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Pollutant Removal in Wastewater from Anaerobic Digesters by Water Lettuce (*Pistia stratiotes* L.) at Both Still-Water and Running-Water Stages

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ABSTRACT

This research investigated the effectiveness of water lettuce (WL; *Pistia stratiotes* L.) in improving the quality of wastewater from biogas systems. Two treatments were designed, one without WL and the other with WL. First, WL were raised in containers that had 15 L of wastewater with an initial ammonium concentration of about 15 mg/L at the still-water stage (days 0–7). Then, at the running-water stage (days 10–22), wastewater with a targeted NH_4^+-N concentration of about 15 mg/L in a 5-L tank was gravitationally delivered continually into terraced Styrofoam containers designed as ponds 1, 2 and 3. Water samples were collected on days 0, 3, 7, 10, 13, 16, 19 and 22, and fresh weights of WL were measured on the same days of sampling the water. The results showed that at the still-water stage, WL contributed to the reduction of chemical oxygen demand (14.74 ± 4.14% and 8.69 ± 0.92%, respectively), total inorganic nitrogen (23.93 ± 2.35% and 12.80 ± 1.30%, respectively), ammonium (25.21 ± 5.44% and 1.12 ± 0.93%), nitrite (59.98 ± 3.22% and 22.37 ± 1.21%, respectively) and phosphate (71.84 ± 0.89% and 61.64 ± 1.65%, respectively) on days 0–3 more than on days 4–7 but did not help decrease nitrate concentrations on days 0–7. WL contributed to reducing organic matter less at the running-water stage than at the still-water stage. WL helped lower ammonium, nitrite and nitrate concentrations at the running-water stage more than at the still-water stage but did so more for ammonium and nitrate compared with nitrite at the running-water stage. No differences in pollutant concentration reductions between the two treatments (without and with WL) were found in ponds 1, 2 and 3. On days 10–22, no clear trend in increasing or decreasing pollutant concentrations emerged, except nitrite concentration, which lessened over time. No significant differences in the relative daily WL fresh biomass increase between the still-water and the running-water days were observed. The findings indicate that WL is an aquatic plant that can be used in treating wastewater from biogas systems, showing a high efficiency in lowering phosphorus concentrations and a potential for removing nitrite.

Keywords: biogas digester, pollutant reduction, water lettuce, wastewater.

INTRODUCTION

Pig waste treatment by biogas systems is one of the practical solutions used to reduce environmental pollution caused by pig farming in Vietnam [Nguyen et al., 2012]. However, compared with the Vietnamese technical standard for livestock wastewater quality, the wastewater from biogas systems still contains higher concentrations of nutrients such as nitrogen and phosphorus, as well as organic matter, thus failing to meet the standard [Tran et al., 2017; Le et al., 2017]. Therefore, wastewater from biogas systems needs further treatment before being discharged into surrounding water bodies. Aquatic macrophytes are known for their high potential of absorbing organic matter and nutrients into their tissues for growth, as well as cleaning polluted water sources [Rezania et al., 2015]. As an invasive species, water lettuce (WL; *Pistia stratiotes* L.) is a floating macrophyte, with high bio-accumulation and good tolerance for ecological-environmental factors [Pandey, 2012]. WL could be used for a phytoremediation process. Several studies have utilised WL to reduce pollutants from surface water sources [Nahar and Hoque, 2021] and treat wastewater [Mukherjee et al., 2015; Victor et al., 2016]. Despite the limited research on using WL to treat wastewater from biogas systems, such use is expected to be a low-cost method [Kumar et al., 2017]. Commonly found in Vietnam's freshwater bodies, WL is a potential green biomass for bio-energy production [Nguyen et al., 2022; Pantawong et al., 2015]. In this laboratory-scale study, WL was tested to determine the reduction of pollutants in wastewater from biogas systems treating pig manure in still-water and running-water systems. The research contributes to introducing a waste circulation model that uses WL to treat wastewater and then utilises collected WL biomass as feedstock for biogas systems.

