

REMOVAL OF AMMONIUM AND NITRATE IN WATER BY AN AQUATIC PLANT: WATER LETTUCE (*Pistia stratiotes* L.)

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Abstract. In rural areas of the Vietnamese Mekong delta, biogas digesters are applied as a potential solution to treat waste from pig rearing. Wastewater from biogas production typically contains a high level of nutrients, which requires appropriate techniques for removal. In order to contribute to making small-scale rural pig rearing in the Vietnamese Mekong Delta more sustainable, this study examines the uptake of inorganic nitrogen by water lettuce under lab scale conditions. Two treatments, including a control (No water lettuce) and with water lettuce (8 plants # 112.6±0.5 g) were conducted in triplicate using plastic tanks (57 x 38 x 30,5 cm³). Each replicate contained 50 L of solutions (nominal concentration: 5 mgN/L NH₄-N + 5 mgN/L NO₃-N). The experiment was set up in a wet lab for 28 d. The environmental parameters of solution and plant weight were monitored every 7 days. The results showed that water lettuce could absorb both ammonium and nitrate, but ammonium uptake was preferential. With approximately 112 g of fresh biomass, over 28 days this biomass was approximately 4 times greater and removed at about 0.26 g nitrogen. The results highlight that water lettuce can be used to remove nutrients from biogas wastewater without aeration.

Keywords: *nutrient removal, water lettuce*

Introduction

The Mekong delta of Vietnam has a dominant role in both agricultural and aquacultural production for the nation. The total area of the delta accounts for approximately 12% of the whole country, and 18.5% of Vietnam's population, with more than 50% residing in rural areas. The livelihood of rural farmers is mainly focused on agricultural activities, including rice and upland crop farming, and livestock rearing. These activities create a large amount of waste (Dinh, 2017) which needs to be managed in an appropriate way in order to minimize environmental degradation.

An integrated farming model termed V-A-C-B (Garden – fishpond – pigsty – biogas) (Ngan and Klaus, 2012) has been developed and widely applied in rural areas of the delta. The integrated farming model is a rather closed system, which has resulted in a dramatic reduction of environmental pollution. However, it still suffers from several limitations such as the large amount of wastewater the system produces. The system is as follows: Starting with the biogas digester (B), the farmers use pig manure from the pigsty (C) to produce biogas for cooking and the wastewater effluent is often used as fish feed (A); residues from both the fishpond and biogas digester can be applied to the garden (V). Parts of the grown vegetable and plants can be used for pig feed (C) (Ngan and Klaus, 2012). However, the cycle of nutrients is not effectively used. To study the life cycle assessment (LCA) of the system, Nhu et al. (2015) found that waste from the pigs or wastes from biogas digester were typically discharged into aquacultural ponds. This waste content is high in nutrients and causes sedimentation of the fishponds or is

reused as fertilizers of agricultural crops and is discharged to surface waterbodies. In anaerobic conditions, inorganic nitrogen forms from biogas effluent are mainly ammonium (NH_4^+), varying between 126 - 421 mg/L (Hong and Lieu, 2012). In alkaline conditions, ammonium exists in ammonia (NH_3), particularly at high temperature (Thurston et al., 1974) as in tropical climates. Ammonium can be converted to nitrite (NO_2^-) and nitrate (NO_3^-) in conditions of available oxygen and existence of *Nitrosomonas* and *Nitrobacter* (Hasan et al., 2021; Rout et al., 2021). Furthermore, high ammonium and nitrate loads combining with phosphorus often cause eutrophication in receiving waterbodies and adverse effects to aquatic organisms. High nitrite in water cause inhibition on transport oxygen of red blood cells (Huertas et al., 2002). Limitation level of nitrite in surface water should be below 5 mg/L for warm water fish (McCoy, 1972) and below 0.06 mg/L for salmonid fishes (Russo and Thurson, 1975). According to the Vietnamese National technical regulation on surface water quality, the official maximum concentrations of ammonium, for protecting aquatic life are 0.3 mgN/L while threshold values for nitrite and nitrate are 0.05 mgN/L and 2 mgN/L, respectively (MONRE, 2015). Therefore, it is necessary to find solutions to remove these forms of inorganic nutrients before discharge into the environment.

