A THESIS FOR MASTER OF SCIENCE

Nitrogen and Phosphorus Removal from Treated Swine Wastewater by Floating Aquatic Plants

부유 수생식물에 의한 돈슬러리 처리수의 질소·인 제거

August 2014

Seoul National University Graduate School

Department of Agricultural Biotechnology

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Nitrogen and Phosphorus Removal from Treated Swine Wastewater by Floating Aquatic Plants

Advisor: Professor Hong Lim Choi

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By

Sartika Indah Amalia Sudiarto

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이 논문을 농학석사학위논문으로 제출함

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2014 년 7 월

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ABSTRACT

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Nitrogen and phosphorus is main pollutants to the environments. Excess nitrogen and phosphorus in water bodies will cause eutrophication that result in oxygen depletion, leads to the death of aquatic plant and animal. The major source of nitrogen and phosphorus contamination to the water bodies are agriculture, sewage, and stormwater (US EPA, 2014). Excess fertilizer and animal feces contains large amount of nitrogen and phosphorus that potentially contaminate the water. Thus excessive nitrogen and phosphorus from agricultural practices need to be removed.

Animal Environment and Bioengineering (AEBE) Laboratory of Seoul National University built a pilot biological wastewater treatment which consists of underground anaerobic digestion (UGAD), activated sludge processes, and membrane filtration at Livestock Experimental Station, Seoul National University, Suwon. The effluent water characteristics have not met the effluent standard imposed by Ministry of Environment, Republic of Korea. According to the standard, TN and TP should not exceed 120 mg/L and 40 mg/L respectively. Therefore, further removal of nitrogen and phosphorus is needed to

meet effluent standard before discharged into water body such as stream or reservoir.

This study was performed to investigate the removal of nitrogen and phosphorus through aquatic plants from treated livestock wastewater. Aquatic plants including, water hyacinth, water lettuce, *Limnobium* sp., and duckweed were used. The objective of this study is to find the floating plant that is able to grow in the effluent of treated swine wastewater as well as able to remove high amount of nitrogen and phosphorus from treated swine wastewater. This study also aimed to quantify the potential biomass production of the floating plants cultured in the effluent.

The experiment was carried out the 13 L volume container with surface area of 0.10 m^2. The experiment was conducted in a batch system with 10 L volume of water substrate. The treatments were 10% effluent and ½-strength modified Hoagland's nutrient solution and control without floating plants. Modified Hoagland's nutrient solution and 10% effluent were obtained by dilution with groundwater. Each treatment was replicated 3 times, so gave total number of 30 experimental units. The experimental unit arrangement was completely randomized design. Water samples were taken at 0, 1, 3, 5, 9, 13, 17, and 21-day. The biomass was harvested at the end of sampling period (Day 21) and the fresh weight was recorded. The parameters for water analysis were total nitrogen (TN), ammonium-N (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), and total suspended solid (TSS). Total suspended solid obtained assumed to reflect the amount of algae that were grown during the experiments. The amount of algae growth can estimate how much nutrient being removed by the algae. This makes better estimation of nutrient removal by plants can be obtained.

The effluent used in this study is diluted to the concentration of 10% because the concentration above 10% turned out to be phytotoxic based on the preliminary germination test. Initial total nitrogen concentration is 151.67 mg/L and 125.71 mg/L for 10% effluent and ½-strength Hoagland's treatment respectively. Meanwhile total phosphorus concentration is 82.77 mg/L and 60.85 mg/L 10% effluent and ½-strength Hoagland's treatment respectively. Water lettuce showed the highest removal of total nitrogen and phosphorus from the 10% effluent. Water lettuce removed 63.15% so the final total nitrogen concentration was 43.33 mg/L which is under the effluent standard of 120 mg/L. Meanwhile, duckweed has the highest phosphorus removal of 36.15% in effluent compared to other plants tested. The final phosphorus concentration is 46.67 mg/L which is still above the effluent standard limit of 40 mg/L.

Duckweed may not be suitable for further use because after more than 9 days, large amount of duckweed were dead because of severe chlorosis (loss of green coloration) that inhibits the photosynthetic activity. As an alternative, water lettuce may be used for optimum nitrogen and phosphorus removal since it has highest relative growth rate of 0.090 ($g\ g^{-1}d^{-1}$) which means growing faster. However, in terms of dry biomass yield, water hyacinth produces the highest amount of dry weight of 5.19 g dry weight/m^2/day and organic matter content of 82% in the effluent treatment. Therefore, if higher biomass production is preferred, water hyacinth is recommended to be used.

There is a tendency that the higher nitrogen and phosphorus uptake, the higher the plant growth. The correlation between nitrogen removal and relative growth rate of the plants was examined. The R^2 value of total nitrogen removal and relative growth rate linear regression was 0.52. This result shows that nitrogen removal is highly correlated with plant relative growth rate. The R^2 value of total phosphorus removal and relative growth rate linear regression was 0.50 which is highly correlated.

Harvested biomass can be used further as a livestock feed, anaerobic digestion feedstock, or bioenergy production such as bioethanol feedstock. However, other strategies need to be developed to increase the removal of nitrogen and phosphorus from the effluent, such as longer retention time and periodic harvesting. Finally, the nutrient removal and biomass growth are higher in all ½-strength Hoagland's treatment. Effluent may have properties that inhibit the growth of and nutrient uptake by the plants, for which further researches are needed.

Keywords: floating aquatic plants, nitrogen removal, phosphorus removal, treated swine wastewater, biomass

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I. INTRODUCTION

Nitrogen and phosphorus are main pollutants to the environment. Excessive nitrogen and phosphorus in water bodies will support the growth of algae causing eutrophication which is harmful to the water ecosystem. Eutrophication will result in oxygen depletion that leads to the death of organism in the water. The major sources of nitrogen and phosphorus contamination in water bodies are agriculture, sewage, and storm-water (US EPA, 2014). Agriculture is the largest source of nitrogen and phosphorus contamination. Excessive fertilizers and animal feces contain a large amount of nitrogen and phosphorus that potentially contaminate the water. Thus, nitrogen and phosphorus from the agricultural practices need to be treated.

Animal Environment and Bioengineering (AEBE) Laboratory of Seoul National University built a pilot biological wastewater treatment which consists of underground anaerobic digestion (UGAD), activated sludge processes, and membrane filtration at Livestock Experimental Station of Seoul National University at Suwon. This biological wastewater treatment has a capacity to treat around 1000 L of swine slurry every day. The effluent contains approximately 1517 mg/L total nitrogen and 828 mg/L total phosphorus. However, the effluent water characteristics have not met the effluent water discharge standard imposed by the Ministry of Environment of the Republic of Korea, which is 120 mg/L and 40 mg/L for total nitrogen and total phosphorus, respectively. Therefore, further removal of nitrogen and phosphorus is needed for the effluent water to be able to discharge to water bodies such as rivers or streams.

Aquatic plants have been widely used for nutrient removal from the wastewater by using constructed wetland systems. Floating and emergent aquatic plants are mostly studied for nutrient removal, especially nitrogen and phosphorus removal from wastewater.In the Republic of Korea, research regarding potential use of aquatic plants for nutrient removal has been started since 1980s (Seo et al., 2010). Lee et al. (1984) studied the removal of water pollutants and heavy metals from water by using water hyacinth (*Eicchornia crassipes*). Meanwhile, Kim et al. (1988) studied about the potential of nutrient removal of several floating and emergent aquatic plants from pig wastewater. The result of his studies was that *Eicchornia crassipes* uptake and removed the largest amount of nitrogen and phosphorus from pig wastewater compared to other tested plants. Other studies were also performed on different kinds of wastewaters (Kim et al, 1991; Ra et al, 1996; Jeong et al, 2000; Shin et al, 2001 and Park, 2002) such as sewage wastewater, synthetic nutrient solution, aquaculture pond effluent, and effluent & livestock wastewaters. Among others, water hyacinth is mostly studied. Iamchaturapatr et al. (2007) studied more diverse types of plants species (21 kinds of floating and emergent aquatic plants). He found that different types of plants performed differently in regard to nitrogen and phosphorus removal.

Despite of an extensive number of studies about nutrient removal through aquatic plants, there are relatively few studies regarding nutrient uptake from treated livestock wastewater by aquatic plants. Treated livestock waste may exhibit different characteristics from the raw wastewater because it has gone through several steps of wastewater treatment. Therefore, a study of potential nitrogen and phosphorus removal through aquatic plants from treated livestock wastewater needs to be performed.

In this study, the potential of nitrogen and phosphorus removal from effluent of treated swine wastewater by water hyacinth, water lettuce, amazon frog bit, and duckweed were investigated. The objective of this study is to find the floating plant that is able to grow in the treated effluent of swine wastewater as well as able to remove high amount of nitrogen and phosphorus from treated swine wastewater effluent. This study also aimed to quantify the potential biomass production of the floating plants cultured in the effluent

II. MATERIALS AND METHODS

II.1. Experimental design

II.1.1. Experimental plant preparation

The plants that are used in this experiment were water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes)*, amazon frog bit *(Limnobium laevigatum*), and duckweed (*Lemna* sp.). This study was started from April to June 2014. The plants were cultured inside a 40 L container and placed in a greenhouse and leave to grow vegetatively for 1 month. The greenhouse temperature, humidity, air speed, and light intensity were range from 25-37°C, 16.9 – 75.8%, 0.03 – 0.19 m^2s^{-1}, and 66.87 – 198.43 $\mu molm^{-2}s^{-1}$ respectively throughout the study. The floating plants, duckweed, water hyacinth, water lettuce, and *Limnobium* sp. (**Figure 1**) are cultured in the ¼-strength modified Hoagland's nutrient solution (Taiz & Zeiger, 2006). The modified Hoagland's nutrient solution provided both sources of nitrogen form for plant, ammonium (NH_4^+) and nitrate (NO_3^-). The Hoagland's stock solution is diluted with ground water and then used for plant culture. Every 3 days, a loss of water by evaporation was replaced by adding ground water. The ¼-strength Hoagland's nutrient solution was added every once a week to ensure adequate supply of nutrients for plants to growth.