MATERIALS AND METHODS

Research materials

WL samples for similar-sized experiments were collected from a freshwater body and then cultured in tap-water tanks (dechlorinated) in a university's wet laboratory for 2 weeks. Wastewater with pig manure from a biogas digester was used as feedstock in this experiment. Some of the parameters that were checked before the experiment were the wastewater's pH level, dissolved oxygen (DO), chemical oxygen demand (COD), ammonium (NH_4^+-N) and phosphate (PO_4^{3-}-P). The wastewater had high concentrations of COD, NH_4^+-N and PO_4^{3-}-P but low DO values (Table 1). Compared with the Vietnamese technical standard for livestock wastewater quality, NH_4^+-N and PO_4^{3-}-P still needed to reach the values required by the standard before the wastewater discharge into water bodies. WL can increase biomass rapidly and remove large amounts of pollutants; however, high concentrations can affect its survival and reduce its pollutant removal efficiency [Nahar and Hoque, 2021; Lu et al., 2010; Sooknah and Wilkie, 2004]. Therefore, wastewater with high concentrations of pollutants must be diluted before testing, so WL can adapt well to pollutants in wastewater. The WL that we studied absorbed plenty of ammonium in the solution we made. Since WL can absorb a lot of ammonium at a concentration of 10 mg/L (Thuan and Cong, 2022), in this experiment, we used wastewater from the biogas digester with NH_4^+-N diluted to 15 mg/L.

Experimental designation

We experimented with two treatments, one without WL as the control and the other with WL, and treatments with three replicates. The Styrofoam containers with a dimension of 600×400×190 cm were filled with 15 L of wastewater. Samples, fifteen WL with a fresh weight of about 100 g, were raised in each container. Since the initial concentration of NH_4^+-N was not high (about 15 mg/L), the experiment was conducted for 22 days. Over the first period (days 0–7) of the experiment, we examined the WL's nutrient removal in wastewater at the still-water stage. Over the second period (days 10–22), wastewater with a concentration of about 15 mg/L of NH_4^+–N was slowly poured into ponds 1, 2 and 3, made of terraced Styrofoam containers, as shown in Figure 1. Small water pipes were connected to the ponds, and the wastewater flow rate was controlled to reach about 15 mL in the Styrofoam containers.

Measurement

Water quality parameters, such as temperature, pH, DO, COD, NH_4^+-N, nitrite (NO_2^--N), nitrate (NO_3^--N) and PO_4^{3-}-P, were measured on days 1, 3, 7, 10, 13, 16, 19 and 22. The WL's fresh weight was measured simultaneously with each day of sampling the water, while dried WL was measured on days 0 and 22.

The pollutant reduction efficiency was calculated according to the following equation:

$$H\ (\%) = \frac{C_{t0} - C_t}{C_t} \times 100 \quad (1)$$

Table 1. Characteristics of wastewater from the biogas digester used in the experiment

Parameter	Unit	Value	Vietnamese technical standard for livestock wastewater quality
pH	-	7.8	6–9
DO	mg/L	0.4	-
COD	mg/L	1,012.8	100
NH_4^+-N	mg/L	161.4	5
PO_4^{3-}-P	mg/L	24.0	-

Figure 1. Experimental system [terraced styrofoam containers designed as pond 1(P1), pond 2(P2) and pond 3(P3)]

where: H(%) denotes the efficiency of reducing pollutants, and C_{t0}(mg/L) and C_t(mg/L) represent the concentrations of the pollutants at the initial time and time t, respectively.

The relative daily reduction efficiency was calculated according to the following equation:

$$H' \left(\frac{\text{mg}}{\text{L}} \times \text{day}^{-1}\right) = \frac{C_{t0} - C_t}{d} \tag{2}$$

where: H' (mg/L × day^{-1}) denotes the relative daily reduction efficiency, C_{t0} (mg/L) and C_t (mg/L) signify the concentrations of the pollutants at the initial time and time t, respectively, and d represents the number of experiment days.

Data analysis

The mean values of the water quality parameters are expressed as (Mean ± SD). Comparisons of the mean differences in water quality parameters, WL weights and pollutants' reduction efficiency levels were analysed using an independent sample t-test in SPSS 22.0 (checked for homogeneity of variance). *One-way* ANOVA on the Ducan test was used to determine whether there were any statistically significant differences in the means of pollutant concentration reduction levels among the ponds; in case homogeneity of variance was not found, a nonparametric test for K-independent samples was used. We tested the statistically significant differences at 5%.

RESULTS

Pollutant removal in still-wastewater condition

Effects of temperature, pH and DO on WL growth

The wastewater temperature range in all treatments was 25.8–27.3 °C (Figure 2). According to Rivers [2002], the optimum temperature range for WL growth is 22–30 °C. Hence, the wastewater temperatures in this experiment were appropriate for WL growth. Additionally, they fell within the optimum temperature range for nitrification in microbiology (25–35 °C) [Guo at el., 2010]. The temperature in the treatment without WL (26.5 ± 0.4 °C) differed insignificantly from that in the treatment with WL (26.9 ± 0.3 °C (n = 27, p = 0.227); therefore, temperature was not a factor that caused a difference in the pollutant concentrations between the two treatment types.

