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Nutrient removal from secondary effluent using filamentous algae in raceway ponds

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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 μmol/m^2·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.

Keywords: filamentous algae; Hydrodictyon reticulatum; nutrient removal; raceway ponds; growth rate

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 *et al*. 2017, Lee *et al*. 2017a). Recently, several studies of algal pond technology, using microalgae to accumulate nitrogen and phosphorus as a tertiary sewage treatment, have been introduced (Park *et al*. 2009).

Algae have been used extensively in the past for sewage treatment using ponds and lagoons (Park *et al*. 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 *et al*. 2013, Chan *et al*. 2013). The wastewater treatment using algae can removal nutrients and produce useful biomass raw materials for organic fertilizer, feed and bio-energy (Wilkiea and Mulbry 2002, Park *et al*. 2013, Lee *et al*. 2014). Meanwhile, unicellular algae species that form colonies 50-200 μm in diameter have been used in wastewater treatment since they can be economically harvested (Craggs *et al*. 2011, Park *et al*. 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 *et al*. 2012, Lee *et al*. 2017a). The studies on filamentous algae have been actively carried out to improve economic efficiency (Hawes and smith 1993, Hall and Payne 1997, Lee *et al*. 2017a, Lee *et al*. 2017b).

Algae is dependent on carbon dioxide and light quantity as an autotrophic organism (Wijffels and Bardosa 2010, Kim *et al*. 2013, Teo *et al*. 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 *et al*. 2013, Teo *et al*. 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

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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 and Wu 2009, Boonchai *et al*. 2012, Choi *et al*. 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 ± 2°C using a low temperature incubator (BI-81, HYSC Inc., Korea). The light quantity and light-dark cycle was maintained 100 μmol/m^2·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).

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 μmol/m^2·s. And the case where light was not irradiated under the same conditions (0 μmol/m^2·s) was tested. A 0.41 m (L) × 0.30 m (W) × 0.20 m (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 ± 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 ± 2°C.

Table 1 Final effluent characteristic from the sewage treatment plant

Parameter	Concentration Average ± S.D.
pH	6.81 ± 0.11
COD (mg/L)	13.38 ± 1.79
TN (mg/L)	7.19 ± 0.68
NH_4^+-N (mg/L)	0.18 ± 0.08
NO_3^--N (mg/L)	5.84 ± 0.62
NO_2^--N (mg/L)	0.04 ± 0.01
TP (mg/L)	1.07 ± 0.81
PO_4^{3-}-P (mg/L)	0.97 ± 0.84

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 ± 5 nm), a green light (525 ± 5 nm), a red light (625 ± 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 ± 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 ± 2°C, and light of 100 μmol/m^2·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 and Craggs 2010) and a light quantity of 121 ± 10 μmol/m^2·s in a water temperature of 26.6 ± 1.4°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, μmol/m^2·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 μmol/m^2·s were 8.70, 8.84, and 8.93, respectively. In general, algae grows during the daytime 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 *et al*. 2010). Chan *et al*. (2013) reported that pH increased during the incubation period when various microalgae were cultured using sewage treatment plant effluent. When pH value increases 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 *et al*. 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.