Nguyen et al. (2015) showed that water hyacinth (*Eichhornia crassipes*) has a high capacity to remove ammonium from tripped catfish (*Pangasianodon hypophthalmus*) farming wastewater. Furthermore, Da et al. (2015) applied an integrated Rice – Fish – Vegetable farming system to treat or reuse nutrients in wastewater from tripped catfish ponds. These techniques had low running costs but required a large surface area. In rural areas of Vietnam, most farmers have larger land areas than in urban areas. Therefore, using plants to remove nutrients is a potential solution. Plants can not only take-up pollutants from the water, but they can also remove carbon dioxide from the air through their photosynthesis, contributing to mitigating greenhouse gas emissions. Effluent from biogas was used as sources of nutrients for up-plant crop as well as for fish farming in the Mekong Delta of Vietnam (Nhu et al., 2015).

Water lettuce (*Pistia stratiotes* L.) is a free-floating plant that is widely distributed in tropical regions. According to Fonkou et al. (2002), when water lettuce grows in favorable environmental conditions, its biomass can double after 5 days, increase 3 times in 10 days, and 9 times after only a month. With such fast growth, the plant may be the ideal candidate for removal of nutrients in aliquot. Two inorganic forms of nitrogen as ammonium and nitrate are up taken by plants. With single ammonium form (9.67 ± 0.36 mgN/L), after 28 d fresh biomass of water lettuce was double (Linh et al., 2021). However, to know what form of inorganic nitrogen plants prefer to uptake is an important requisite for creating optimal conditions (Boudsocq et al., 2012). The present study aims to test what form of inorganic nitrogen water lettuce prefers to uptake under lab scale conditions and provide a foundation for the application of water lettuce in reducing pollution of livestock wastewater and effluent from biogas.

Materials and methods

Materials

Water lettuce was collected from Phong Dien district, Can Tho city and then transported to the College of Environment and Natural Resources, Can Tho University and stored in fiberglass tanks for two weeks before commencement of the experiment.

Ammonium Chloride (Merck) and Sodium Nitrate (Merck) chemicals were used to create a mixture of NH₄-N and NO₃-N solution for the experiment.

Experimental design

The experiment was conducted with a completely randomized design with two treatments, including a control (without water lettuce) and with water lettuce (8 plants # 112.6±0.5 g). The experiment was triplicated using plastic tanks (57 x 38 x 30,5 cm³). Each replicate contained 50 L of mixture solutions (nominal concentration: 5 mgN/L NH₄-N + 5 mgN/L NO₃-N). The air temperature during the experiment varied between 25.4°C and 31.4°C. Time of light and dark condition was approximately 12:12. The experiment was setup in a wet lab which was covered by a transparent roof, which allowed light, but prevented rainwater, and lasted for 28 d.

Sampling frequency and analytical methods

After preparation of the solution, the water in the tanks was mixed for 10 minutes and water samples were collected for detecting the actual concentration of NH₄-N and NO₃-N. Thereafter, water temperature, pH and dissolved oxygen (DO) were measured weekly using a portable meter (TOA-DKK DO-31P and TOA-DKK HM-31, Japan). In addition, water samples were collected weekly and analyzed for NH₄-N, NO₂-N, NO₃-N following the procedures from Standard Method (APHA, 2012, *Table 1*).