The wastewater pH levels in all treatments ranged from 7.1 to 7.6 (Figure 2). WL grows well in water with pH = ca. 7 [Pieterse et al., 1981]. Hence, the wastewater pH level in this experiment was suitable for WL growth. The difference in the pH levels between the treatment

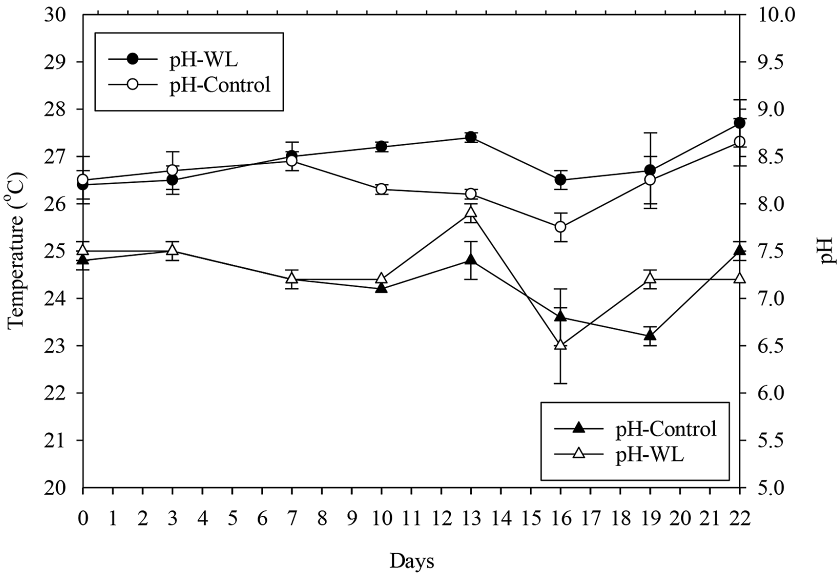

Figure 2. Average values of temperature and pH on days 1, 3 and 7 of the experiment

without WL (7.3 ± 0.1) and the treatment with WL (7.4 ± 0.2) was not significant (n = 27, p = 0.077); therefore, pH was not a factor that caused a difference in pollutant concentrations between the two treatment types. The changes in the DO values between the treatments ranged from 3.0 to 5.3 mg/L (Figure 3). In general, the DO values in this experiment were within the safe range for living aquatic plants (> 2 mg/L), and thus, also safe for WL growth. The DO values in the treatment without WL (4.5 ± 0.6 mg/L) differed insignificantly from the treatment with WL (4.4 ± 0.8 mg/L) (n = 27, p = 0.261). The DO values in the treatment without WL decreased gradually (5.0 ± 0.2, 4.3 ± 0.8 and 4.2 ± 0.0 mg/L on days 0, 3 and 7, respectively), and the differences in the DO values among the days were significant (n = 27, p < 0.01). The changes in the DO values over time in the treatment with WL showed a trend similar to those in the treatment without WL (5.1 ± 0.1, 4.2 ± 0.6 and 4.0 ± 0.1 mg/L on days 0, 3 and 7, respectively). No significant differences in the DO values between the two treatment types were found on day 3 (n = 9, p =

