. (2007), "Removal of heavy metals from aqueous solution by common freshwater filamentous algae", *World J. Microbiol. Biotechnol.*, **23**(8), 1115.
- Su, Y., Mennerich, A. and Urban, B. (2012), "Comparison of nutrient removal capacity and biomass settleability of four high-potential microalgal species", *Bioresour. Technol.*, **124**, 157-162.
- Su, Y., Mennerich, A. and Urban, B. (2011), "Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal–bacterial culture", *Water Res.*, **45**, 3351–3358.
- Tas, B. (2011), "Bloom and eutrophication of *Hydrodictyon reticulatum* (Chlorophyceae) at Civil and Kacalı Stream, Ordu, Turkey", *Ener. Educ. Sci. Tech-A.*, **28**(1), 319-330.
- Teo, C.L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A.M. and Idris, A. (2014), "Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths", *Bioresour. Technol.*, **162**, 38-44.
- Tremblin, G., Cannuel, R., Mouget, J.L., Rech, M.O and Robert, J.M. (2000), "Change in light quality due to a blue-green pigment, marennine, released in oyster-ponds: effect on growth and photosynthesis in two diatoms, *Haslea ostrearia* and *Skeletonema costatum*", *J. Appl. Phycol.*, **12**(6), 557-566.
- Wijffels, R.H. and Barbosa, M.J. (2010), "An Outlook on Microalgal Biofuels", *Science*, **329**(5993), 796-799.
- Wilkie, A.C. and Mulbry, W.W. (2002), "Recovery of dairy manure nutrients by benthic freshwater algae", *Bioresour. Technol.*, **84**(1), 81-91.
- Zhang, Y., Safa, N.J. and Angelidaki, I. (2011), "Simultaneous organic carbon, nutrients removal and energy production in a photomicrobial fuel cell (PFC)", *Energy Environ. Sci.*, **4**, 4340–4346.