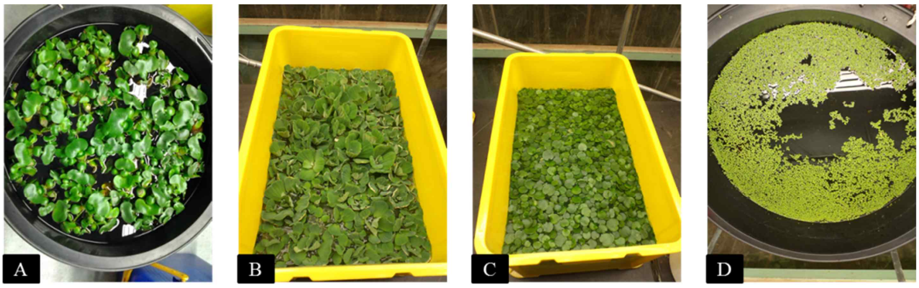

Figure 1. Floating plants stock culture A) Water hyacinth; B) Water lettuce; C) *Limnobium* sp; D) Duckweed

II.1.2. Experimental procedure

The experiment was carried out in a 13 L volume container with the surface area 0.10 m^2. The experiment was conducted on a batch system with 10 L volume of water substrate. The treatments are 10% effluent and ½-strength modified Hoagland' nutrient solution and control without floating plants. Modified Hoagland's nutrient solution and effluent were diluted until 10% concentration with groundwater. Before being used, the groundwater was passed through 65μm sieves to remove the contaminants. Each treatment is replicated 3 times, giving a total number of 30 experimental units.

The experimental unit arrangement was a completely randomized design. Initial biomass (fresh weight) of water hyacinth, water lettuce, *Limnobium* sp., and duckweed are 60, 30, 20, and 7.5 g, respectively. The initial biomass fresh weight was adjusted to gives the ½ coverage total surface of experimental units.

The experimental units were placed inside a greenhouse with the temperature ranging between 30 – 37 °C. Water samples were taken at 0, 1, 3, 5, 9, 13, 17, and 21st days after plants cultured. The dissolved oxygen, temperature, and pH were measured directly before taking the samples by using portable equipment. The water was mixed before taking the samples to ensure the homogeneity of the samples. The loss of water by evaporation was compensated by adding groundwater to the marked point made in the container before the experiment started. The samples were put inside a refrigerator of 4°C until an analysis was performed. The analysis was performed within 1 week from the sampling date.

Environmental parameters measurement of air speed, temperature, relative humidity and photo-synthetically active radiation (PAR) conducted at the same day of water sampling. Air speed, temperature, and relative humidity were measured by portable instruments (Kanomax) while photo-synthetically active radiation was measured with a light meter (LI-COR LI-250A) at the leaf surface.

The biomass was harvested at the end of sampling period (day 21). The plant then was washed to remove attached algae and contaminants and then weighed for fresh weight. The root and shoot were separated and then air-dried for further analysis.

II.2. Analysis and assessment

II.2.1. Physicochemical characteristics of water

Dissolved Oxygen (DO) and pH were measured directly during sampling by portable measurement equipment (Hanna Instruments, Ltd., Italy). Total nitrogen, ammonia, nirate, nitrite, and total phosphorus were determined by persulfate digestion method (No.10072), salicylate method (No.10031) cadmium reduction method (No.8039), diazotization method (No.8507), and molybdovanadate method with acid persulfate digestion (No.10127), respectively, with Hach chemical reagents according to Hach protocols manual (DR5000, Hach USA). Total suspended solid was determined by filtration of the sample with CHMLab glass microfiber filter with the pore size of 1.2 µm and then dried at 105°C according to APHA standard method 2540 D (APHA, 2005). The elemental content of effluent and Hoagland's nutrient solution was analyzed by ICP-MS.

II.2.2. Determination of leaf chlorophyll content

Chlorophyll was extracted from 1 g fresh plant leaf tissues with 96% ethanol. Plant chlorophyll content was determined by spectrophotometry of ethanol extract at 649 and 665 nm and calculated by formula developed by Winterma.Jf and Demots (1965). The concentration of chlorophyll is based on the fresh weight.

$$Chlorophyll\ a = 13.7\, A_{665\ nm} - 5.76\, A_{649\ nm} \quad (1)$$

$$Chlorophyll\ b = 25.8\, A_{649\ nm} - 7.4\, A_{665\ nm} \quad (2)$$

$$Chlorophyll_{a+b} = 20\, A_{649\ nm} + 6.1\, A_{665\ nm} \quad (3)$$

II.2.3. Plant biomass and Relative Growth Rate (RGR) determination

Plant fresh weight was determined by blotting the plant to the tissue paper and leaving for 5 minutes to absorb the water. The remaining water on the plant surface was wiped with tissue paper and then the fresh weight is measured by analytical balance. The plant is air dried in the ambient temperature for 1-2 day. Air-dried plant was ground and then passed through 1 mm sieve. A sub-sample was used further to determine the moisture content by oven drying at 103-105°C for 5 hours following AOAC standard method No. 935.29. The organic and ash contents were determined by ignition at 550°C for 2 hours. Relative Growth Rate (RGR) of the plant biomass was calculated by using the formula (4) with W_2 and W_1 is the weight of final and initial plant biomass and t_2 and t_1 is referred to the time.

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \qquad (4)$$

III. RESULTS AND DISCUSSIONS

III.1. Characteristics of treated swine wastewater (effluent)

The effluent (E) and Hoagland's nutrient solution used in this study was diluted with groundwater. The Hoagland's nutrient solution was used as a comparison for the effluent because Hoagland's nutrient solution contains all of the known mineral elements for rapid plant growth without toxic effect for plant. So, it is expected that the plant growth and nutrient removal will be near optimum in the Hoagland's nutrient solutions. By comparing these two treatments, the possible factors influencing the plant growth and nutrient removal can be figured out if there is any differences in plant growth and nutrient removal in the effluent treatment. Effluent was diluted to the concentration of 10%. It is because without dilution, the effluent exhibits phytotoxic effect based on the preliminary germination index test. The 10% dilution is sufficient to reduce the phytotoxic effect

Table 1. Chemicals characteristics of E-10 and H-0.5 treatment solutions

Chemicals	E-10 (mg/L)	H-0.5 (mg/L)
TN	151.67	125.71
NO_3^-	120.33	76.47
NO_2^-	18.47	0.26
TAN	18.93	14.07
TP (PO_4^{3-})	82.77	60.85
pH	6.76	6.91
DO	8.73	9.30

Table 1 shows a comparison of 10% effluent (E-10) with half-strength Hoagland's nutrient solution (H-0.5). The pH of E-10 and H-0.5 nutrient solution is comparatively the same. Generally, total nitrogen (TN), NO_3^-, NO_2^-, Total Ammonia Nitrogen (TAN), and Total Phosphate (TP) concentration is higher in the E-10 than H-0.5 treatment. This indicates that E-10 has more inorganic nutrients, especially macronutrients that may have beneficial effect for plant growth. **Table 2** shows mineral elements composition of E-10 and H-0.5. Ca, K, and S are comparable between E-10 and H-0.5. However, Cu, Zn, Mn, Na and Mg are higher in the E-10 than in the H-0.5 nutrient solutions. Meanwhile iron is about 4 times less in E-10 than in the H-0.5 nutrient solutions.

Table 2. Elements content of E-10 and H-0.5 treatment solutions

Elements	E-10 (mg/L)	H-0.5 mg/L)
Cu	0.147	0.025
Zn	1.116	0.102
Mn	0.789	0.004
Fe	0.029	0.126
Ca	70.558	89.316
Na	36.856	9.481
Mg	38.844	17.181
Mo	0.111	0.364
K	17.964	13.104
S	32.358	33.260

The difference between mineral element contents may exhibit different effects on the plants. For example, the lower content of iron in the E-10 compared to the standard nutrient solution (H-0.5) can cause nutrient deficiency in plants. Iron deficiency can lead to chlorosis (loss of green coloration of leaves) that will influence the photosynthetic activity (Taiz & Zeiger, 2006). In other hand, a high amount of Na and Mg may exhibit salinity stress on the plants. Salinity stress can inhibit plant functions because salinity stress cause disruption of cell membrane integrity and metabolism, production of reactive oxygen species, and death of cells (Parida & Das, 2005). So, if the plant experience salinity stress, the nutrient uptake may also be influenced. It can affect the efficiency of plants to remove nitrogen and phosphorus from the E-10. However, some types of plants are able to exhibit salt tolerance in the high salinity environment.