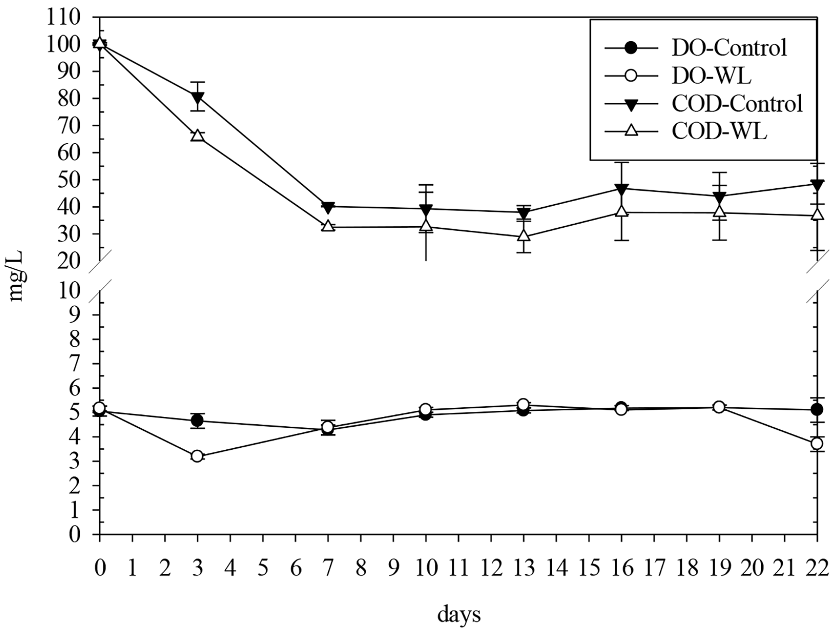

Figure 3. Mean values (± SD) of DO and COD concentrations on days 1, 3 and 7 of the experiment

0.115); however, the DO values in the treatment without WL were significantly higher than those in the treatment with WL on day 7 (n = 27, p = 0 < 0.01). Based on this result, DO was a factor that might affect the differences in pollutant concentrations between the two treatment types on day 7. The decrease in the DO values on days 3 and 7 can be explained by the utilisation of microorganisms for oxidation. Additionally, aquatic plants contribute to oxygen reduction through their respiration and decomposition [Lesiv et al., 2020; Sikawa and Yakupitiyage, 2010; Fox et al., 2008]. Hence, WL's respiration and decomposition also contributed to oxygen reduction during the treatment with WL. It can be the main reason for less DO in the treatment with WL compared with the treatment without WL.

Removal of pollutants

Removal of organic matter

The COD concentration decreased gradually over time in both treatment types (Figure 3 and Table 2). In the treatment without WL, the COD reduction efficiencies on days 3 and 7 were 19.42 ± 5.17% and 59.95 ± 0.49%, respectively, versus 34.16 ± 1.65% and 69.65 ± 4.51%, respectively, in the treatment with WL. The COD reduction efficiencies of WL in this study are less than those reported in Sooknah and Wilkie's (2004) study (72.2–74.0%). The difference can be explained by the longer experiment period (31 days) in Sooknah and Wilkie's study than in ours. The COD reduction efficiencies in the treatment with WL were significantly higher than those in the treatment without WL on days 3 and 7 (n = 9, $p < 0.01$ for both cases. WL contributed to COD reduction at 14.74 ± 4.14% in the first 3 days and 8.69 ± 0.92% on days 4–7. The COD reduction over time can be explained by the fact that WL roots filter suspended solids and absorb soluble nutrients in wastewater [Shah et al., 2014]. In the treatment without WL, the relative daily COD reduction on days 1–3 (6.48 ± 1.72 mg/L × d^{-1}) was less significant than that on days 4–7 (15.00 ± 0.08 mg/L × d^{-1}) (n = 9, $p < 0.01$). Such a trend was also found in the treatment with WL; the relative daily COD reduction on days 1–3 (11.40 ± 0.56 mg/L × d^{-1}) was less significant than that on days 4–7 (19.91 ± 0.16 mg/L×d^{-1}) (n = 9, $p < 0.01$). Other factors without WL contributed to COD reduction more on days 4–7 compared to days 1–3.

Removal of nutrients

Changes in NH_4^+-N concentrations

There was a decline in NH_4^+-N concentrations over the experiment period in both treatment types (Figure 4 and Table 2). In the treatment without WL, NH_4^+-N reduction efficiencies were 31.00 ± 2.46% and 75.25 ± 12.67% on days 3 and 7, respectively. In the treatment with

Table 2. Reduction levels of COD, NH_4^+-N, NO_2^--N, NO_3^--N, TIN and PO_4^{3-}-P concentrations on days 3 and 7 compared with day 0

Parameters	Day	Changes in pollutant concentrations on days 3 and 7 compared with day 0 (mg/L)	
		Control	With WL
COD	3	-19.44 ± 5.17**	-34.16 ± 1.69**
	7	-59.99 ± 0.32**	-67.63 ± 0.64**
NH_4^+-N	3	-4.68 ± 0.38**	-6.26 ± 0.35**
	7	-12.38 ± 0.14ns	-12.35 ± 0.13ns
NO_2^--N	3	3.382 ± 0.389**	1.357 ± 0.210**
	7	9.097 ± 0.960**	7.060 ± 0.065**
NO_3^--N	3	0.000 ± 0.000ns	0.000 ± 0.000ns
	7	0.020 ± 0.001**	0.116 ± 0.021**
TIN	3	-1.297 ± 0.400**	-4.906 ± 0.348**
	7	-3.250 ± 0.153**	-5.171 ± 0.162**
PO_4^{3-}-P	3	-0.088 ± 0.004**	-0.313 ± 0.005**
	7	-0.154 ± 0.001**	-0.400 ± 0.009**

Note: *negative data represent decreased concentrations on day 3 or 7 compared with day 0. Positive data represent increased concentrations on day 3 or 7 compared with day 0. ** Denotes a significant difference between the two treatments ($p < 0.01$); ns indicates no significant difference between the two treatments ($p > 0.05$).

