An experimental study on using water lettuce (*Pistia stratiotes* L.) to treat anaerobic effluent and feedstock

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Background: Aquatic plants play a crucial role in nature-based wastewater treatment and provide a promising substrate for renewable energy production from anaerobic digestion. The study describes the potential of using water lettuce to reduce pollutants from husbandry anaerobic digester effluent and feed back to the biogas digester.

Methods: The first experiment was piloted using styrofoam boxes containing husbandry anaerobic digester effluent. Water lettuces were arranged by 50%, 25%, and 12.5% surface coverage. Each treatment was conducted in 5 replicates through natural conditions. In the second experiment, WL was fed into an existing continuous anaerobic digester to examine biogas production on a farm scale.

Results: The effluent treatment efficiency of TSS, BOD_5, COD, TKN, and TP was 93.75 - 98.44%, 79.65 - 85%, 79.6 - 83.62%, 64.5 - 68.25%, and 91.07 - 91.93%, respectively. The doubling time of water lettuce was estimated at around 12 days for all treatments. For the second experiment, the biogas yield varied between 190.6 and 333.3 L.kg VS^{-1}. The CH_4 content reached over 54%.

Conclusions: Water lettuce offered better management in effluent recirculation through a plant-based pollutant removal system and produced biomass for biomethanation.

1 *Water lettuce (Pistia stratiotes L.) increases biogas effluent*
2 *pollutant removal efficacy and proves a positive substrate for*
3 *renewable energy production*

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Abstract

28 **Background:** Aquatic plants play a crucial role in nature-based wastewater treatment and provide
29 a promising substrate for renewable energy production using anaerobic digestion (AD) technology.
30 This study aimed to examine the contaminant removal from AD effluent by water lettuce (WL)
31 and the biogas production from WL biomass co-digested with pig dung (PD) in a farm-scale biogas
32 digester.

33 **Methods:** The first experiment used styrofoam boxes containing husbandry AD effluent. WLs
34 were initially arranged in 50%, 25%, 12.5%, and 0% surface coverage. Each treatment was
35 conducted in five replicates under natural conditions. In the second experiment, WL biomass was
36 co-digested with PD into an existing anaerobic digester to examine biogas production on a farm
37 scale.

38 **Results:** Over 30 days, the treatment efficiency of TSS, BOD_5, COD, TKN, and TP in the effluent
39 was 93.75%–97.66%, 76.63%–82.56%, 76.78%–82.89%, 61.75%–63.75%, and 89.00%–89.57%,
40 respectively. Higher WL coverage increased the pollutant elimination potential. The WL biomass
41 doubled after 12 days for all treatments. In the farm-scale biogas production, the biogas yield
42 varied between 190.6 and 292.9 L kg VS_{added}^{-1}. The methane content reached over 54%.

43 **Conclusions:** WL removed AD effluent nutrients effectively through a phytoremediation system
44 and generated significant biomass for renewable energy production in a farm-scale model.

Introduction

46 Agricultural production is the main economic activity in the Vietnamese Mekong Delta (VMD),
47 of which livestock production constitutes more than 20% (GSO, 2021). Within this sector, pig
48 livestock dominates in most local areas. It is estimated that the VMD has 2.08 million pigs (GSO,
49 2022). Mostly, pig farms temporarily raise livestock on a small scale (< 10 pigs) without suitable
50 waste treatment measures (Nam *et al.,* 2021). Therefore, waste management from pig raising is a
51 significant challenge owing to the considerable amount of pig excrement directly released to the
52 external environment (Ngan, 2012; Nam *et al.,* 2022). Environmental issues have emerged
53 seriously in rural areas, where local communities use surface water sources from rivers/canals as
54 the primary water supply source for agriculture production and domestic utilization (Roubík *et al.*,
55 2018; Kamya *et al.,* 2022). Consequently, sustainable livestock production requires responsible
56 waste management to minimize negative influences on the surrounding environment and
57 ecosystems.
58 Anaerobic digestion (AD) is an effective technology for treating biodegradable waste and
59 producing eco-friendly energy (Markphan *et al.,* 2020; Ye *et al.,* 2013; Szaja *et al.,* 2020; Wang
60 *et al.,* 2022). In the 1990s, this technology was introduced in the VMD to treat animal waste and
61 recapture biogas for cooking and heating (Ngan *et al.*, 2012). However, the scale of raising
62 livestock of the VMD's households is modest, leading to a shortage of feedstock substrate for
63 producing biogas (Nam *et al.,* 2022). Accordingly, substitute or additional feedstocks, such as rice
64 straw, water hyacinth, and potential biomass, are encouraged for use as co-substrates for biogas
65 digesters to achieve higher biogas yields (Nam *et al.*, 2023). The co-digestion of animal waste and
66 biomass provides a more flexible digestion process in small livestock household digesters. This
67 approach potentially enhances biogas production by adjusting the carbon/nitrogen (C/N) ratio to a
68 more favorable range for anaerobic biodegradation regression (Nam *et al.,* 2021; Yadvika *et al.,*
69 2004).
70 Although biogas production is offered not only for livestock waste treatment but also for renewable
71 energy production, the AD process shows limitations in waste treatment efficiency and outlet

72 effluent quality when solely substrate is applied. The AD effluent quality regularly does not meet
73 the national technical regulations on husbandry wastewater (QCVN 62-MT:2016/BTNMT) before
74 it is released into the exterior environment (MoNRE, 2016). It is commonly accepted that treatment
75 efficacy from AD is about 30% of the organic matter, while the rest remains as digestate and sludge
76 (Gurung, 1997). Consequently, AD effluent contains much more indigestible matter and high
77 concentrations of identified nutrients, such as nitrogen, phosphorous, and potassium (Ngan, 2012;
78 Le, 2008, Abe *et al.,* 2016; Wang *et al.,* 2018; Kalaimurugan *et al.,* 2022). As such, reutilizing
79 nutrients from wastewater or AD effluent to grow aquatic plants is a promising option for
80 bioremediation because it is cheap and eco-friendly (Nor *et al.,* 2023).
81 In the rural areas of the VMD, households typically own a large plot of land to develop a traditional
82 farming system known as VAC (V - garden, A - pond, and C - livestock cage) or VACB (V -
83 garden, A - pond, C - livestock cage, and B - biogas plant) (Ngan, 2011). The pond is commonly
84 used as a buffer unit for receiving AD effluent before discharging it into the external environment.
85 Ordinarily, several types of aquatic plants, such as water lettuce (*Pistia stratiotes*), water hyacinth
86 (*Eichhornia crassipes*), and duckweed (*Lemna minor*), are grown on the pond to eliminate onsite
87 AD effluent nutrients. These plants are identified as invasive plants that are grown popularly in
88 the tropical conditions of the VMD (Coelho *et al.*, 2005; Šajna *et al.*, 2007). In addition, these
89 plants are known for their efficient nutrient-absorbing ability, metal and toxic elimination, and
90 biogenic element accumulation (Lu *et al*., 2010; Brix, 1991). Aquatic plant biomass could be
91 valuable for promoting renewable energy production through AD technology (Barua *et al.,* 2018;
92 Nam *et al.,* 2021).