III.2. Physiochemical parameters of water

III.2.1. Dissolved oxygen

Dissolved oxygen tends to decrease over the experimental period. Because the growth of floating plants covering the surface area results in a lower possibility of oxygen exchange between water surface and atmosphere. The control treatment has the highest dissolved oxygen content (**Figures 2** and **3**) compared to other treatments because no plants cover the surface area. Changes in plants covering on the surface of water are shown in Figures **18 – 21**.

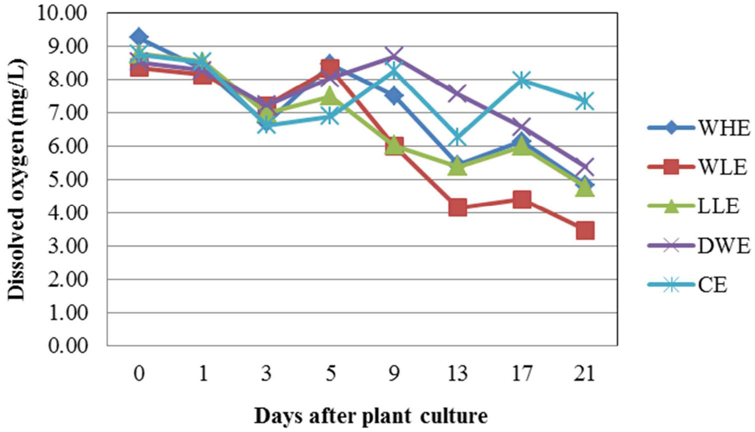

Figure 2. Dissolved oxygen dynamics during experiments in the E-10 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

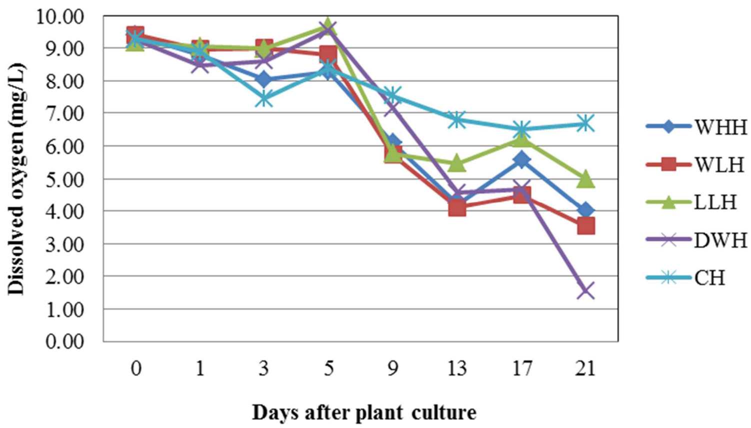

Figure 3. Dissolved oxygen dynamics during experiments in the H-0.5 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

In the H-0.5 treatment, the duckweed experimental unit has the lowest dissolved oxygen content. The duckweed size is small (4-5 mm) and thus the surface area is tightly covered by duckweed layer, so that almost no water surface is in direct contact with the atmosphere. Oxygen is also required for respiration of living things. A reduction in the dissolved oxygen concentration may also because of the plant respiration, especially in the root system. The dissolved oxygen reduction in the E-10 treatment is between 1.41 - 4.42 mg/L, lower than in the H-0.5 treatment which is between 2.59 – 5.38 mg/L because the plants are growing more in the H-0.5 than in the E-10 treatment (**Figures 18-21**).

III.2.2. pH

The acidity of water can influence the availability of the nutrients. For example, in the alkaline condition ammonium ion tends to change into ammonia which cannot be taken up by the plants. Moreover, almost all plant nutrient elements are available at pH 5.5 – 6.5 (Taiz & Zeiger, 2006).

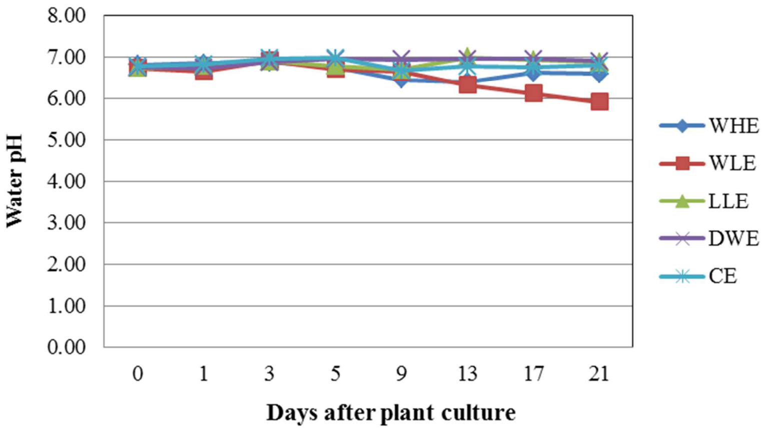

Figure 4. Water pH dynamics during experiments in the E-10 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

The pH of the water in both E-10 and H-0.5 treatment are almost constant during the experimental period. The pH of water ranges between 5.91 – 7.19, so almost all nutrients is available to be taken up by the aquatic plants.

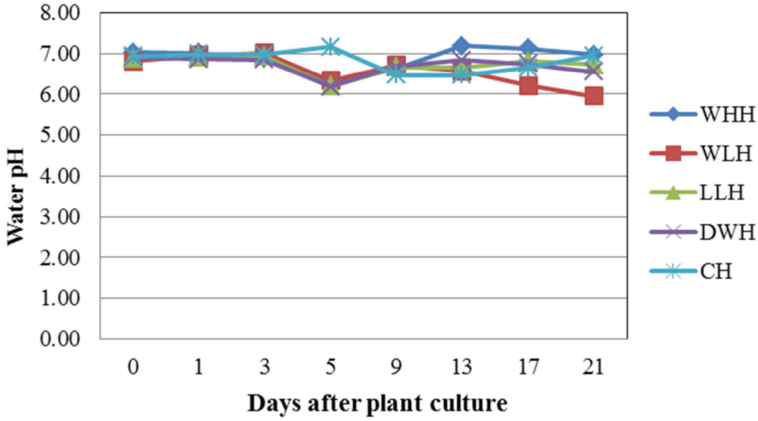

Figure 5. Water pH dynamics during experiments in the H-0.5 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

III.2.3. Total suspended solids

Total suspended solids (TSS) are the portion of total solids that is retained on a filter after filtration of wastewater samples (Metcalf & Eddy. et al., 2003). In this experiment, the filter pore size that is used for the total suspended solid determination is 1.2 µm. Thus it means that any particle that is retained in the filter and measured as TSS has a particle size more than 1.2 µm. Bacteria, cyanobacteria, and archaea have the cell size of 0.2-0.3 µm while single cell algae has a size of more than 2 µm (Metcalf & Eddy. et al., 2003). So bacteria, cyanobacteria, and archaea will pass through the filter while the algae is retained in the filter and makes the total suspended solid values obtained in this experiment assumed to reflect the amount of algae growing in the solution during the experiments. The algae may also contribute to the total nutrient removal. By knowing the TSS and nutrient removal in the control treatment (without plant), the nitrogen and phosphorus removal by the algae can be estimated so that the actual nutrient removal by the plant can be obtained. **Table 3** shows TSS of control treatment of both E-10 and H-0.5 treatment.

Table 3. Total suspended solids (TSS) of control treatment before and after experiments and estimation of mg nitrogen and phosphorus removed per mg TSS.

Treatments	TSS initial (Day 0) (mg/L)	TSS final (Day 21) (mg/L)	TN removed (mg)/ mg TSS	TP removed (mg)/ mg TSS
C-E	27.33	190.74	0.23	0.13
C-H	2.22	526.67	0.16	0.10

Abbreviations: -E/H: 10% effluent/ ½-strength Hoagland's; and C: Control

This result does not exactly measure the total nitrogen and phosphorus that are removed by algae because the algae species that grow in the control may be different from the algae that grow in the experimental units with plants and may have a different nitrogen and phosphorus removal capacity. In addition, TSS only resembles the algae that is floating in the water and does not measure the amount of algae biomass that is attached in the plant organs and surface of experimental units. However, it is still a good approach to estimate the nitrogen and phosphorus removal by the algae to obtained better estimation of nitrogen and phosphorus uptake by the plant only.

III.3. Water chemical analysis

III.3.1. Nitrogen

The total nitrogen concentration is fluctuating during the experiments (**Figure 6** and **7**). However, at the end of the experimental period, the total nitrogen is lower compared to the initial concentration. The initial concentration of total nitrogen is 151.67 mg/L and 125.71 mg/L in E-10 and H-0.5 treatment, respectively. The final nitrogen concentration is in the range of 10 - 113.33 mg/L. This shows some plants are able to reduce the total nitrogen down to below the standard set by the Ministry of Environment which is 120 mg/L.