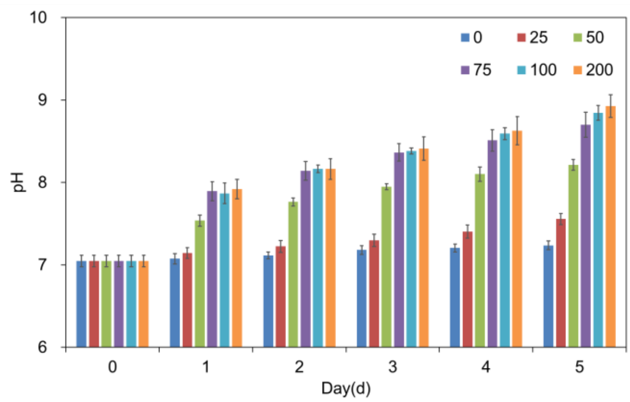
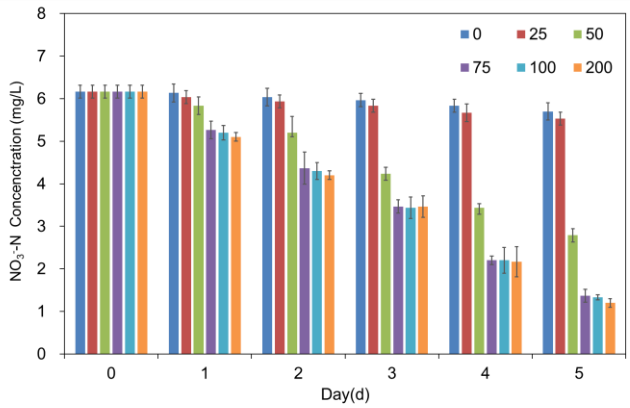
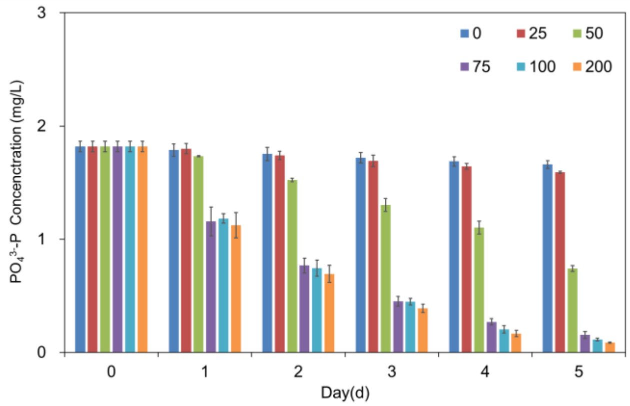


Fig. 1 pH changes according to light quantity



(a)



(b)

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

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 *et al*. 2011). Therefore, Hyenstrand *et al*. (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.

The average influent concentration of PO_4^{3-}-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 μmol/m^2·s during initial 3 days which showed a linear decreasing tendency. This is due to the fact that phosphorous can be stored intracellularly (Su *et al*. 2012), whereas nitrogen cannot (Perez-Garcia *et al*. 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 *et al*. 2010, Chan *et al*. 2013). However, in this experiment, as the pH was only 8.93 at the highest light quantity (200 μmol/m^2·s), it seems that the most of

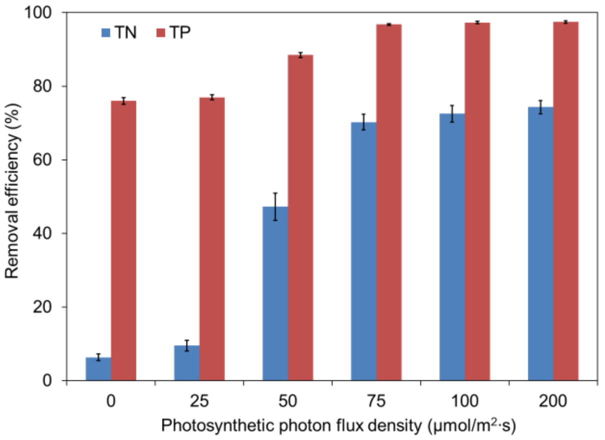
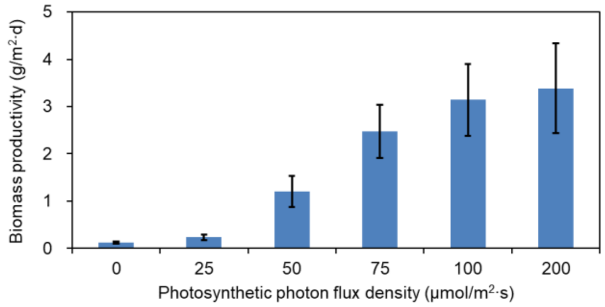
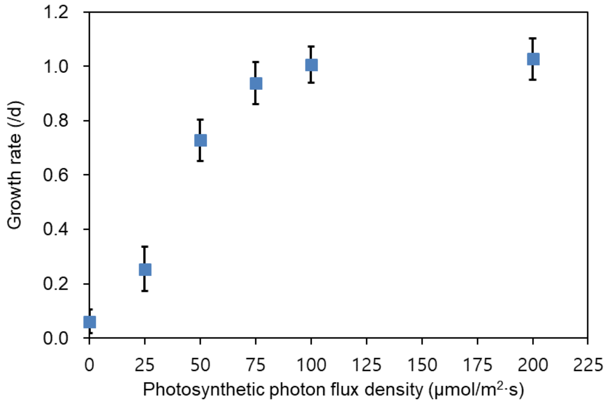


Fig. 3 TN and TP removal for each level of light quantity



(a)



(b)

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

phosphorus was not removed by abiotic precipitation (Su *et al*. 2011, Zhang *et al*. 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 μmol/m^2·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 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 μmol/m^2·s, but when considering the cost, it is considered preferable to irradiate only 75 μmol/m^2·s.