WL, NH_4^+-N concentrations decreased by 41.58 ± 2.13% and 69.69 ± 18.98% on days 3 and 7, respectively, compared with day 0. The NH_4^+-N reduction in the treatment with WL was significantly higher by 1.54 ± 0.38 mg/L (25.21 ± 5.44%) than that in the treatment without WL on day 3 (n = 9, $p < 0.01$), but such significant difference was not found on day 7 (a difference by only 0.02 ± 0.185mg/L; 1.12 ± 0.93%) (n = 9, $p = 0.894$). NH_4^+ is an inorganic nitrogen that aquatic plants absorb for growth. On the first 3 days, WL contributed to NH_4^+-N reduction by 25.21 ± 5.44%. Hence, WL mainly contributed to NH_4^+-N reduction in wastewater on the first 3 days at the still-water stage. In the treatment without WL, the relative daily NH_4^+-N reduction on days 1–3 (0.10 ± 0.01 mg/L × d^{-1}) was less significant than that on days 4–7 (0.21 ± 0.01 mg/L × d^{-1}; n = 9, $p < 0.01$). In the treatment with WL, the relative daily NH_4^+-N reduction on days 1–3 (0.14 ± 0.01 mg/L×d^{-1}) was also less significant than that on days 4–7 (0.21 ± 0.01 mg/L × d^{-1}; n = 9, $p < 0.01$). The findings indicated that other factors without WL contributed to NH_4^+-N reduction more on days 4–7 compared with days 1–3.

Changes in NO_2^--N and NO_3^--N concentrations

The changes in NO_2^--N and NO_3^--N concentrations over the experiment period are shown in Figure 4 and Table 2. The initial concentrations of NO_2^--N and NO_3^--N were very low; the concentration of NO_2^--N (0.023 ± 0.001 mg/L) was significantly higher than that of NO_3^--N (0.003 ± 0.000 mg/L; n = 18, $p < 0.01$). NO_2^--N and NO_3^--N concentrations tended to increase from day 0 to day 7, with the increase in the former always higher than that in the latter, implying that ammonium oxidation occurred stronger than nitrite oxidation.

On day 3, NO_2^--N concentration in the treatment without WL was significantly higher by 2.027 ± 0.004 mg/L compared with that in the treatment with WL (n = 9, $p < 0.01$). The insignificant differences in NO_3^--N concentrations between day 3 and day 0 in both treatment types implied that nitrite oxidation might not occur on days 0–3. Hence, the 59.98 ± 3.22% difference in NO_2^--N concentrations between the treatment with WL and that without WL was caused by WL. Although plants can definitely use nitrite as a nitrogen source, there are few scientific reports on plants' absorption of nitrite. When duckweed (*Spirodela oligorrhiza*) was grown in media containing nitrite and nitrate, the plant clearly took up the former, preferring it over the latter [Walstad, 1999]. Additionally, when the researcher grew *Spirodela oligorrhiza* in media containing ammonium and nitrite, the plant removed both ions at approximate rates. These results suggest that aquatic plants may remove both ammonium and nitrite equally, favouring them over nitrates [Walstad, 1999]. In our study, we found that WL contributed to nitrite reduction. However, there is a need for further experiments on using WL for nitrite removal in other wastewaters.