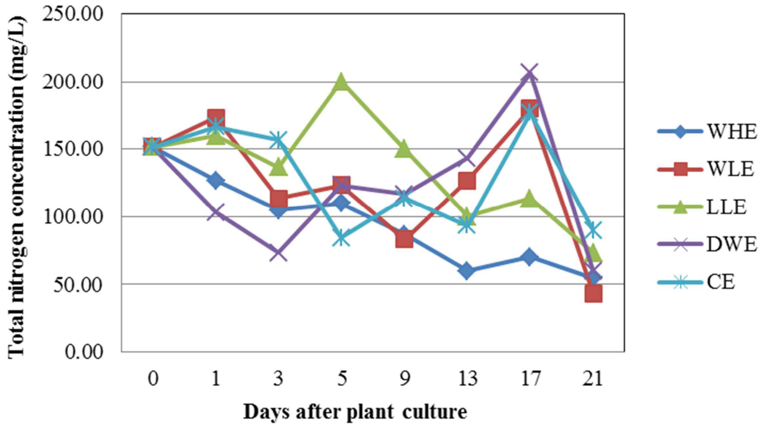

Figure 6. Total nitrogen concentration during experiments in the E-10 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

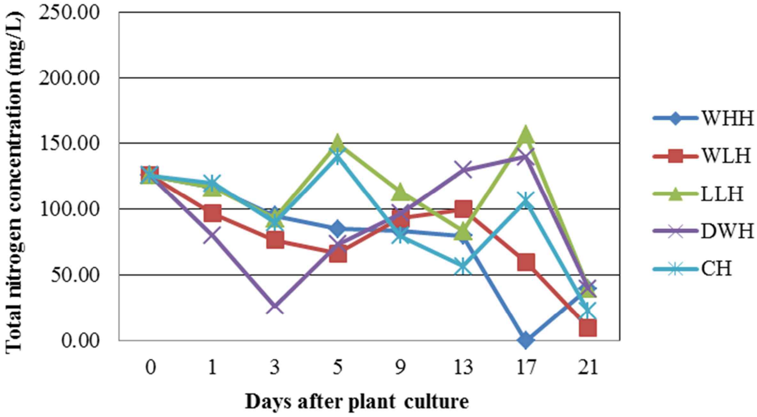

Figure 7. Total nitrogen concentration during experiments in the H-0.5 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

The E-10 treatment control which is without plants has the highest final total nitrogen concentration of 113.33 mg/L. This shows that aquatic plants play an important part in the nitrogen removal from E-10. However, even without any plants, control treatment has a lower total nitrogen final concentration than the initial total nitrogen concentration.

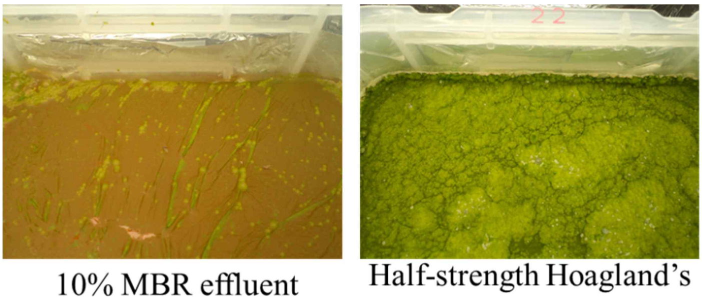

Figure 8. Microalgae growth in the control treatments (without plants)

Figure 8 shows the algae growing in the control treatment. Algae are believed to be responsible for the nitrogen removal in the control treatment. Algae are photosynthetic organisms which also assimilate inorganic forms of nitrogen like plants. Algae are growing massively in the control treatment because of no competition of light and nutrient with the plants. In the E-10 and H-0.5 treatment algae also grow, but after the plant cover the surface of experimental unit, the algae growth is inhibited.

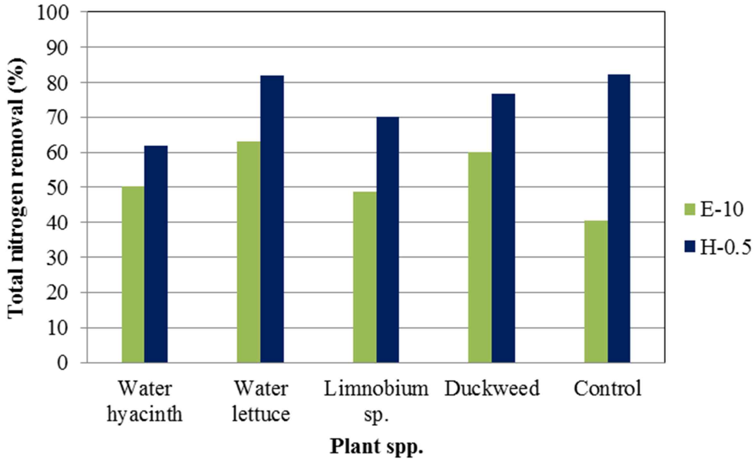

Figure 9. Total nitrogen removal comparison between E-10 and H-0.5 treatment of four types of plants and control (without plants)

The removal of total nitrogen (**Figure 9**) generally is higher in the H-0.5 treatment. This indicates E-10 characteristics influence the performance of plants to uptake nutrient. Among other plants, water lettuce removes the highest total nitrogen of 63.15 and 82.12% in E-10 and H-0.5 treatment, respectively. This value is obtained after subtracting the total nitrogen removal from the water with estimation of total nitrogen removal by algae (TSS). The higher removal in the H-0.5 treatment is because the biomass production is higher. The biomass production of water lettuce in H-0.5 treatment is 7.55 g dry weight/m^2/day while the biomass production in the E-10 treatment is

4.26 g dry weight/m^2/day. More plant biomass increases the uptake of nitrogen from the water.

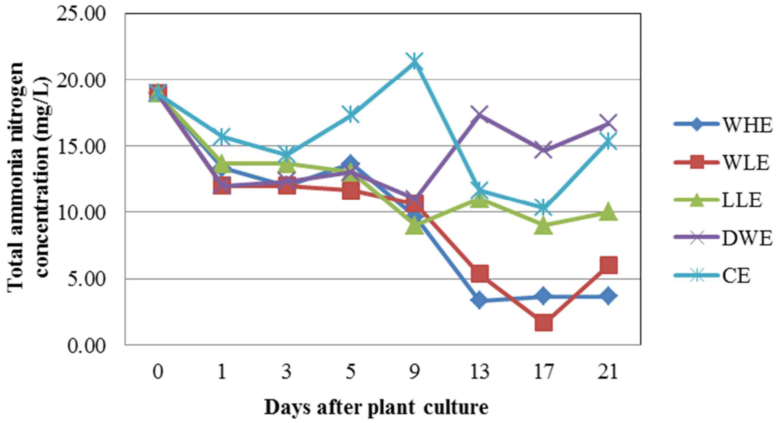

Figure10. Ammonium concentration dynamics during experiments in the E-10 treatment (WH: Water hyacinth; WL: Water lettuce; LL: Limnobium sp.; DW: Duckweed; and C: Control)

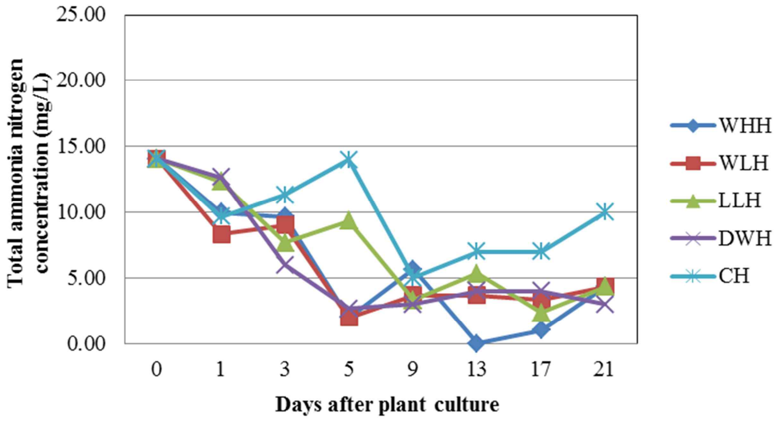

Figure 11. Ammonium concentration dynamics during experiments in the H-0.5 treatment (WH: Water hyacinth; WL: Water lettuce; LL: Limnobium sp.; DW: Duckweed; and C: Control)

There is a difference in the dynamics of ammonium concentration between E-10 effluent and H-0.5 treatment. In the H-0.5

treatment (**Figure 11**) the ammonium is gradually decreased until the end of experiments. However, the ammonium concentration in the E-10 treatment (**Figure 10**) is more fluctuating at the end of the experiments. At 13 days after plant culture, the ammonium concentration in the Duckweed-E-10 treatment is increased. This may be a result of plant material breakdown since on the 13th day some of the duckweed biomass is died and sink at the bottom of the experimental unit. The dead plant biomass may undergo a decomposition process that releases ammonium to the water as a result of protein breakdown.