93 Although water hyacinth is a good candidate for nutrient removal and biomass harvest, its high
94 content of hemicellulose (30.8%–48.0%) slows biogas production, resulting in overloading in a
95 long-term operation (Nam *et al.,* 2023; Lin *et al.,* 2015; Bote *et al.,* 2020). The possibility of
96 duckweed biomass production is slight, albeit with high biomethanation potential, resulting in an
97 inadequate substrate for household-scale biogas production. In contrast, water lettuce (WL) has
98 demonstrated its effectiveness for domestic wastewater contaminant removal of BOD, NH_3, TN,
99 and TP by 83.5%–97.53% in a 5-day treatment (Gaballah *et al.,* 2019). The rapid elimination of
100 nutrients in wastewater can reduce the risk of water body eutrophication. Moreover, WL has great
101 biomass production potential due to its fast-growing nature. WL's biomass production is about 2.4
102 tons of dry biomass per hectare per year, indicating stable substrate-providing feasibility for biogas
103 production (Sutaryo *et al.,* 2022). This plant can also grow in various environments, including
104 clean water and wastewater, signifying its adaptability for AD effluent nutrients in varying
105 amounts (Whangchai *et al.,* 2021). WL's chemical composition is 34.4% cellulose, 11.6% lignin,
106 and 26.3% hemicellulose, which is promising for renewable energy production from
107 biodegradation compared to other agricultural terrestrial plants (Güngören Madenoğlu *et al.,*
108 2019). Previous studies have demonstrated the concordance of biogas production from WL
109 exclusively and from co-digestion with cow dung and waste sludge (Cong *et al.,* 2022; Sutaryo *et*
110 *al.,* 2022; Güngören Madenoğlu *et al.,* 2019). Biogas yields of WL range from 533–707 L kg VS^{-1}
111 (Güngören Madenoğlu *et al.,* 2019), while the potential of biogas production from water hyacinth

112   and duckweed varies from 102 L kg VS$_{added}^{-1}$ to 478 L kg VS$_{added}^{-1}$ (Nam *et al.,* 2017; Patil *et al.,*
113   2014; Gunnarsson & Petersen, 2007) and from 250 L kg VS$_{added}^{-1}$ to 390 L kg VS$_{added}^{-1}$,
114   respectively (Tonon *et al.,* 2017). As such, WL is promising for contaminant removal, as well as
115   its availability and sustainable use for biogas production at a farm scale. Although WL's potential
116   has been identified, the effectiveness of removing contaminants in AD effluent and biogas
117   production from co-digestion of WL with pig manure at the household scale has remained unclear.
118   This study, therefore, aimed to *(i)* examine the contaminant removal of WL applied to AD effluent
119   and *(ii)* investigate the biogas production potential of WL co-digested with pig manure in a farm-
120   scale biogas digester.

Materials & methods

121

Study site

122

123   All the experiments were implemented in a farmer household in Thanh My village (9°59'24.44",
124   105°47'43.57"), Cai Rang district, Can Tho city, Vietnam. The household had raised pigs for a
125   long time and owned a 2-year-old HDPE (high density polyethylene) biogas tube plant of 7.64 m^3
126   (Fig. 1), but only one pig of 40 kg was being raised in the pigsty at the study implementation time.
127   The field experiments were approved by the College of Environment and Nature Resources, Can
128   Tho University (ID 101/KMT).

Experimental design

129

130   *Experiment 1: water lettuce phytoremediation performance*
131   The WL was collected from the pond adjacent to the experiment site. The plants chosen were from
132   4–6 cm in length, and each plant had from 5 to 7 leaves at the initiation. We removed all the bad
133   parts from the plants and rinsed them with tap water to eliminate any debris of other macrophytes.
134   Then, the WL was placed into a plastic barrel filled with husbandry anaerobic digester effluent
135   (HADE) for one week, allowing it to adapt to its environmental conditions.
136   The experiment was designed in a batch system using styrofoam boxes (60 cm long × 45 cm wide
137   × 41 cm high). Each box contained 30 L of HADE, which was not added to during the experiment.
138   The WL was placed into boxes for different treatments by the plants arranged in 50%, 25%, and
139   12.5% of the box's surface area. In parallel, a control treatment (0%) was set up without WL. Each
140   treatment was replicated in five boxes. Accordingly, a total of 20 boxes were prepared. All the
141   experimental boxes were randomly arranged on a scaffold under natural conditions. A plastic film
142   was set up to cover the experiment boxes if it rained. The experiment ended in 30 days. The
143   pollution elimination percentage was calculated as shown in the basic equation (1):

144   $$\text{Removal efficiency (\%)} = \frac{C_i - C_e}{C_i} \times 100 \tag{1}$$

145   in which C_i is the initial concentration of pollutants and C_e is the final concentration of pollutants
146   remaining in the effluent.
147   The HADE was collected from the existing PE biogas plant at the experiment site and installed in
148   the boxes without any dilution. The HADE was examined before the initial experiment and after
149   the phytoremediation tests at 6, 12, 18, 24, and 30 days. The water samples were collected as mixed