On day 7, NO_2^--N concentration in the treatment without WL was significantly higher by 2.037 ± 0.005 mg/L compared with that in the treatment with WL (n = 9, $p < 0.01$). Hence, the

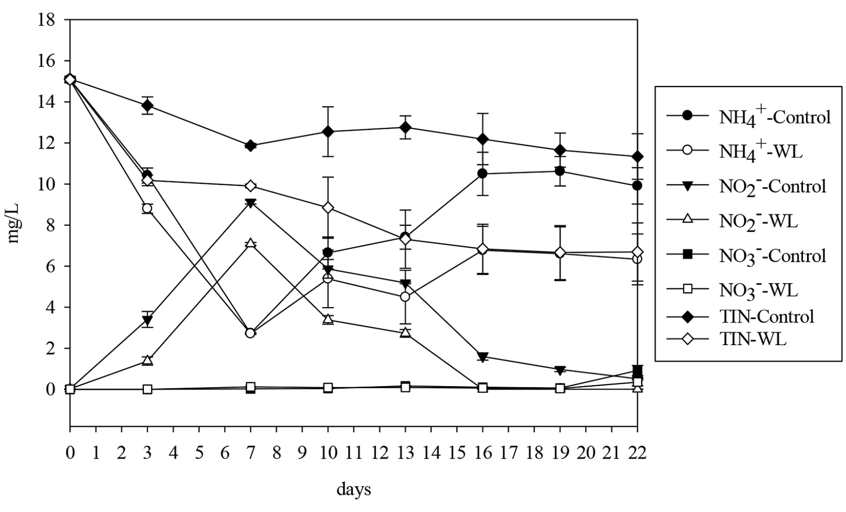

Figure 4. Mean values (± SD) of NH_4^+-N, NO_2-N, NO_3^--N and TIN concentrations on days 1, 3 and 7 of the experiment

22.37 ± 1.21% difference in NO_2^--N concentrations between the treatment with WL and that without WL was caused by WL. NO_3^--N concentration in the treatment without WL was significantly lower by 0.095 ± 0.002 mg/L compared with that in the treatment with WL (n = 9, $p < 0.01$). The results indicated that nitrite oxidation in the treatment with WL was higher than that in the treatment without WL. In other words, WL enhanced nitrification on day 7. A simple calculation showed that WL contributed to nitrite reduction by 1.941 ± 0.130 mg/L (95.30 ± 0.40% NO_2^--N produced). Additionally, WL did not seem to contribute to nitrate reduction.

Nitrification is an essential process in the nitrogen cycle, and the nitrogen formed in this process is a nutrient for plants [Angove et al., 2018]. Nitrate concentration is deficient, so WL used NH_4^+ and NO_2^- for its growth. It confirms the role of WL in reducing NH_4^+-N in wastewater, especially in the first 3 days. We also performed another study [Thuan and Cong, 2022] on the absorption of NH_4^+ in a prepared solution of NH_4^+ and NO_3^-; the results showed that WL preferred to absorb NH_4^+. The role of WL for nitrate reduction was not observed at the still-water stage. Nitrate is the end product of the nitrification process through two stages of ammonia oxidation and subsequent nitrite oxidation. In the experiment reported in this paper, ammonia oxidation prevailed over nitrite oxidation, leading to low NO_3^--N concentrations. Therefore, NO_3^- uptake by WL was not observed.

Changes in TIN ($NH_4^+ + NO_2^- + NO_3^-$) concentrations

The TIN concentrations gradually decreased in both treatment types (Figure 4 and Table 2). In the treatment without WL, it dropped by 8.58 ± 2.64% and 21.49 ± 0.87% on days 3 and 7, respectively, versus 32.51 ± 2.11% and 34.28 ± 0.84% on days 3 and 7, respectively, in the treatment with WL. The contribution of WL to total nitrogen reduction on the first 3 days was 23.93 ± 2.35% compared with only 12.80 ± 1.30% on days 4–7. The findings indicated that nitrogen removal efficiency depended on the nitrogen input concentration, with the efficiency decreasing if the concentration was less than 15 mg/L.

Phosphorus removal

The decrease in PO_4^{3-}-P concentration in the treatment without WL was significantly smaller than that in the treatment with WL on days 3 and 7 (n = 9, $p < 0.001$ for both cases) (Figure 4 and Table 2). The results demonstrated the contribution of WL to reducing PO_4^{3-}-P concentration in wastewater. Phosphate is the form of phosphorus that plants absorb for growth. The more significant decrease in PO_4^{3-}-P concentration in the treatment with WL compared with the treatment without WL is due to the phosphorus absorption by WL. On day 3, the decrease in PO_4^{3-}-P concentration caused by WL was 0.224 ± 0.005 mg/L (equivalent to 71.84 ± 0.89%), while on day 7, it was 0.248 ± 0.004 mg/L (61.64 ± 1.65%). Unlike the nitrogen results, the decrease in the daily PO_4^{3-}-P concentration on days 1–3 was significantly lower than on days 4–7 (n = 9, $p < 0.01$); a similar trend was also found in the treatment with WL (n = 9, $p < 0.01$).