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 and Smith 1993, Hall and Payne 1997).

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

Where B_1 and B_2 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/m^2·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 and Payne (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 *et al*. (2010) reported that biomass concentrations were significantly reduced when *Hydrodictyon reticulatum* used NH_4^+-N compared to NO_3^--N as a nitrogen source. It seems that NO_3^--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 and Smith (1993) found that the compensation point of *Hydrodictyon reticulatum* was lower than 35 μmol/m^2·s and that the light saturation point is 160 μmol/m^2·s at 20°C. In this experiment, we found that the light saturation point was 100 μmol/m^2·s at the water temperature of 23°C and that the compensation point was >25 μmol/m^2·s. This low optimum light quantity is consistent with the view of Raven *et al.* (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

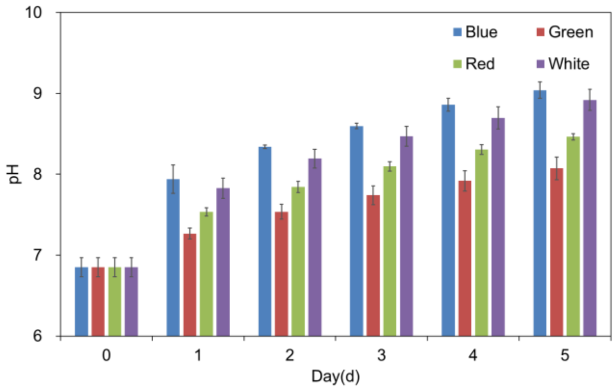
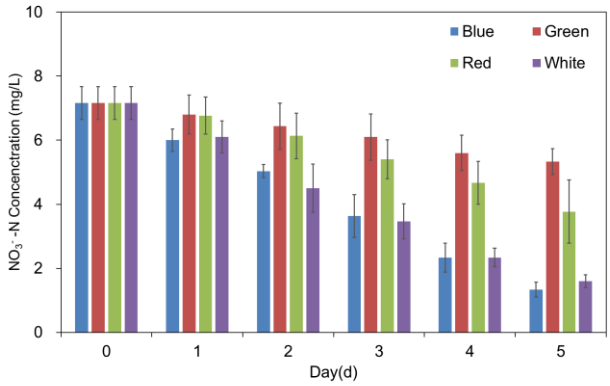
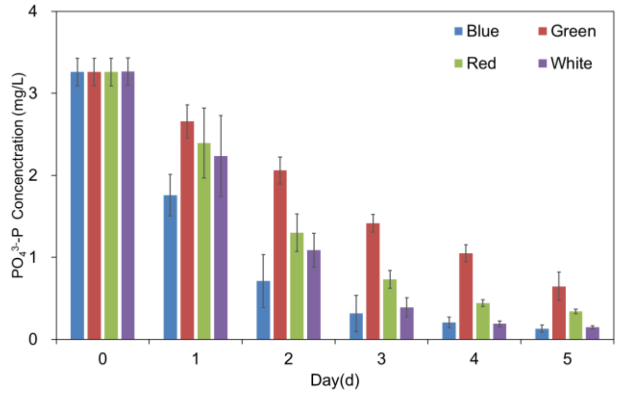


Fig. 5 pH changes according to light wavelength



(a)



(b)

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

(green light, 525 ± 5 nm), 8.46 (red light, 625 ± 5 nm), 8.92 (white light, 450-750 nm) and 9.04 (blue light, 470 ± 5 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 *et al*. 2013). Energy uptake in photosynthetic organisms depends on the chemical nature of the constituent pigments (Carvalho *et al*. 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 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.