150   samples from each treatment to achieve a pooled sample. The physicochemical parameters of pH,
151   TSS, BOD_5, COD, total Kjeldahl nitrogen (TKN), and total coliforms were chosen as required by
152   the QCVN 62-MT:2016/BTNMT National Technical Regulation on Livestock Effluent. The
153   collected HADE samples were analyzed according to the Standard Methods for the Examination
154   of Water and Wastewater (APHA, 1998).
155   The growing ability of the WL was recorded before and after the phytoremediation process every
156   5 days for the 30 days of the experiment. The plants' fresh weight was determined using a digital
157   balance to weigh all the plants within each box. The root length and leaf length were measured by
158   randomly choosing three plants from each box and recording the average value of the three
159   maximum values of the root and leaf (Fig. 2).
160   *Experiment 2: anaerobic digestion performance*
161   WL from the nearby pond was collected and fed into the existing PE biogas plant as supplementary
162   material. After each collection time, the WL was dried within a day in shady conditions to avoid
163   nutrient loss, and it was then fed into the biogas digester. The quantity of WL co-fed every 2 days
164   into the PE biogas plant was 10 kg wet weight within the first 10 days, 40 kg wet weight in the
165   next 30 days, and 24 kg wet weight in the last 20 days. At the start, the volatile solids (VS) content
166   of the WL was determined by drying a sample of the biomass at 105°C for 24 hours and then
167   heating it at 550°C for 2 hours. The VS value was then calculated based on the weight of the dried
168   sample.
169   The daily produced biogas was recorded onsite by a VIGADO G1.6 gas meter (PDM 095-2008,
170   Vietnam). For the gas components, biogas samples were collected in an aluminum bag after
171   feeding the WL every 4 days for 60 days. The gas samples were transported to the biogas
172   laboratory at Can Tho University and analyzed using a portable biogas analyzer (Biogas Pro 5000,
173   Landtec, USA) for the biogas components, including CH_4, CO_2, and other compositions (Fig. 3).
174   **Data processing**
175   The data were plotted using the RStudio software package (R Foundation for Statistical
176   Computing, R version 4.1.3 for Windows). The Pearson correlation coefficient was employed to
177   assess the correlation between the HADE pollutant variables (TSS, BOD_5, COD, TKN, and TP)
178   and the WL biomass (leaf length, root length, and fresh weight biomass). The correlation
179   significance is indicated as follows: * represents $0.05 < P < 0.01$, ** represents $0.01 < P < 0.001$,
180   and *** represents $P < 0.001$.

Results and discussion

181

Initial HADE characteristics

182
183   The HADE collected initially and used for the phytoremediation experiment had a high organic
184   concentration compared to the National Technical Regulation on Livestock Effluent (Table 1).
185   Compared to a previous study on the effluent of similar PE biogas plants operated in the VMD
186   (Ngan, 2012), the tested values were lower, as only one pig was being raised at the time of the
187   study. This HADE concentration is well suited for the WL phytoremediation process. Thus, the
188   collected HADE was initially applied to set up the experiment without dilution. Within the study
189   period, the daily ambient temperatures (recorded randomly between 08:00 and 16:00) at the

190 experiment site ranged from 27.3°C to 30.7°C (Fig. 4). The variation was appropriate for the
191 optimum temperature (22°C–30°C) for growing WL (Pettet & Pettet, 1970). The water levels in
192 the experimental boxes were also recorded to ensure the growing conditions for the WL. At the
193 beginning, all the experimental boxes were filled with a water level of 21 cm. At the end of the
194 experiment time, the water levels were 14.54 cm, 15.54 cm, 15.52 cm, and 16.40 cm for the WL
195 surface cover of 50%, 25%, 12.5%, and 0%, respectively. Thus, the water loss was estimated at
196 0.93 cm day^{-1}, 0.82 cm day^{-1}, 0.81 cm day^{-1}, and 0.72 cm.day^{-1} under 50%, 25%, 12.5%, and 0%
197 WL surface cover (Fig. 5).

198 **Phytoremediation process experiment**

199 *Pollution removal*

200 Figure 6a shows the phytoremediation performance of the HADE using WL every 6 days for 30
201 days. The pH levels slightly decreased during the phytoremediation process for all treatments.
202 Although the treatment with 12.5% surface cover showed the largest change in pH values between
203 days 12 and 18, the difference in pH across all treatments was negligible.

204 The concentration of contaminants (TSS, BOD$_5$, COD, TKN, and TP) rapidly reduced in the first
205 6 days of running the experiment (Fig. 6b–f). There was a slight decrease for the rest of the period.
206 The higher coverages of WL displayed better treatment efficacy of HADE. All the WL treatments
207 demonstrated a higher potential for pollutant elimination than the control treatment (0%) (Fig. 6).
208 Figure 7 shows the pollutant treatment efficiency of WL in the effluent from the digester. In the
209 first 6 days, there was a sharp decrease in the treatment efficiencies of the WL on the HADE, but
210 then a slight decrease between days 6 and 30 was shown. The TSS removal efficiency in the first
211 6 days of TSS was highest at 80.47%–94.53% (Fig. 7a), followed by TP (68.36%–82.86%, Fig.
212 7e), BOD$_5$ (44.19%–59.30%, Fig. 7b), COD (44.3%–59.73%, Fig. 7c), and TKN (38.25%–
213 46.75%, Fig. 7d). After 30 days, the reduction efficiency of TSS and TP did not continuously
214 increase much compared to the first 6 days, while the treatment efficiencies of BOD$_5$, COD, and
215 TKN rose much higher than during the first 6 days (Fig. 7).

216 Recent studies have confirmed high treatment efficiency on BOD$_5$ (83.3%–92.8%) and COD
217 (79.2%–85.9%) when the phytotreatment of polluted river water, palm oil mill wastewater, and
218 sewage wastewater using macrophytes was applied (Shahid *et al*., 2019; Wei, 2019; Schwantes *et*
219 *al*., 2019). However, other research has reported lower removal efficiencies of phytotreatment.
220 Jyotsna *et al*. (2015) confirmed removal efficiencies of 44.8% of TSS, 62.4% of BOD$_5$, and 63.8%
221 of COD for pulp and paper mill effluent after 30 days by lesser duckweed. Tang *et al*. (2009)
222 recorded reduction efficiencies of 33.2% of COD and 21.8% of TKN for wetland constructed to
223 treat eutrophic river water. However, the organic concentrations in the pulp and paper mill effluent
224 and eutrophic river water were much higher than in this study. Treatment effectiveness can vary
225 depending on various factors, such as the initial pollutant levels, the duration of treatment, and the
226 specific conditions of the treatment environment.

227 In our study, the removal efficiency of COD was slightly lower than that of BOD$_5$, which is similar
228 to the study by Hamzah *et al*. (2016) of phytoremediation testing on palm oil mill final discharge
229 wastewater (84.7% of BOD$_5$ and 22.3% of COD). In fact, recalcitrant compounds, such as high