Table 1. Analytical methods for water and plant parameters

Parameter	Method
Parameters in water	
pH	TOA-DKK HM-31, Japan
DO/Temperature	TOA-DKK DO-31P, Japan
N-NO ₃ ⁻ (mg/L)	SMEWW-4500 NO ₃ ⁻ . E:2012
N-NH ₄ ⁺ (mg/L)	SMEWW 4500-NH ₃ . F:2012
N-NO ₂ ⁻ (mg/L)	SMEWW 4500-NO ₂ . B:2012
Parameters in water lettuce	
Fresh biomass (g)	Let water drops out 10 min and weight
Dried biomass of water lettuce	Dry at 105°C to constant weight
N _{total} in water lettuce (%)	Kjeldahl method

For plants, the initial fresh weight of total water lettuce in each replication was measured before placement into each tank. This measurement then was also repeated at 7 d intervals during experiment. At each sampling time, the plants were removed out of the tested solution, water was allowed to drip out for 10 mins before the fresh biomass was weighed. Afterwards, they were placed back into the same tank. At the end of 28 d, the water lettuces were dried at 105°C until a constant weight was achieved for calculation of water content and determine total nitrogen using common methods (*Table 1*).

Statistical analysis

The environmental parameters of water and fresh biomass weight of water lettuce were compared over sampling intervals by applying analysis of variance (ANOVA) and

the Duncan test using the SPSS 20.0 software. Paired-samples T test was applied for comparing between control and water lettuce treatments. Significant differences were considered at $p < 0.05$.

Results

Variation of water temperature, DO and pH during the experimentation

Results showed that water temperature varied between 26.4°C and 31.4°C in the control treatment and between 27.0°C and 31.2°C in the treatment with water lettuce (Table 2). DO varied between 5.5 mg/L and 5.9 mg/L in the control treatment, while this variation was between 4.3 mg/L and 5.9 mg/L in the water lettuce treatment. Water pH fluctuated between 7.5 and 7.7 in the control treatment and from 5.4 to 7.6 in the other treatment.

Table 2. Temperature, DO and pH of water during the experimentation

Time (Day)	Temperature (°C)		DO (mg/L)		pH	
	Control	Water lettuce	Control	Water lettuce	Control	Water lettuce
0	31.4±0.1 ^e	31.2±0.1 ^d	5.9±0.0 ^c	5.9±0.0 ^b	7.5±0.1 ^a	7.6±0.0 ^e
7	27.9±0.1 ^c	28.1±0.3 ^b	5.5±0.0 ^a	4.8±0.2 ^{a*}	7.7±0.1 ^a	7.0±0.1 ^{d*}
14	27.2±0.1 ^b	27.0±0.2 ^a	5.8±0.2 ^{abc}	4.6±0.0 ^{a*}	7.7±0.4 ^a	6.5±0.2 ^{c*}
21	26.4±0.2 ^a	26.4±0.3 ^a	5.9±0.1 ^{bc}	4.9±0.8 ^{a*}	7.5±0.4 ^a	5.9±0.3 ^{b*}
28	29.7±0.1 ^d	29.1±0.8 ^c	5.6±0.3 ^{ab}	4.3±0.2 ^{a*}	7.5±0.4 ^a	5.4±0.1 ^{a*}

Data is presented mean ± standard deviation. Following column, data follows at least the same a letter is insignificant difference ($p > 0.05$). Following row, data follows an asterisk (*) indicates significant difference to parallel control ($p < 0.05$)

Variation of dissolved inorganic nitrogen in water during the experimentation

In this study, both ammonium and nitrate were mixed in a solution. Concentrations of ammonium and nitrate decreased significantly towards the end of the experiment (Table 3). Ammonium decreased from 4.69 mg/L to 1.92 mg/L at the end 28 d in the control, while in the treatment with water lettuce, it decreased from 4.56 mg/L to 0.11 mg/L. In contrast, nitrate increased from day zero to day 28 in the control and to day 14 in the water lettuce treatment. Hereafter, it decreased and at the end on 28 d reached 5.66 mg/L in the control, while in the water lettuce treatment, it was 41.2% of the concentration in the control (2.29 mg/L). Nitrite was detected at very low concentrations, varying between 0.001 and 0.037 mg/L in the control, and between 0.001 and 0.309 mg/L in the water lettuce treatment.

Biomass and characteristic of water lettuce

Fresh biomass of water lettuce increased greatly towards the end the experiment (Figure 1). At the beginning, the fresh biomass was 112.6 g ± 5 and was 440.5 g ± 26.1 at the end 28 d.