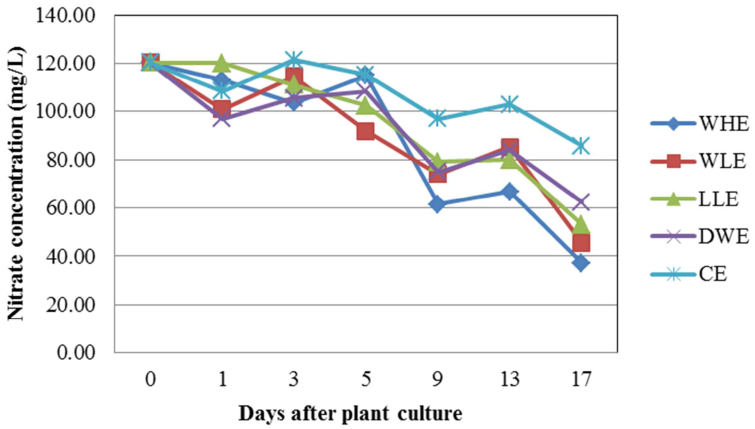

Figure 12. Nitrate concentration dynamics during experiments in the E-10 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; C: Control)

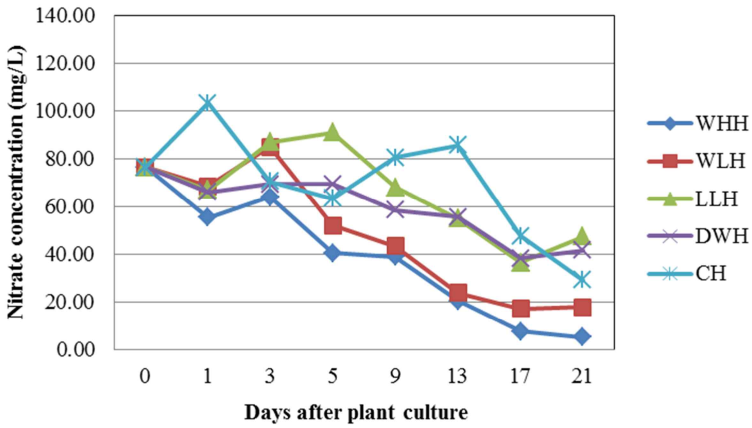

Figure13. Nitrate concentration dynamics during experiments in the H-0.5 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; C: Control)

The nitrate concentration both in E-10 and H-0.5 treatment is decreased until the end of the experiment. The nitrate concentration starts to decrease in a high amount since day 5. During early period, the nitrate concentration does not change much (**Figure 12** and **13**). Meanwhile the ammonium concentration starts to decrease sharply since day 0 to day 1 (**Figure 10** and **11**). This different phenomenon indicates that all the plants in this study prefer to uptake ammonium-nitrogen as the first source of nitrogen. It has been observed that aquatic plants grow well with ammonium ion as source of nitrogen because less energy is required to uptake and assimilate ammonium nitrogen rather than nitrate-nitrogen (Jampeetong et al., 2012). Ammonium can be also volatilized as ammonia so that the ammonium in the water is reduced. Ammonium was volatilized as ammonia at pH more than 7 (Metcalf & Eddy. et al., 2003). The average pH of E-10 and H-0.5 is 6.74 with the maximum and minimum pH of 7.19 and 5.19, respectively. Because of that, the ammonia volatilization is not the main pathway for ammonium reduction in this experiment. Ammonium uptake by the plants is mainly responsible for ammonium removal.

III.3.2. Total Phosphorus

The total phosphorus is reduced during the experimental period both in E-10 and H-0.5 treatment and control (**Figure 14** and **15**). However the reduction of total phosphorus concentration in the H-0.5 treatment tends to be more rapid than in the E-10 treatment. This is may be the result of a lower growing capacity of the plant in the E-10 that results in the lower phosphorus uptake by the plants.

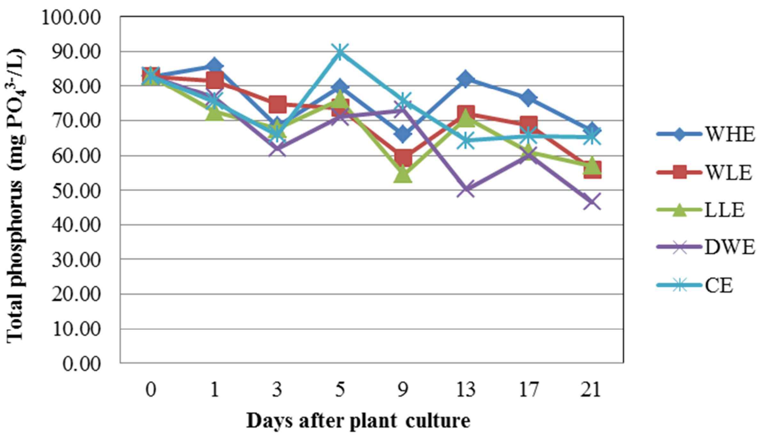

Figure 14. Total phosphorus concentration during experiments in the E-10 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

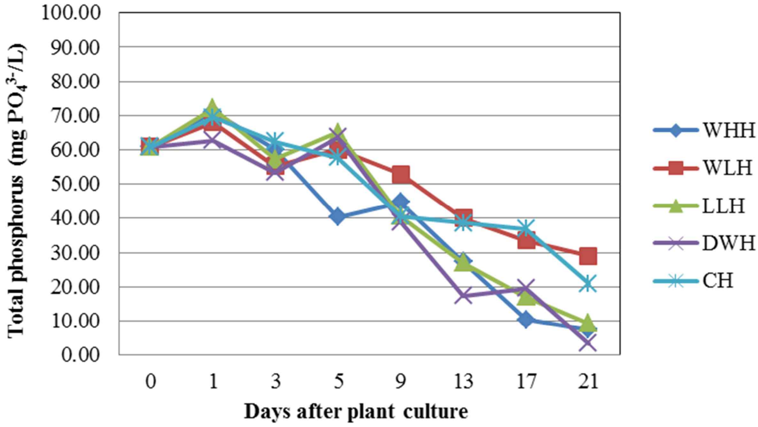

Figure 15. Total phosphorus concentration during experiments in the H-0.5 treatment (WH: Water hyacinth; WL: Water lettuce; LL: *Limnobium* sp.; DW: Duckweed; and C: Control)

The final concentration of total phosphorus is in the range of 3.33 – 67.00 mg/L. Duckweed removes the highest amount of total phosphorus both in E-10 and H-0.5 treatment (**Figure 16**). However, the final phosphorus concentration is 46.67 mg/L which is still above the effluent standard limit of 40 mg/L. To meet the effluent standard limit, a longer treatment time can be employed. A longer residence time results in better nutrient removal in the experiment with duckweed (Chaiprapat et al., 2005; Xu & Shen, 2011). In addition, harvesting plant biomass regularly may improve the nutrient removal from the E-10. Xu and Shen (2011) experiments showed that harvesting duckweed more frequent improve the total nitrogen and phosphorus removal from swine lagoon wastewater. This is also related with duckweed growth which is inhibited in the high density duckweed mat (Xu & Shen, 2011). Harvesting plant biomass periodically also can reduce the possibility of phosphorus to release again from the dead plant tissues decomposition.

Total phosphorus final concentration in the H-0.5 treatment meets the effluent standard limit of 40 mg/L since the 13th day. Thus the 10% effluent properties may have influence on the capacity of plants to uptake phosphorus from the water.

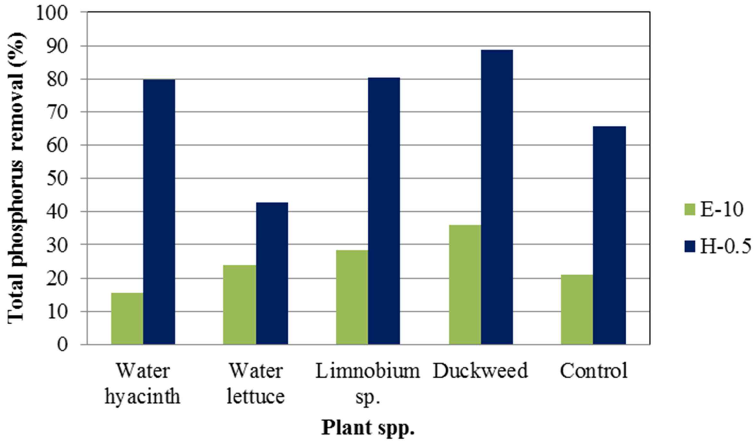

Figure 16. Total phosphorus removal comparison between E-10 and H-0.5 treatment of four types of plants and control (without plants)

Water hyacinth has the phosphorus removal of 15.42% in the E-10 treatment, which is the lowest among other plants. Meanwhile, duckweed has the highest phosphorus removal of 36.15% in the E-10 treatment. Water lettuce has the phosphorus removal of 23.78%. Water lettuce and water hyacinth has a better adaptability to grow in the 10% effluent. But, duckweed and *Limnobium* sp. growth is inhibited in the E-10 treatment. So, water lettuce and water hyacinth is more suitable for further phosphorus removal from the effluent even though the removal of total phosphorus is lower than duckweed and *Limnobium* sp.

III.4. Plant biomass

III.4.1. Plant visual appearance and observation

The plant growth is an important parameter to determine whether the floating plants can be used or not in the nutrient removal from the effluent. Every plant has a different growing capacity and adaptability to grow and remove nutrients in the effluent.

The difference in biomass amount and appearance was noticeable between treatments. All the plant in this experiment showed chlorosis leaves after growing in the E-10 as is shown in the **Figures 17, 18, 20,** and **21**. Chlorosis leaves may be caused by either nutrient deficiency or toxicity (Marschner, 1995).