230   humic acid in pig dung (PD), are known to cause low treatment efficiency of COD of the
231   phytoremediation (Norulaini, 2001; Zhang *et al.*, 2012).
232   High removal efficiencies of over 89% of TP were recorded for all the WL treatment coverages
233   after 30 days. The utility of phosphorus in aquatic plants is essential in various physicochemical
234   and biological processes. In addition, values from 61.8% to 65.0% were the reduction efficiencies
235   of TKN after 30 days of phytoremediation. In a similar approach using swine wastewater, Sudiarto
236   *et al.* (2019) documented that TKN and TP removals within 21 days were 71.4% and 32.2%,
237   respectively. Similarly, Parwin and Paul (2019) reported the highest removal efficiencies of 94.4%
238   of TKN and 98.1% of TP using water hyacinth to treat kitchen wastewater within 4 weeks. In
239   contrast, the lowest reduction efficiencies of BOD_5, COD, and TKN using water caltrop to treat
240   municipal wastewater have been recorded as 18.3%, 14.2%, and 27.2%, respectively (Kumar &
241   Chopra, 2018).
242   *Water lettuce biomass*
243   Previous findings disclosed that the biomass of WL increased up to the first 10 days and then
244   diminished as the basal leaves decayed (Fonkou *et al.*, 2002) or due to their maximum uptake limit
245   of nutrients (Hamzah *et al.*, 2016). Our study showed that the biomass gradually increased through
246   the experiment period (Fig. 8). The difference compared to the previous report was due to the
247   required space for the growth of WL. Based on the produced WL, the doubling time for the wet-
248   weight biomass from the treatments of 50%, 25%, and 12.5% surface coverage were estimated at
249   12.32 days, 12.11 days, and 12.28 days, respectively (Table 2). The rapid increase in WL's biomass
250   indicates its potential for eliminating pollutants and providing valuable material for biogas
251   production.
252   Fig. 9a shows that the root lengths of the WL increased over time. Root lengths were recorded
253   from 6.66 cm to 8.08 cm (an increase of 21.3%), 6.52 cm to 8.06 cm (an increase of 23.6%), and
254   6.16 cm to 8.04 cm (an increase of 30.5%) after 30 cultivated days for the treatments of 50%, 25%,
255   and 12.5% surface coverage, respectively. The maximum leaf length of growth recorded in the
256   treatment of a surface cover of 12.5% WL show that with fewer bodies (Fig. 9b). The nutrients
257   were absorbed and formed up to the root system instead of the leaves. During the first 15 days, the
258   length of the WL's leaves increased in all treatments but then decreased as a result of the decay of
259   the lower leaves and the emergence of new leaves. Consequently, a high amount of biomass was
260   recorded as the WL continuously grew in the experimental containers.
261   Figure 10 depicts the Pearson correlation of the pollutant parameters in the HADE among the
262   treatments, as indicated by the color and size of the circles. The magnitude of the correlation is
263   reflected by these visual cues. A strong positive relationship was found between pollutant
264   concentrations, with r values ranging from 0.8 to 1.0. The BOD_5 and COD displayed the highest
265   correlation. The Pearson correlation revealed adequate interdependence between the pollutant
266   composition and the prospect of WL to effectively eliminate pollutant levels in the HADE.
267   Similarly, leaf length and root length showed a moderate correlation (r = 0.53), while root length
268   and fresh biomass exhibited a high correlation (r = 0.85) (Fig. 11). No relationship was found
269   between leaf length and fresh biomass. As such, the root length played a crucial role in increasing

270 the WL's biomass. Macrophyte roots are vital for reducing contaminants in the aboveground part
271 of their bodies. Moreover, macrophytes also secrete biopolymer from their roots, which assist in
272 flocculation (Sharma, Singh, & Manchanda, 2015). Non-settling and colloidal particles are also
273 removed, at least partially, by bacterial growth, which results in the removal of some colloidal
274 solids and the microbial decay of other organic pollutants.

275 **Biogas production from water lettuce biomass**

276 The biogas produced from the HDPE digester is shown in Fig. 12. The analyzed WL result showed
277 that the VS value was 4.9%, which was higher than the VS value reported by Abbasi & Nipaney
278 (1991). For a co-substrate of WL and PD, feeding the material to the HDPE digester every 2 days
279 is recommended (Ngan et $al.$, 2018). In the current study, 10 kg of WL (approximately 0.49 kg of
280 VS) was fed every 2 days for the first 10 days, allowing microorganisms to adjust slowly to the
281 new feed. Afterwards, 40 kg of WL (about 1.96 kg of VS) was fed every 2 days for the next 30
282 days, and 24 kg of WL (equivalent to 1.18 kg of VS) was fed each time during the final 20 days.
283 The loading rates of WL were low compared to those suggested by Eder & Schulz (2007) for the
284 AD process, but PD was also fed into the biogas plant.

285 On the first day of the WL feeding, 365 L day^{-1} of biogas production was recorded, which was
286 transformed solely from the PD. On day 2, the produced biogas was slightly reduced (321 L day^{-1}
287), announcing a small "shock" within the fermentation process. From days 3 to 10, biogas
288 production increased and ranged from 266 L day^{-1} to 597 L day^{-1} (411.7 ± 104.4 L day^{-1} on
289 average). The biogas production volume increase due to the WL addition was estimated to be 46.7
290 L day^{-1}. This amount is equivalent to a biogas yield of 190.6 L kg VS$_{added}^{-1}$.

291 During the second period, biogas production decreased after increasing the feeding amount, as the
292 microorganisms required some time to adjust to the large feedstock. The results show that a large
293 variation in biogas production was recorded from 234 L day^{-1} (day 14) to 1280 L day^{-1} (day 32).
294 Several peaks in biogas production appeared during the period. The average volume of biogas
295 produced in this period was 676.2 ± 293.4 L day^{-1}. One pig was being raised in the pigsty at that
296 time. We assumed that the amount of PD increased by 5% compared to the first period due to the
297 pig's increasing weight. It is estimated that an amount [365 + (365 × 5%)] = 383.3 L day^{-1} of
298 biogas was produced solely from the PD in the second period. Therefore, the biogas production
299 volume increased due to the WL contribution by around 292.9 L day^{-1}. As such, the biogas yields
300 for the period were estimated at 292.9 L kg VS$_{added}^{-1}$.

301 During the third period, biogas production gradually decreased compared to the second period.
302 The maximum and minimum biogas productions were recorded on days 46 (1039 L day^{-1}) and 57
303 (260 L day^{-1}). The average biogas production in this period was 518.6 ± 224.1 L day^{-1}. If the
304 amount of PD increased by 10% due to the growth of the pig, it is estimated that the biogas
305 produced solely from the PD would amount to 401.5 L day^{-1} in the second period, calculated as
306 [365 + (365 × 10%)] = 401.5 L day^{-1}. Consequently, the average biogas volume produced from
307 the WL was estimated to be approximately 117.1 L day^{-1}. This resulted in an estimated biogas
308 yield of 198.5 L kg VS$_{added}^{-1}$.