Pollutant removal in running-water condition

Removal of organic matter

The difference in COD concentrations between the treatments with WL and without WL at the still-water stage (11.27 ± 4.79 mg/L) was significantly lower than that at the running-water stage (8.53 ± 4.32 (n = 27, $p < 0.01$). WL contributed to reducing organic matter at the running-water stage less than at the still-water stage. Additionally, at the running-water stage, no significant differences in the reduction of organic matter in pond 1 (4.04 ± 2.02 mg/L), pond 2 (9.23 ± 2.03 mg/L) and pond 3 (10.79 ± 4.58 mg/L) were found (n = 5, $p < 0.05$). On days 0–22, there was no clear trend of changes in COD concentrations over time (Figure 4).

Removal of nitrogen

Inversely to the COD results, the difference in NH_4^+-N between the treatments without WL and with WL at the still-water stage (0.82 ± 0.80 mg/L) was significantly lower than that at the running-water stage (3.10 ± 1.16 mg/L) (n = 27, $p < 0.01$). It indicated that WL contributed more to reducing NH_4^+-N at the running-water stage than at the still-water stage. As discussed above, ammonium removal depended on ammonium input concentration. A very low NH_4^+ -N concentration (< 3 mg/L) was observed on days 4–7, leading to its low removal, in contrast to a higher concentration (> 5 mg/L) on days 10–22, leading to its higher removal. At the running-water stage, no significant difference in the reduction of organic matter in pond 1 (4.04 ± 2.02 mg/L), pond 2 (9.23

± 2.03 mg/L) and pond 3 (10.79 ± 4.58 mg/L) was found (n = 5, p = 0.304). On days 0–22, there was no clear trend in the increase or decrease of organic matter over time (Figure 4).

Regarding NO_2^--N concentrations, the difference between the treatments without WL and with WL at the still-water stage (2.032 ± 0.184 mg/L) was significantly higher than that at the running-water stage (1.600 ± 0.817 mg/L; n = 27, $p < 0.01$). The findings illustrated that WL contributed more to nitrite reduction at the still-water stage than that at the running-water stage. No significant differences in NO_2^--N concentrations among pond 1, pond 2 and pond 3 were found (n = 5, p = 0.707). There was a decrease in NO_2^--N concentrations over time during the running-water stage (2.484 ± 0.299, 2.340 ± 0.012, 1.722 ± 0.151, 0.957 ± 0.112 and 0.509 ± 0.065 mg/L for days 10, 13, 16, 19 and 22, respectively; Figure 4).

As for NO_3^--N concentrations, on day 3, no significant differences between the treatments without WL and with WL were observed, as discussed above. On days 7 and 10, NO_3^--N concentrations in the treatment without WL were lower than those in the treatment with WL; inversely, on days 13, 16, 19 and 20, NO_3^--N concentrations in the treatment without WL were higher than those in the treatment with WL. The results indicated that WL contributed to nitrate reduction at the running-water stage on day 13. On days 13–22, no significant differences in NO_2^--N concentrations among pond 1, pond 2 and pond 3 were found (n = 4, p = 0.956). No certain pattern for the differences in NO_2^--N concentrations between the treatments without WL and with WL at the running-water stage emerged over time (0.071 ± 0.005, 0.033 ± 0.008, 0.021 ± 0.001 and 0.563 ± 0.021 mg/L for days 13, 16, 19 and 22, respectively; Figure 4).

Similar to the NH_4^+-N trend, the difference in TIN between the treatments without WL and with WL at the still-water stage (2.803 ± 0.901 mg/L) was significantly lower than that at the running-water stage (4.831 ± 0.871 mg/L; n = 27, $p < 0.01$). In other words, WL contributed to the TIN reduction at the running-water stage more than at the still-water stage. In the running-water condition, WL contributed more to the reduction in ammonium and nitrate, favouring them over nitrite. WL likely prefers to absorb ammonium and nitrate over nitrite in the running-water condition. In contrast, WL chooses to absorb ammonium and nitrite over nitrate at the still-water stage.