Table 3. Variation of ammonium, nitrite and nitrate during the experimentation

Time (day)	N-NH ₄ ⁺ (mgN/L)		N-NO ₂ ⁻ (mgN/L)		N-NO ₃ ⁻ (mgN/L)	
	Control	Water lettuce	Control	Water lettuce	Control	Water lettuce
0	4.69±0.15 ^d	4.56±0.27 ^d	0.002±0.001 ^a	0.002±0.001 ^a	4.14±0.05 ^b	4.24±0.16 ^c
7	3.87±0.10 ^c	2.93±0.25 ^{c*}	0.020±0.002 ^a	0.309±0.055 ^{b*}	5.11±0.06 ^c	5.10±0.09 ^d
14	3.62±0.07 ^c	0.66±0.01 ^{b*}	0.037±0.035 ^b	0.206±0.144 ^b	5.22±0.02 ^a	6.10±0.00 ^{a*}
21	2.95±0.43 ^b	0.26±0.17 ^{a*}	0.026±0.003 ^{ab}	0.003±0.002 ^{a*}	5.77±0.02 ^e	4.44±0.17 ^{c*}
28	1.92±0.39 ^a	0.11±0.03 ^{a*}	0.037±0.006 ^b	0.003±0.001 ^{a*}	5.66±0.17 ^d	2.29±0.24 ^{b*}

Data is presented mean ± standard deviation. Following column, data follows at least the same a letter is insignificant difference ($p > 0.05$). Following row, data follows an asterisk (*) indicates significant difference to parallel control ($p < 0.05$)

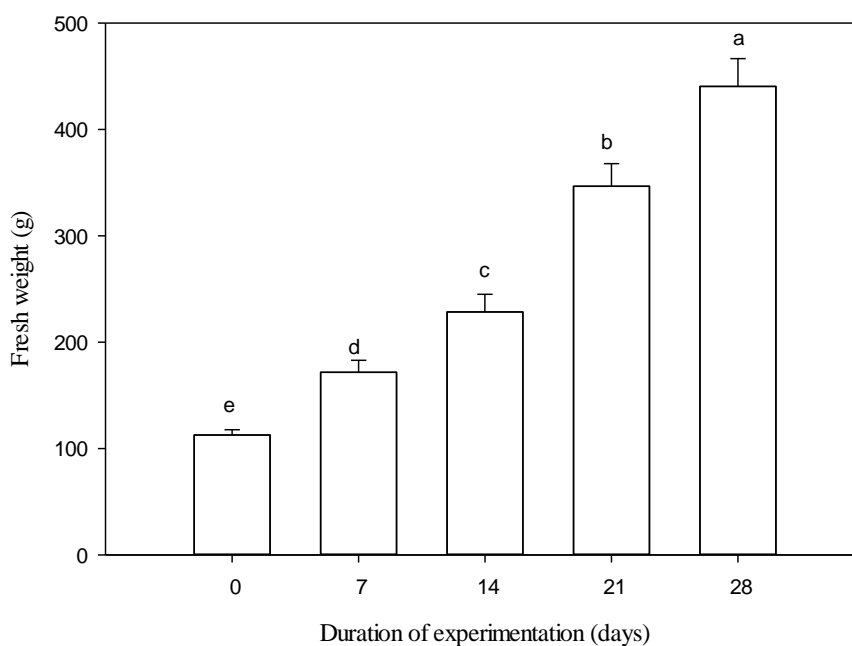


Figure 1. Change in fresh biomass of water lettuce during the experiment. Different letter indicates significant difference ($p < 0.05$, Duncan test)

The nitrogen content in the water lettuce was 2.93% at the beginning and 3.09% at the end 28 d of the experiment (Table 4). Dried biomass was calculated to be 2.93 g for the beginning and 3.09 g for the end the experiment. Whilst total N was calculated as 0.09 g at the beginning and 0.35 g for the end.