309 The biogas yields increased gradually as the feeding material from the WL increased,
310 demonstrating WL's potential for renewable energy production. However, changes in the feeding
311 amount can result in temporary disruptions, as microbes need time to break down the organic
312 matter and nutrients in WL and convert them into biogas. It is worth noting that only a portion of
313 the WL fed into the digester during the first 10 days was converted into biogas. In the next 30 days,
314 the remaining WL from the previous period combined with the new feeding was digested,
315 increasing biogas production in the second and third periods.
316 As shown in Fig. 12, the cumulative biogas production exhibits sigmoid growth curves, as
317 described above. In the first phase, the curve showed a slow increase (lag phase) at the start of the
318 co-feeding with WL. The second and third periods showed a rapid increase that approached
319 exponential growth (log phase) due to the significant amount of WL added to the digester.
320 In this study, the biogas yields from WL fermentation ranged from 190.6 L.kg VS^{-1} to 292.9 L.kg
321 VS^{-1}, comparable to previous reports involving the co-digestion of WL and cow manure and
322 digestion of solely WL substrate (Phuong *et al.*, 2015; Cong *et al.,* 2022). It should be mentioned
323 that these previous experiments were implemented in a lab-scale batch anaerobic digester with
324 strict control over operating parameters, while this study was carried out in a farm-scale digester.
325 Despite the differences in scale, this study demonstrated that WL is a promising material for biogas
326 production.
327 *Biogas composition*
328 Figure 13 shows the change in biogas composition over time. The biogas composition recorded on
329 the first day was 55.3% CH_4 and 30.5% CO_2. During the experiment, the CH_4 content varied from
330 54.1% to 59.9% and the CO_2 content varied from 21.9% to 31.7%. Nam *et al*. (2015) reported
331 47.7%–56.6% and 46.5%–54.4% methane content for onsite experiments of co-digestion of rice
332 straw and water hyacinth, respectively, with PD using PE biogas plants in the VMD. Another study
333 on co-digestion of cow dung and WL found CH_4 content from 50.6% to 54.8% after 2 weeks of
334 fermentation (Phuong *et al*., 2015), while Cong *et al.* (2022) discovered that solely digesting WL
335 produced the highest concentration of methane by 62.2% on day 35. This study noted a high
336 methane content from the start of the experiment, as the HDPE plant had been in operation for two
337 years and was being fed with PD. The biogas could be applied to energy consumption due to its
338 high methane content, particularly for household cooking in the VMD.
339 *Feasibility of farm-scale biogas production and AD effluent removal systems*
340 Biogas production can be considered a renewable energy source in most localities. Unlike other
341 renewable energy sources, such as solar and wind power, biogas energy sources are used directly
342 by households. Accordingly, biogas plants are more appropriate for decentralized energy
343 production areas in the VMD. It is notable that bio-gasification from AD depends on several
344 factors, such as carbon/nitrogen (C/N), hemicellulose content, pH, and the buffering capacity of
345 the substrate (Güngören Madenoğlu *et al.,* 2019). In farm-scale experimentation, WL showed great
346 biogas production potential for co-digestion with PD. CH_4 concentration was achieved by more
347 than 50% after 2 weeks, implying conformity for household cooking and heating (Ngan *et al.*,
348 2020).

349   Under these circumstances, WL source availability is the main challenge in expanding the system
350   for households. In the VMD, many household farms have sufficient land area to install biogas, as
351   well as a pond for growing WL combined with aquacultural activities. WL can be grown on a pond
352   with a nutrient source provided by the livestock biogas plant. The contaminant-eliminating
353   productivity of WL was reliable throughout the current study. Nutrient removal by WL involves
354   not only eutrophication reduction of surrounding water bodies, but also significant biomass
355   production for renewable energy generation. WL biomass can be used to feed the biogas plant
356   directly or applied as simple bio-pretreatment technologies (soaking in biogas effluent or anoxic
357   mud for a 5-day period) to enhance the biogas yield, as suggested by Nam *et al.* (2021) and Nam
358   *et al.* (2023). The co-digestion of WL and PD could solve common issues concerning insufficient
359   animal waste sources for biogas production, because many farms in various localities typically
360   have a smaller standing stock of animals (permanently or temporarily) (Nam *et al.,* 2017; Nam *et*
361   *al.,* 2021). Our study encourages the co-digestion of WL with PD to enhance renewable energy
362   production. Notably, the use of renewable energy produced from AD allows for energy
363   independence in the context of high energy consumption demand. Moreover, the application of
364   household biogas plants to produce low-cost energy increases household economic efficacy owing
365   to saving electrical use, liquefied petroleum gas (LPG) and firewood, while reducing greenhouse
366   gas emissions from agricultural activities (Jan, Truc & Nam, 2018).

Conclusions

368   This study applied WL as a post-treatment method to AD effluent and achieved impressive
369   removal efficiencies of pollutants after 6 days, including 84.4%–94.5% TSS, 80.0%–82.9% TP,
370   50.3%–59.7% COD, 50.0%–59.3% BOD_5, and 38.3%–46.8% TKN. Over 30 days, the treatment
371   efficiencies of TSS, BOD_5, COD, TKN, and TP in the effluent were 93.8%–97.7%, 76.6%–
372   82.6%, 76.8%– 82.9%, 61.8%– 63.8%, and 89.0%– 89.6%, respectively. Based on the wet
373   weight biomass, the doubling times for WL grown on surface coverages of 50%, 25%, and
374   12.5% were 12.32 days, 12.11 days, and 12.28 days, respectively. Thus, WL could be used as a
375   supplementary substrate for biogas production. The biogas yield was recorded from 190.6 to
376   292.9 L kg VS_{added}^{-1} when converted solely from WL. The CH_4 content was verified from 54.1%
377   to 59.9%, making it suitable for biogas consumption. In general, WL could be applied in the
378   post-treatment stage for AD effluent and as an additional substrate for biogas plants to produce
379   renewable energy. Further research should explore the potential of using WL as a substitute
380   substrate for livestock waste to generate energy.
381

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385

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575

The physiochemical properties of husbandry anaerobic digester effluent.
1 **Table 1.** Physiochemical properties of HADE

Parameter	Unit	Concentration	Reference	Standard‡
pH	-	7.33	$6.5 - 7.4^\dagger$	$5.5 - 9.0$
TSS	mg L^{-1}	128	$70 - 12200^\dagger$	50
BOD$_5$	mg L^{-1}	86	$360 - 1125.4^\ddagger$	40
COD	mg L^{-1}	149	$84 - 5864^\dagger$	100
TKN	mg L^{-1}	40	$16.8 - 548.8^\dagger$	50
TP	mg L^{-1}	14	$5.5 - 122.3^\dagger$	NA
Total Coliform	MPN 100mL^{-1}	7.5×10^5	$4.6 \times 10^4 - 9.3 \times 10^{7\ddagger}$	3000

2 †Ngan & Hoang (2020), ‡Ngan (2012), ‡QCVN 62-MT:2016/ BTNMT (referred to A column)

Doubling time of water lettuce among treatments

Table 2. Doubling time of water lettuce among treatments

Treatments	Initial day	Final day	Doubling time (days)
50%	70.8	536.4	12.32
25%	39.2	307.6	12.11
12.5%	19.2	146.4	12.28
Average	-	-	12.24