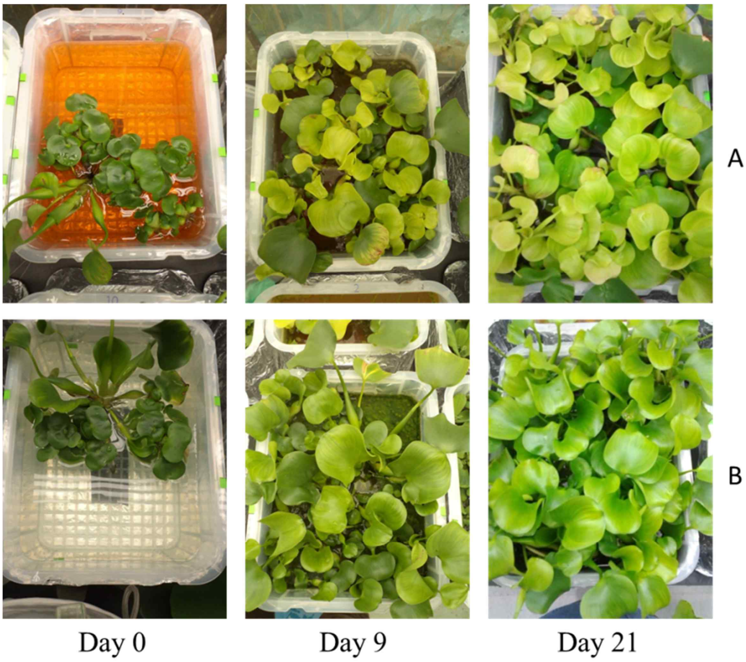

Figure 17. Water hyacinth growth during experiment (A. E-10; B. H-0.5)

Day 0 Day 9 Day 21

Figure 18. Water lettuce growths during experiment (A. E-10; B. H-0.5)

Chlorosis leaves have less photosynthetic pigments content (**Figure 19**), chlorophyll. Chlorophyll is important for plant photosynthetic activities. From visual observation it is obvious that plants with E-10 treatment were yellow in color. Figure 18 shows the chlorophyll content of plants. Water hyacinth has the highest total chlorophyll content compared to the other plants which is 519.06 mg/kg fresh weight. The chlorophyll content in the H-0.5 treatment is generally higher than the E-10 treatment. The difference in the chlorophyll content between plant grown in the E-10 and H-0.5 treatment was statistically noticeable ($p<0.05$). Thus means, E-10 highly influence the chlorophyll content.

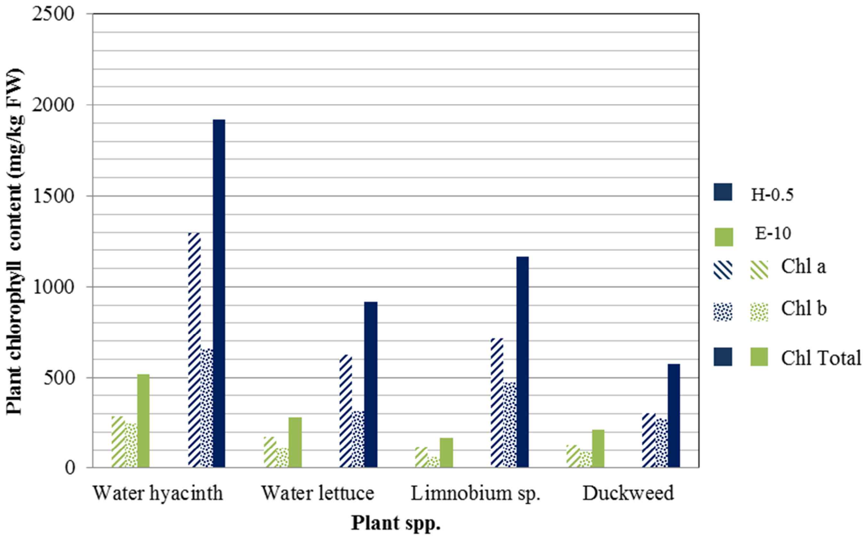

Figure 19. Plant chlorophyll contents

The chlorosis leaves appear especially in new leaves. This kind of chlorosis may be caused by iron deficiency because iron is immobilized mineral nutrient that cannot be transferred to younger leaves (Taiz & Zeiger, 2006). Iron is an essential nutrient that is important in the synthesis of chlorophyll-protein complexes in the chloroplast (Taiz & Zeiger, 2006).). Moreover, the content of iron in E-10 treatment is more than 4 times less concentration compared with H-0.5 treatment (**Table 2**). However, chlorosis may also result from the toxicity of some mineral nutrients and response of physiological stress.

As shown in **Figures 17**, **18**, **20**, and **21**, water lettuce and water hyacinth are more capable to adapt and grow in the 10% effluent based on their visual appearance which shows a less chlorotic and necrotic incidence. In addition, according to Relative Growth Rate (RGR) calculation, water lettuce has the highest RGR value of 0.09 (g $g^{-1}d^{-1}$)

and water hyacinth has the second highest RGR value of 0.06 (g $g^{-1}d^{-1}$) in the E-10 treatment. This adaptability is because water hyacinth and water lettuce have more complex tissues compared with *Limnobium* sp. and duckweed. Water hyacinth and water lettuce have more extensive root systems as well as leave structures. The leaves of water lettuce and water hyacinth mostly above the water surface reduce the contact between leaves and 10% effluent. In other hand, *Limnobium* sp. and duckweed leaves are in direct contact with the 10% effluent.

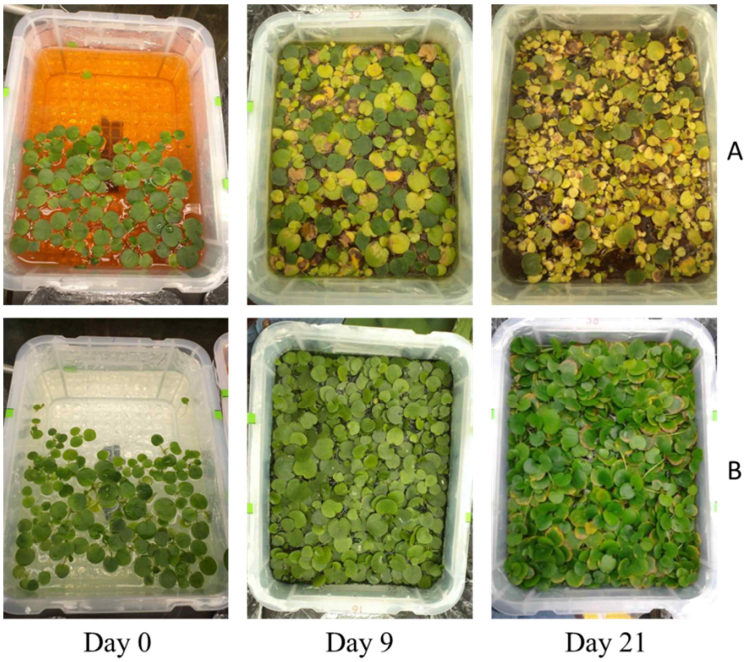

Figure 20. *Limnobium* sp. growth during experiment (A. E-10; B. H-0.5)

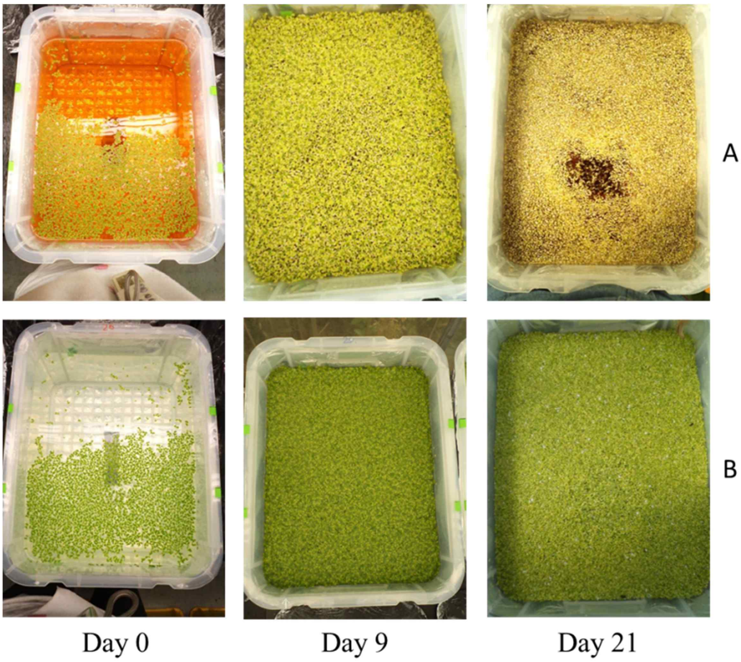

Figure 21. Duckweed growth during experiment (A. E-10; B. H-0.5)

On the 21st day, some of the duckweed biomass was sinking to the bottom of the experimental unit which contributes to the loss of a large amount of final biomass production. The chlorosis effect of E-10 treatment continues until the duckweed cannot tolerate and becomes dead. The duckweed fronds totally lost their pigments and become transparent in color (**Figure 21**). Therefore, the relative growth rate of duckweed in the E-10 treatment is low, which is only 0.05 (g $g^{-1}d^{-1}$) (**Figure 22**). *Limnobium* sp. has the lowest growth rate of 0.04 (g $g^{-1}d^{-1}$) because, as can be seen in the **Figure 20**, some of the plant biomass experienced chlorosis which may inhibit the photosynthetic activity thus results in the death of plant tissues.

III.4.2. Plant growth and biomass

Water lettuce has the highest relative growth rate in the E-10 treatment which is 0.09 (g $g^{-1}d^{-1}$), followed by water hyacinth which is 0.06 (g $g^{-1}d^{-1}$) (**Figure 21**). Water lettuce is considered to have a better adaptability to grow in the E-10 treatment. The difference in relative growth rate is because water lettuce plant tissues are denser than water hyacinth tissues. Duckweed performed the best but in the H-0.5 treatment. Based on this result, for further removal of nitrogen and phosphorus from effluent, *Limnobium* sp. and duckweed may not be suitable.