Removal of phosphorus

No significant difference in PO_4^{3-}-P concentrations between the treatments without WL and with WL at the still-water stage (0.239 ± 0.012 mg/L) and at the running-water stage (0.238 ± 0.033 mg/L) was found (n = 27, p = 0.879). No significant differences in PO_4^{3-}-P concentrations among pond 1 (0.245 ± 0.056 mg/L), pond 2 (0.251 ± 0.018 mg/L) and pond 3 (0.228 ± 0.019 mg/L) were observed (n = 5, p = 0.617). A certain pattern for the differences in PO_4^{3-}-P concentrations between the treatments without WL and with WL at the running-water stage emerged over time (0.918 ± 0.030, 0.227 ± 0.023, 0.266 ± 0.002, 0.258 ± 0.029 and 0.256 ± 0.028 mg/L for days 10, 13, 16, 19 and 22, respectively).

Among aquatic macrophytes, WL stands out as a highly effective and sustainable option for phytoremediation of inorganic contaminants in wastewater. Unlike chemical treatments or advanced biological techniques that can be environmentally harmful and costly, the application of WL for removing inorganic pollutants is considered eco-friendlier and more economical. This finding aligns with previous research by Yildiz (2004) and Tsuji (2002). Remarkably, WL not only excels in absorbing inorganic parameters such as nitrate nitrogen, nitrite nitrogen, and phosphate but also demonstrates exceptional ability to concentrate and remove various toxic contaminants from aquatic environments, as reported by Mishra and Tripathi (2009). When compared to other commonly used aquatic plants like water hyacinth (*Eichhornia crassipes*) and duckweeds (*Lemna* sp. and *Spirodella* sp.), the phytoremediation application of WL offers a more environmentally friendly and cost-effective solution for mitigating inorganic contaminants in wastewater. This unique combination of high phytoremediation efficiency, versatility in removing both inorganic and toxic pollutants, and its eco-friendly and economical nature makes WL a promising candidate for sustainable wastewater treatment and environmental remediation efforts.

WL biomass

There was a gradual increase in fresh WL biomass over time. In general, except on days 10 and 19, the differences in the increase of the relative daily fresh biomass throughout the study period were not significant (n = 9, $p > 0.05$; 6.18 ± 3.52, 5.62 ± 2.39, 4.88 ± 1.76, 5.68 ± 1.09, 6.16 ± 0.88, 7.24 ± 1.60 and 6.89 ± 1.34 g/day for days 0–3, 4–7, 8–10, 11–13, 14–16, 17–19 and 10–22, respectively). In general, no significant differences in the relative daily fresh biomass increase between still-water days and running-water days were found. The increase in WL's relative daily dried biomass over the 22 days of the experiment was 0.32 ± 0.14 g/day. Further studies must be performed at higher pollutant concentrations and over longer experiment periods for using WL biomass. WL density was not tested in this experiment, but this factor should be considered in future experiments. Research on the WL re-contamination period should also be conducted to determine the appropriate times for harvesting the plant.

The WL is currently recognized as a promising green material for biogas production (Cong et al., 2022; Pantawong et al., 2015). The study demonstrated the potential of WL in removing contaminants from wastewater, making it suitable for scaling up in real conditions, particularly in households where farmers use biogas digesters. Water lettuce can reduce pollutant levels in ponds or lakes before the treated water is discharged into canals or rivers, contributing to environmental protection and promoting a closed-loop system in the traditional Vietnamese VAC/VACB farming model. Furthermore, WL biomass can be effectively utilized for producing value-added manures through vermicomposting, where it is mixed with cow dung (Suthar et al., 2017). This approach offers a viable solution for treating WL after it has absorbed contaminants from water bodies.

CONCLUSION

Our study showed that WL contributed to reducing pollutants, including organic matter and the nutrients ammonium, nitrite and phosphate, in wastewater from a biogas digester at the still-water stage, with the highest reduction efficiency accounted for in phosphorus. At the initial ammonium concentration of about 15 mg/L, the contribution of WL to pollutant reduction was better on days 0–3 than on days 4–7 of the experiment without wastewater supply. At the still-water stage, no contribution of WL to nitrate was found, but it appeared at the running-water stage. Additionally, WL contributed more to the reduction of ammonium, nitrite and nitrate in the running-water condition than in the still-water condition, but the inverse trend was observed in organic matter. No significant differences in the decrease in pollutant concentrations between the treatments without WL and with WL among pond 1, pond 2 and pond 3 were found. On days 0–22, no clear trend in an increase or decrease emerged over time, except NO_2^--N reduction. The results also indicated no significant differences in the relative daily fresh WL biomass increase between the still-water and running-water days. Our study's important finding is WL's removal of nitrite, which future research should further examine for broader applications of aquatic plants.

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