Figure 1

The existing polyethylene biogas digester

Figure 2

Water lettuce biomass measurement

Figure 3

Measurement of biogas volume and biogas composition

Figure 4

Ambient temperature during experiment
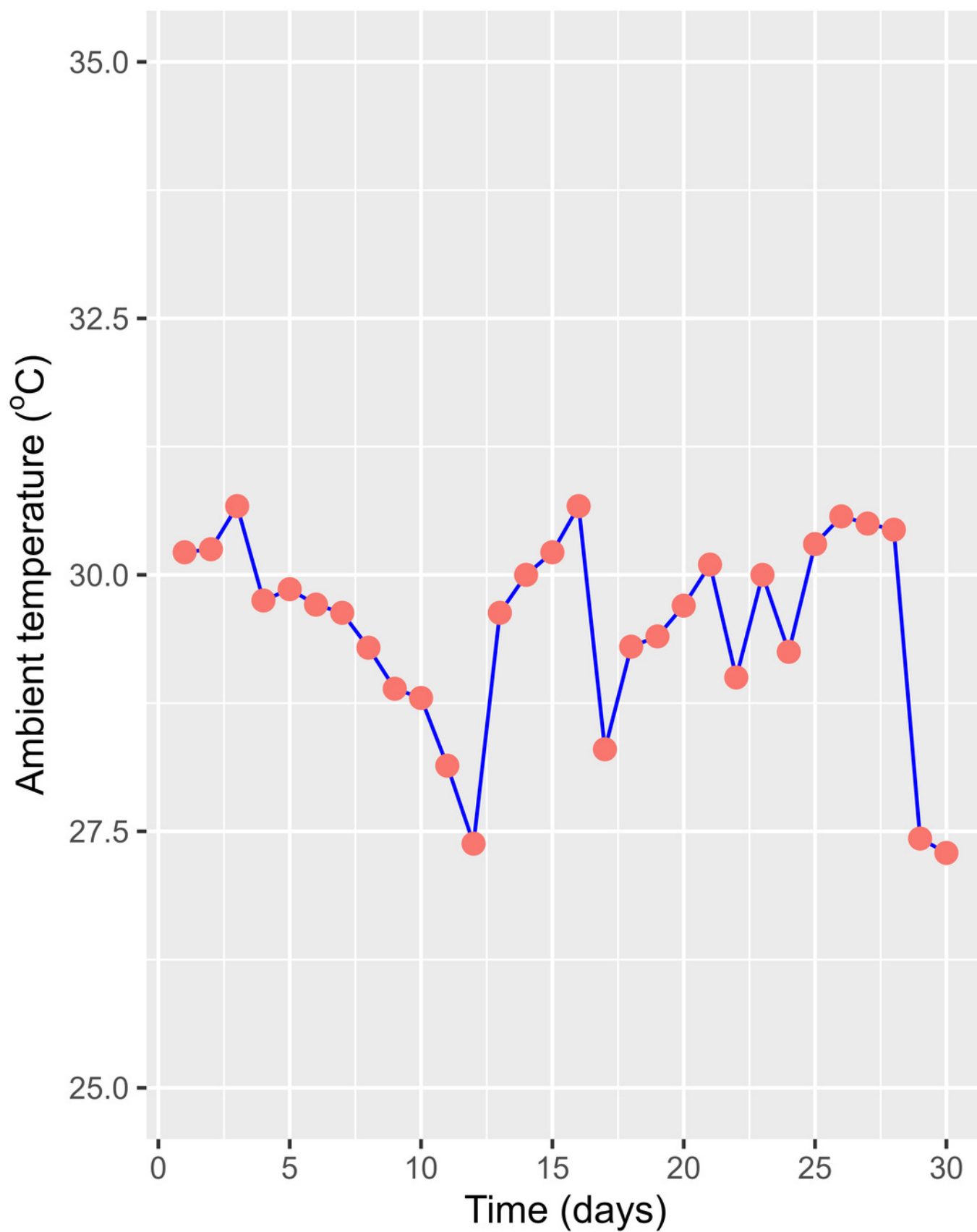
Figure 5

The variation of husbandry anaerobic digester effluent in treatments over times
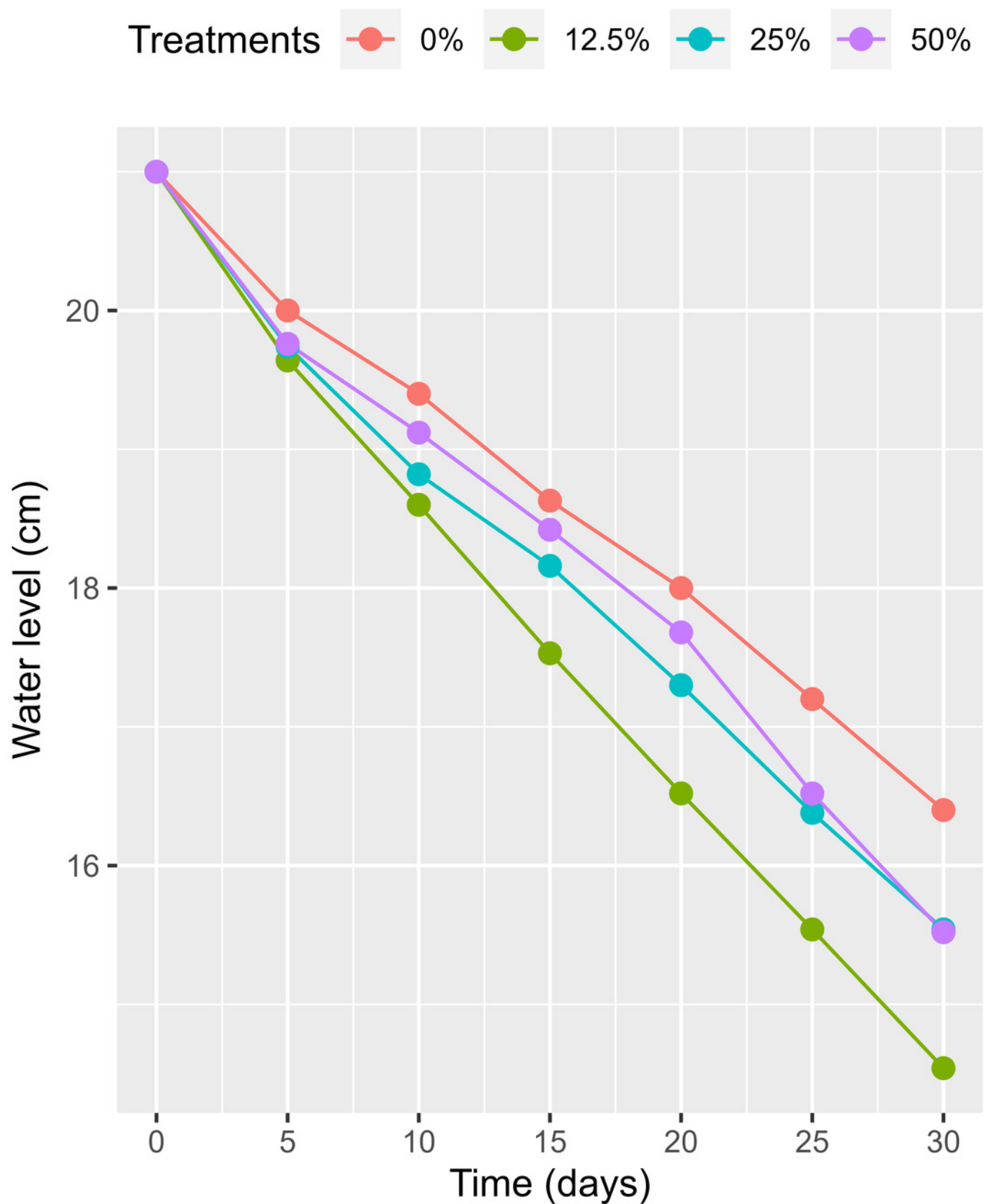
Figure 6