Table 4. Characteristics of water lettuce at the beginning and the end experiment

Items	At the beginning	At the end 28 d
N content in water lecture (%)	2.93 ± 0.1	3.09 ± 0.2
Dried biomass (g/rep)	2.0 ± 0.3	11.4 ± 0.7
Total N (g/rep)	0.09 ± 0.01	0.35 ± 0.02

Data is presented mean ± standard deviation, n=3

Discussion

Variation of inorganic nitrogen during the experiment could have resulted from the transformation among ammonium, nitrite and nitrate, etc., and functions of organisms (algal, water lettuce, bacteria). Ammonium can exist in the form of NH_4^+ or NH_3 depending on pH and temperature; the higher pH or temperature is the greater the percentage of NH_3 (Thurston, 1974). With the condition of temperature (26.4 - 31.4) and pH (7.5-7.7) in the control treatment (Table 2), the highest NH_3 was estimated at about 1.8%. In this treatment, after 28 d, the remaining concentration was 40.9% or equaling a reduction of 59.1%. In contrast, concentration of nitrate was higher than that at the beginning. The lowest DO in the control was 5.5 mg/L (Table 2), indicating that the treatment was in anaerobic condition. With available DO, nitrification process could have occurred (Rout et al., 2021; Hasan et al., 2021) and led to reduce ammonium and increased nitrate.

In the water lettuce treatment, with temperature (26.4 - 29.1) and pH (5.4-5.9) (Table 2), NH_3 may account for 0.057%; This suggests that NH_4^+ was dominant and after 28 d, the remaining concentration was 2.4% from that of the beginning or a 97.6% reduction. Although the DO in the water lettuce treatment was lower than in the control (Table 2) the lowest measured DO was 4.3 mg/L. This indicates that water in this treatment was also in anaerobic conditions. In this treatment, the biological roles may be very important for the nutrient removal. Water lettuce is none winter-hard plant, minimum temperature for growth is 18°C (Gupta et al., 2012). Therefore, water lettuce may not be suitable for frigid areas due to their sensitivity to cool temperature (Clough et al., 1987). Temperature in the present study (The air temperature 25.4°C - 31.4°C, water temperature 26.4°C - 29.1°C) is typical for tropical region and suitable for growth of water lettuce. The roots of the water lettuce may have contained bacteria. The seen reductions in ammonium and nitrate in this treatment could be explained by effects of both bacteria and water lettuce. Total inorganic nitrogen (ammonium, nitrite, nitrate) at the beginning in this treatment was approximately 8.8 mgN/L, which continuously decreased to the end of the experiment. After 21 d, the concentration of ammonium in the water lettuce treatment was very low (0.11 mg/L). This level may not be sufficient for the requirements of water lettuce, and then after the plant shifted to uptake nitrate. In other word, water lettuce prefers to uptake ammonium first. This is in agreement with the earlier findings by Aoi and Hayashi (1996). Inorganic nitrogen forms in biogas effluent were found almost in NH_4^+ (Hong and Lieu, 2012). This form is the first priority for uptalking. Therefore, water lettuce can be used to remove ammonium form from biogas effluent without any further oxidation.

Fonkou et al. (2002) found that the biomass of water lettuce doubled after 5 days, increased 3 times in 10 days, and 9 times after only a month. In the present study, the solution which was used for growing water lettuce was mixed NH_4Cl and NaNO_3 , without any other additional nutrients such as phosphorus, calcium (Ca), Fe, K, and Mn, etc. Therefore, after 28 d, the fresh biomass in the present study was approximately 4 times that of the beginning. To maintain optimal growth density, water lettuce is also recommended to be periodically harvested (Fonkou et al., 2002) to maintain the effective removal of nitrogen and phosphorus (Lu et al., 2011). The harvested biomass can be used for producing compost (Cong et al., 2021) or used as additional feedstock material for producing biogas (Cong et al., 2022).

Conclusions and recommendations

Water lettuce could uptake both ammonium and nitrate, but ammonium was preferentially up taken. With approximately 112 g of fresh biomass, after 28 days, biomass increased by approximately 4 times and removed about 0.26 g nitrogen from the tested solution. The results suggest that water lettuce can be used to remove nutrients from biogas wastewater without aeration.

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