The changes in nitrogen concentration and phosphate phosphorus concentration during the experiment at each light wavelength are shown in Fig. 6. The NH_4^+-N concentrations of effluent water were lower than 0.1 mg/L in all experimental conditions because the average NH_4^+-N concentration of influent was low at 0.24 mg/L. The concentration of NO_3^--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 *et al*. (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 PO_4^{3-}-P concentration of the effluent tended to decrease as pH increased. The PO_4^{3-}-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 *et al*. 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 NO_3^--N under white and red light was 30.1%, but the removal efficiency difference of PO_4^{3-}-P was only 4.7%. This is a significant increase compared to phosphorous removal in previous studies. The removal efficiency of PO_4^{3-}-P was particularly higher in the green light treatment, where the removal efficiency of NO_3^--N was the lowest. Therefore, *Hydrodictyon reticulatum* can be considered to have relatively high phosphorus removal capacity.

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 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 $g/m^2 \cdot d$, 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

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

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

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

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

microalgae, but it shows the opposite trend in red algae (Mercado *et al*. 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.

However, in the case of some benthic diatoms (Gabriel and Sánchez-Saavedra 2001), *Heterocapsa pygmaea* (Nelson and Prézelin 1990) and diatoms *Skeletonema costatum* (Tremblin *et al.* 2000), there was no difference in growth rate under multiple light wavelengths, even under blue light. Teo *et al*. (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 *et al*. (2013) reported that the *Chlorella vulgaris* growth rate was best under blue light as a result of evaluating 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 *et al.* (2000) reported that when NH_4^+-N is used as a nitrogen source, H^+ is released in the synthesis process, decreasing pH. However, when NO_3^--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 NO_3^--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 ± S.D.	Effluent (mg/L) Average ± S.D.	Removal Efficiency (%) Average ± S.D.
pH	HRT 4 d	6.76 ± 0.08	10.87 ± 0.91	-
	HRT 8 d	6.80 ± 0.08	10.73 ± 1.05	-
COD	HRT 4 d	13.44 ± 1.79	11.94 ± 1.65	11.11 ± 4.10
	HRT 8 d	13.31 ± 1.85	11.63 ± 1.45	12.39 ± 4.95
TN	HRT 4 d	6.84 ± 0.51	1.94 ± 0.41	71.46 ± 6.27
	HRT 8 d	7.23 ± 0.57	2.33 ± 0.41	67.50 ± 6.82
NH_4^+-N	HRT 4 d	0.15 ± 0.04	0.10 ± 0.03	31.47 ±15.51
	HRT 8 d	0.23 ± 0.07	0.11 ± 0.03	46.99 ±19.63
NO_3^--N	HRT 4 d	5.59 ± 0.49	0.93 ± 0.37	83.24 ± 6.66
	HRT 8 d	5.78 ± 0.49	1.16 ± 0.45	79.50 ± 9.01
NO_2^--N	HRT 4 d	0.04 ± 0.01	0.03 ± 0.01	-
	HRT 8 d	0.03 ± 0.01	0.05 ± 0.02	-
TP	HRT 4 d	0.76 ± 0.09	0.12 ± 0.10	84.61 ± 12.25
	HRT 8 d	0.76 ± 0.11	0.17 ± 0.11	77.86 ± 13.28
PO_4^{3-}-P	HRT 4 d	0.67 ± 0.09	0.09 ± 0.09	86.53 ± 12.20
	HRT 8 d	0.67 ± 0.11	0.13 ± 0.09	80.19 ± 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 feasible.

The average removal efficiency of TN in HRTs (4 and 8 d) were 71.5 and 67.5%, respectively. In the case of NH_4^+-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 NH_4^+-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 NH_4^+-N is considered to be removed by assimilation of *Hydrodictyon reticulatum*. In the case of NO_3^-- 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 NO_3^--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 and Craggs 2011). From this experiment, we concluded that the tertiary treatment of the sewage treatment plant using *Hydrodictyon reticulatum* in a raceway pond could effectively remove NO_3^--N and reduce the TN concentration of effluent.

In the case of TP, the influent concentration in HRTs (4 and 8 d) 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 PO_4^{3-}-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/m^2·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/m^2·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 μmol/m^2·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	1.05	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 and Smith 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 is 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 μmol/m^2·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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