Husbandry anaerobic digester effluent polluted parameters removal by times
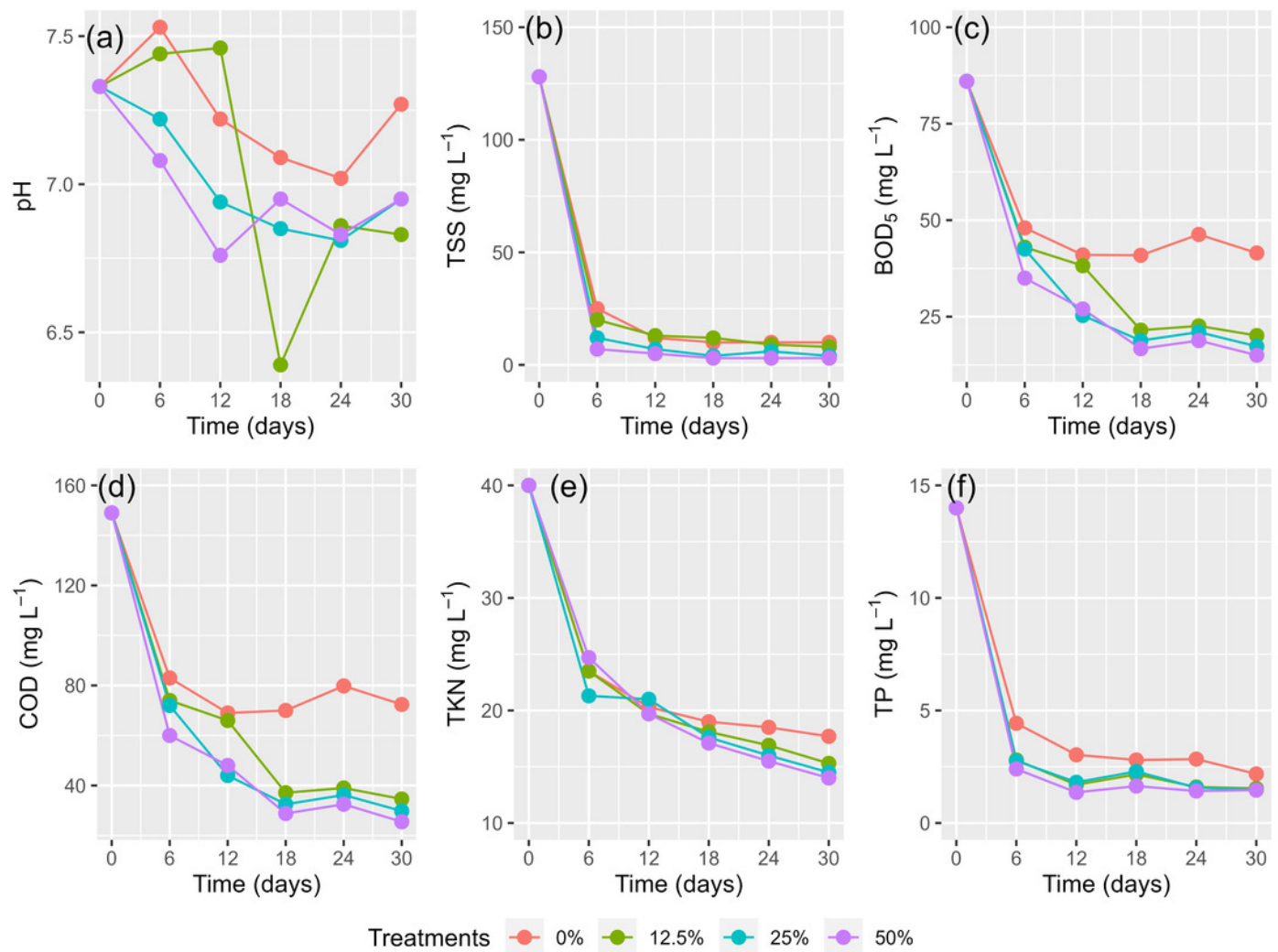
Figure 7