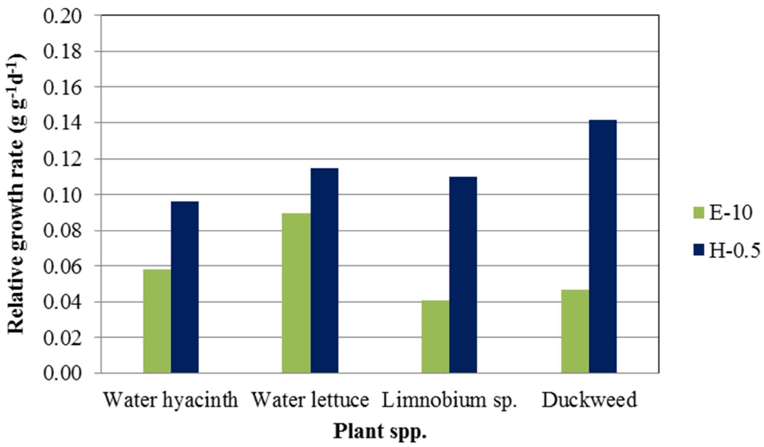

Figure 22. Relative growth rate of floating aquatic plants

Table 4 shows the relative growth rate and biomass production during the experiment. Water hyacinth produces the highest amount of dry weight biomass. Water hyacinth produces 5.19 g dry weight/m^2/day in the E-10 treatment (**Figure 23**). However the relative growth rate of water hyacinth is lower than that of water lettuce.

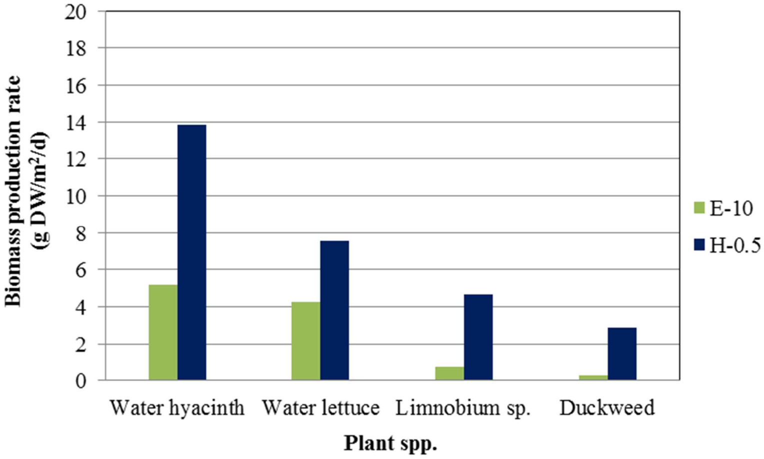

Figure 23. Production of biomass in dry weight basis

Table 4. Relative growth rate and biomass yield of the floating aquatic plants in E-10 and H-0.5 treatment

		Water hyacinth	Water lettuce	*Limnobium* sp.	Duckweed
RGR (d^{-1})	E-10	0.06	0.09	0.04	0.05
	H-0.5	0.10	0.12	0.11	0.14
Final biomass fresh weight (g)	E-10	237.77	214.20	56.52	21.86
	H-0.5	468.21	333.54	200.86	147.42
Final biomass dry weight (g)	E-10	15.32	10.59	2.65	0.91
	H-0.5	33.56	17.56	10.93	6.36
DW/FW ratio	E-10	0.06	0.05	0.05	0.04
	H-0.5	0.07	0.05	0.05	0.04

RGR: Relative growth rate; DW: Dry weight; FW: Fresh weight; E-10: 10% effluent; H-0.5: ½-strength Hoagland's

The relative growth rate is final biomass relative to the initial biomass weight during a certain period of time, in this case, 21 days. A higher relative growth rate indicates that the plant is growing faster.

The higher dry weight to fresh weight ratio (DW/FW ratio) indicates the plants have more dry matters of the fresh weight. Water hyacinth has a higher DW/FW ratio compared to water lettuce. This is because water lettuce contains more water than water lettuce (**Table 5**). So, even though water hyacinth has a lower relative growth rate than water lettuce, it produces more dry matters than water lettuce. This indicates the water hyacinth might be more suitable to maintain the sustainability of a nutrient removal system because it produces biomass that can be further utilized for another purposes, such as anaerobic digestion substrate or animal feed. However, modification in the system is needed so that the removal of total nitrogen and phosphorus from effluent can be increased.

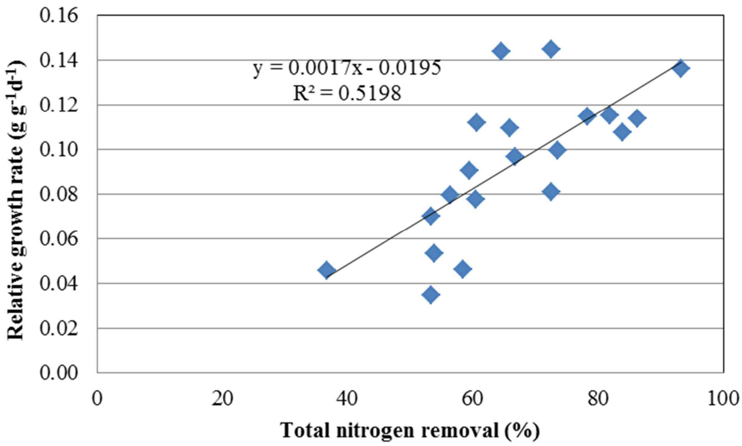

Figure 24. Correlation of relative growth rate and total nitrogen removal by linear regression

There is a tendency that the higher nitrogen and phosphorus uptake, the higher the plant growth. The correlation between nitrogen removal and relative growth rate of the plants was examined. Single linear regression analysis was conducted and shown in the **Figure 24** and **25**. The R^2 value of total nitrogen removal and relative growth rate linear regression was 0.52 which means the Pearson's correlation coefficient is about 0.72. This result shows that nitrogen removal is highly correlated with plant relative growth rate. This is clear that both of parameters are highly correlated since nitrogen is the macronutrients that is needed by the plants for new cell synthesis and lack of nitrogen inhibit growth of the plant. The R^2 value of total phosphorus removal and relative growth rate linear regression was 0.50 which means the Pearson's correlation coefficient is about 0.70 which is highly correlated. Phosphorus also the main important nutrient of plant cells. Phosphorus in needed for membrane cell synthesis as well as the building blocks of ATP which is cell energy source. Lack of phosphorus will result in stunted growth.

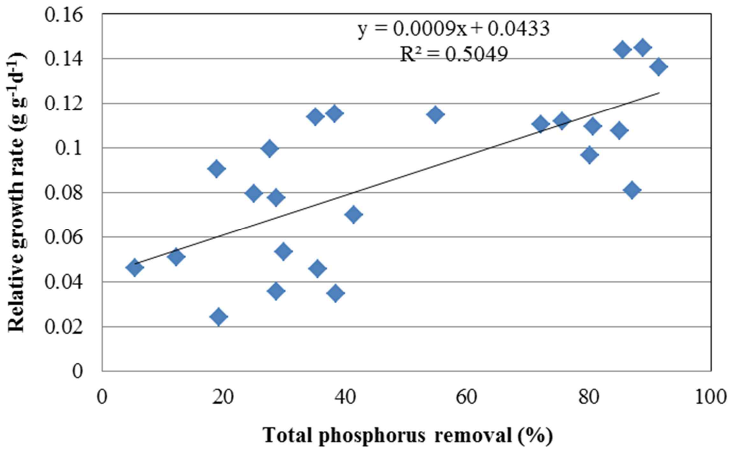

Figure 25. Correlation of relative growth rate and total phosphorus removal by linear regression

The moisture content of the floating aquatic plant biomass is quite high which range from 92% up to about 96% (**Table 5**). The ash content of the plants generally is higher in the E-10 treatment, except for *Limnobium* sp. which is the opposite. This indicates that water hyacinth, water lettuce, and duckweed are able to uptake more metals compared to *Limnobium* sp. from the E-10. Water lettuce has the highest ash content compared to other plants. Water lettuce has been known to uptake a high amount of manganese (Mn) from manganese-contaminated water (Hua et al., 2012), Zn, and Cd. However, the ash content is not statistically noticeable between the E-10 and H-0.5 treatment ($p > 0.05$).

Table 5. Plant biomass proximate analysis

		Water hyacinth	Water lettuce	*Limnobium* sp.	Duckweed
MC (%± SD)	E-10	93.62±0.40	95.06±0.40	94.73±1.22	95.92±0.67
	H-0.5	92.80±2.22	94.73±0.19	94.55±0.04	95.69±0.16
DM (%±SD)	E-10	6.38±0.40	4.94±0.40	5.27±1.22	4.08±0.67
	H-0.5	7.2±2.22	5.27±0.19	5.45±0.04	4.31±0.16
OM (% DM±SD)	E-10	82±1.48	77.78±0.88	87.15±7.82	79.93±7.56
	H-0.5	83.16±3.02	80.13±1.1	76.97±3.51	88.54±7.56
ASH (% DM±SD)	E-10	17.69±1.48	22.22±0.88	12.85±7.82	20.07±6.02
	H-0.5	16.84±3.02	19.87±1.1	23.03±3.51	11.46±7.56

MC : Moisture content; DM: Dry material; OM: Organic material; E-10: 10% effluent; H-0.5: ½-strength Hoagland's

IV. CONCLUSION

Among the plants used, water lettuce gives the highest removal of total nitrogen and phosphorus from the effluent. Wlettuce which removes total nitrogen gives the final total nitrogen concentration of under the effluent standard limit of 120 mg/L. All the plants prefer to uptake ammonium in the early period of time. Nitrate is up-taken 5 days later after ammonium ion is decreased; therefore, all the aquatic plant used in this experiment can be also used for high-ammonium wastewater.

Duckweed and *Limnobium* sp. have more simple structures compared to water hyacinth, and water lettuce does not seem to be suitable for nutrient removal from the effluent. However, duckweed has the highest phosphorus removal compared to other plants. However, the final total phosphorus concentration is still above the effluent standard limit of 40 mg/L. In addition, from observation, a large amount of duckweed fronds is dead because of chlorosis so it is believed that duckweed cannot be used for further treatment. As an alternative, water lettuce can be used for further effluent phosphorus removal. Even though the phosphorus removal efficiency is lower than that of the duckweed, water lettuce has a better adaptability to grow in the effluent. Therefore, water lettuce can be used for further N and P removal from effluent. In addition, water lettuce has the highest relative growth rate. Based on linear regression analysis, relative growth rate is highly correlated with nitrogen and phosphorus removal with R^2 value of 0.52 and 0.50 respectively.

The ash content of plants is higher from effluent treatment except for *Limnobium* sp. This indicates water hyacinth, water lettuce, and duckweed can uptake more metals from the effluent. Based on the biomass yield, water hyacinth gives the highest dry matter biomass production of 5.19 g dry weight/m^2/day in the effluent treatment. This indicates the water hyacinth might be more suitable to maintain the sustainability of a nutrient removal system because it produced biomass that can be further utilized for another purposes such as anaerobic digestion substrate or animal feed or bioethanol raw materials. However, a new strategy is needed so that the removal of total nitrogen and phosphorus from effluent by water hyacinth can be increased through a longer retention time or periodic harvesting. Finally, the nutrient removal and biomass growth is higher in all ½-strength Hoagland's treatment. Effluent may have properties that inhibit the growth and nutrient uptake by the plants, which requires further researches.

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초록

부유 수생식물에 의한 돈슬러리 처리수의 질소·인 제거

질소와 인은 주요 환경오염원이다. 물 속의 과도한 질소와 인은 부영양화를 초래하여, 산소 고갈로 인한 수생식물 및 물고기 등의 폐사를 가져올 수 있다. 물 속 질소와 인 오염의 주요원인은 영농(축산 포함)행위, 오폐수, 빗물에 기인한다고 보고되어 있다 (US EPA, 2014). 그러므로 가축분뇨를 처리 후 정화방류할 때도 방류수의 질소, 인을 함량을 수질기준으로 반드시 저감해야 한다.

서울대학교 동물환경생체공학(AEBE) 연구실에서는 지난 5 년 동안 pilot 생물학적 축산폐수 처리시스템을 농생대 부속목장에 축조하여 운영하고 있다. 이 시스템은 지하 혐기성소화(UGAD)공정과 활성슬러지 공정(activated sludge process) 및 막 여과(membrane filtration)공정으로 구성되어 있다. 일반적으로 처리수는 우리나라 환경부가 정한 공공처리장의 방류수 수질기준 TN, TP 가 각각 120 mg/L 과 40 mg/L 을 초과해서는 안된다. 우리 나라 대부분의 축산폐수처리시설은 이를 만족시키지 못하기

때문에 하천이나 저수지와 같은 수역으로 방류시키기 전에 질소와 인을 추가적으로 제거하여 그 기준을 충족시킬 필요가 있다.

최근 LID (low Impact Development)기법의 하나로 축산폐수의 무기물(T,P)를 제감에 수생식물들을 널리 사용하고 있지만, 관련연구는 극히 한정적이다. 그러므로 본 연구는 수생식물에 의한 가축분뇨 처리수의 질소와 인의 저감효율에 분석하였다. 실험 수생식물로는 물부레옥잠(water hyacinth), 물상추(water lettuce), *Limnobium* sp., 및 좀개구리밥(duckweed) 등 네 종(種)을 사용하였다.

표면적이 0.10 m^2 인 13 L 들이 용기에 10 L 의 물기질을 담아 배치(batch) 시스템으로 실험을 수행하였다. 10% 처리수를 처리구로 ½농도-Hoagland’s 양액재배를 대조구로 설계하여 연구를 수행하였다. ½농도-Hoagland’s 양액 및 10%-방류수는 지하수로 희석하였다. 각각의 처리시료를 3 반복하여 전체 30 개의 실험단위 (2 trt. x 5 plants x 3 rep.= 30ea)로 구성하였다. 실험결과는 완전임의배치법(completely random distribution method)을 사용하여 통계분석하였다. 물 표본들은 0, 1, 3, 5, 9, 13, 17, 및 21 번째 실험일에 채취하였다. 바이오매스(biomass)는 물 시료채취 기간 말미(Day 21)에 채취하였으며 전(全) 건조중량을 측정하였다. 물 분석변수들, TN,

NH_4^+, NO_3^-, NO_2^-, TP, 및 TSS(total suspended solid)등을 분석하였다. TSS 의 변화는 실험기간 동안의 조류(藻類)성장량을 나타낸다. 전체 양분제거량에서 조류에 의해 양분제거량을 감(減)하면 순(純)수생식물에 의한 양분저감량을 산정할 수 있다.

예비 발아실험에 의하면 처리수의 N, P 농도가 10%-방류수를 넘으면 식물에게 위해(危害)한 것으로 나타나 본 연구에서는 10%-방류수 농도까지 희석시킨 희석수를 treatment (처리)수로 사용하였다. 농도를 절반으로 희석한 1/2- Hoagland's 양액과 10%-방류수를 비교 실험결과, 10%-방류수 및 ½-농도 Hoagland's 양액의 초기 총 질소 농도는 각각 151.67 mg/L 및 125.71 mg/L 로 나타났다. 한편 10%-방류수 및 ½-Hoagland's 양액의 총인 농도는 각각 82.77 mg/L , 60.85 mg/L 로 분석되었다. 물상추(water lettuce)의 경우, 10%-방류수에서 다른 식물보다 많은 총질소, 총인을 제거하는 것으로 분석되었다. 물상추(water lettuce)의 경우, 총질소의 제거율은 63.15%로서 최종 질소농도는 43.33 mg/L 로 분석되었다. 이는 방류수 수질기준인 120 mg/L 보다 낮았다. 한편, 좀개구리밥 (duckweed)은 유출수의 경우, 인의 제거율은 36.15%로서 다른 식물보다 높게 분석되었다. 총인

농도는 46.67 mg/L 로서 방류수 수질기준인 40 mg/L 보다 높게 나타났다.

실험시작 후 9 일 이상이 되자 많은 양의 좀개구리밥(duckweed)이 광합성 활성을 억제하는 심각한 백화현상(녹색채색의 손실)으로 폐사되었기 때문에 좀개구리밥은 더 이상 사용할 수가 없었다. 물상추는 최대 상대성장률이 0.090(g $g^{-1}d^{-1}$)으로 다른 수생식물보다 질소와 인 제거율이 높게 나타났다. 하지만 biomass 양을 고려해 볼 때 물부레옥잠은 유기물질 함량 82%와 건물중량 5.19 g/m^2/day 으로 최대값을 보여 바이오매스 발생량만 본다면 물부레옥잠 선발이 적합할 것으로 사료된다.

수생식물이 질소와 인을 더 많이 흡수할수록 수생식물의 성장은 더 증가하는 경향을 보인다. 질소 제거와 식물들의 상대적 성장율 간 상호관계를 살펴보면 총질소 제거 및 상대적 성장률 선형 회귀의 R^2 값은 0.52 로 나타났다. 이는 질소 제거가 식물 상대적 성장률과 크게 상호 연관되어 있다는 것을 보여준다. 마찬가지로 총인 제거 및 상대적 성장율 선형 회귀 R^2 값은 0.50 으로 상호관계가 높은 것을 알 수 있었다.

그러므로, 만일 더 많은 biomass 생산을 원한다면, 물부레옥잠을 선택하는 게 좋을 수 있다. 수확된 biomass 는 가축사료나 혐기성 소화 기질원료, 또는 바이오 에너지 (바이오 에탄올 등) 생산을 위한 원료로 사용 가능하다. 하지만, 유출수로부터 질소와 인의 제거효율을 높이기 위해서, 더 긴 체류시간(HRT) 적용과 수확방법 등 다른 전략들도 개발할 필요가 있다. 본 실험에서는 상대적으로 ½-농도 Hoagland's 대조구에서의 양분 제거 및 바이오매스 성장율이 더 높은 것으로 나타났다. 10%-방류수 처리구는 식물의 성장 및 양분 섭취를 방해하는 특성이 있을 것으로 추정되나 이의 원인을 구명하는 데 향후 심도 있는 연구가 필요할 것으로 사료된다.

핵심 용어: 부유 수생식물, 질소 제거, 인 제거, 돈슬러리 처리수, 바이오매스

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