Treatment efficiency of husbandry anaerobic digester effluent
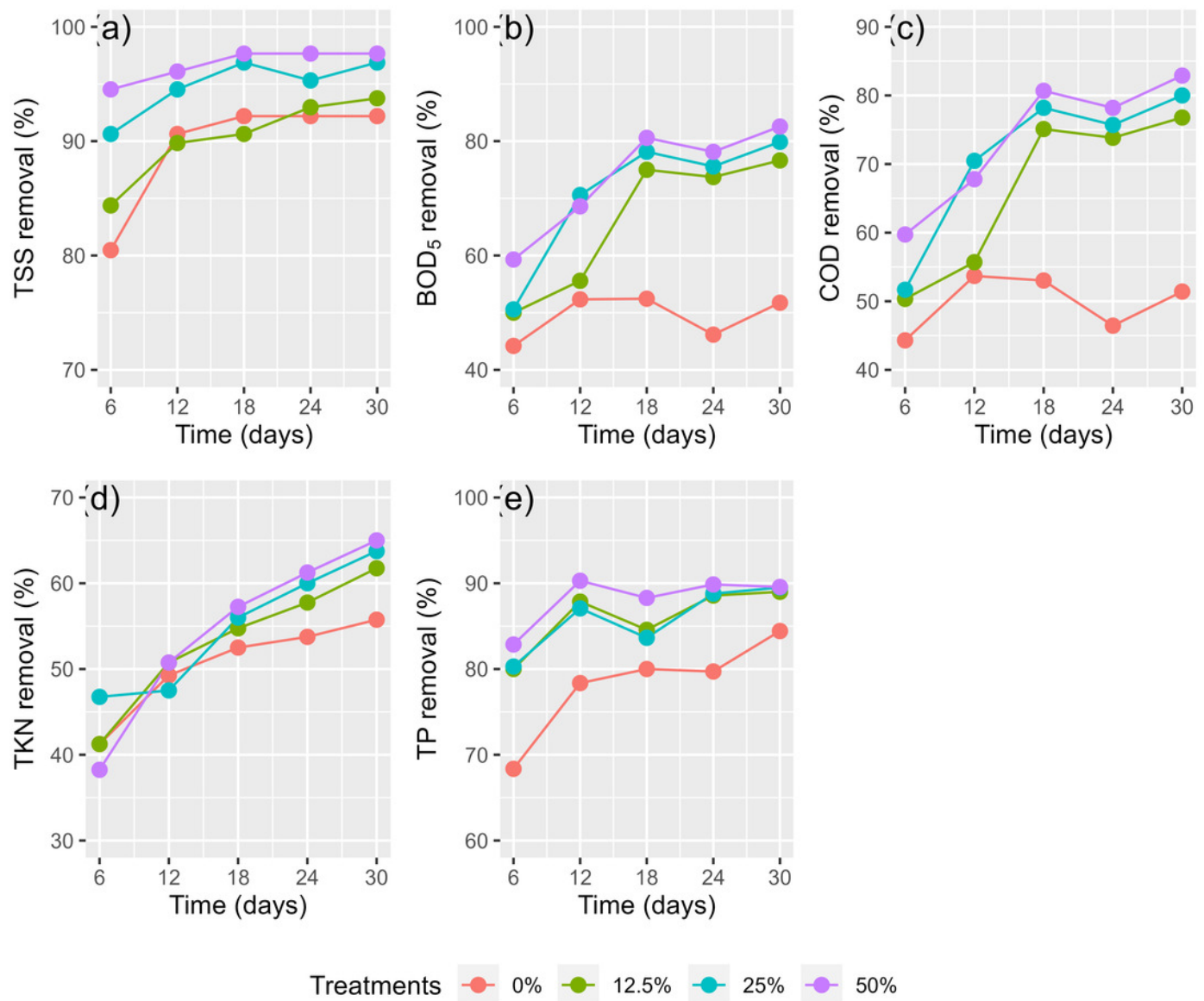
Figure 8

Water lettuce biomass change among treatments
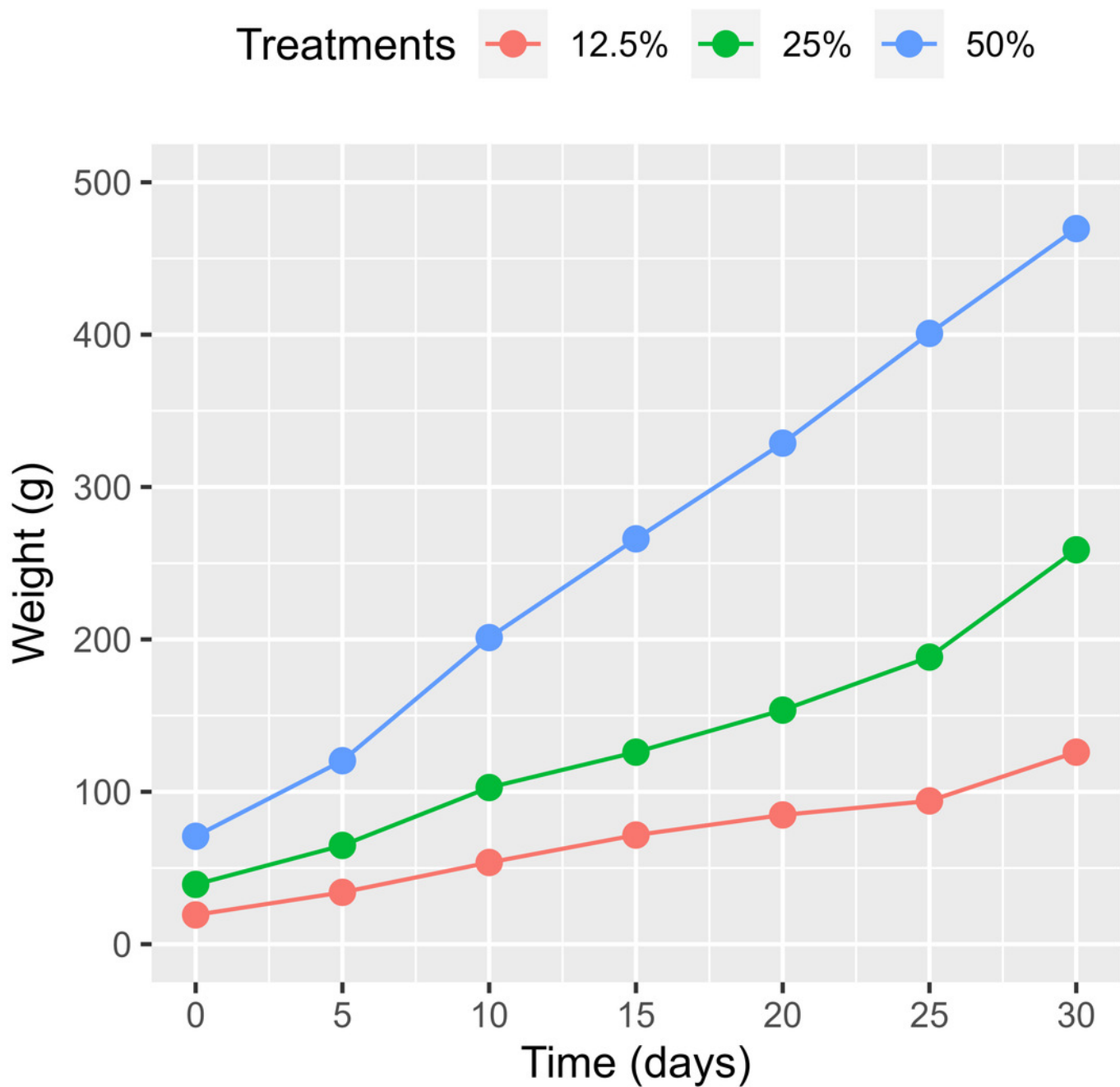
Figure 9

The root length and leaves length of water lettuce
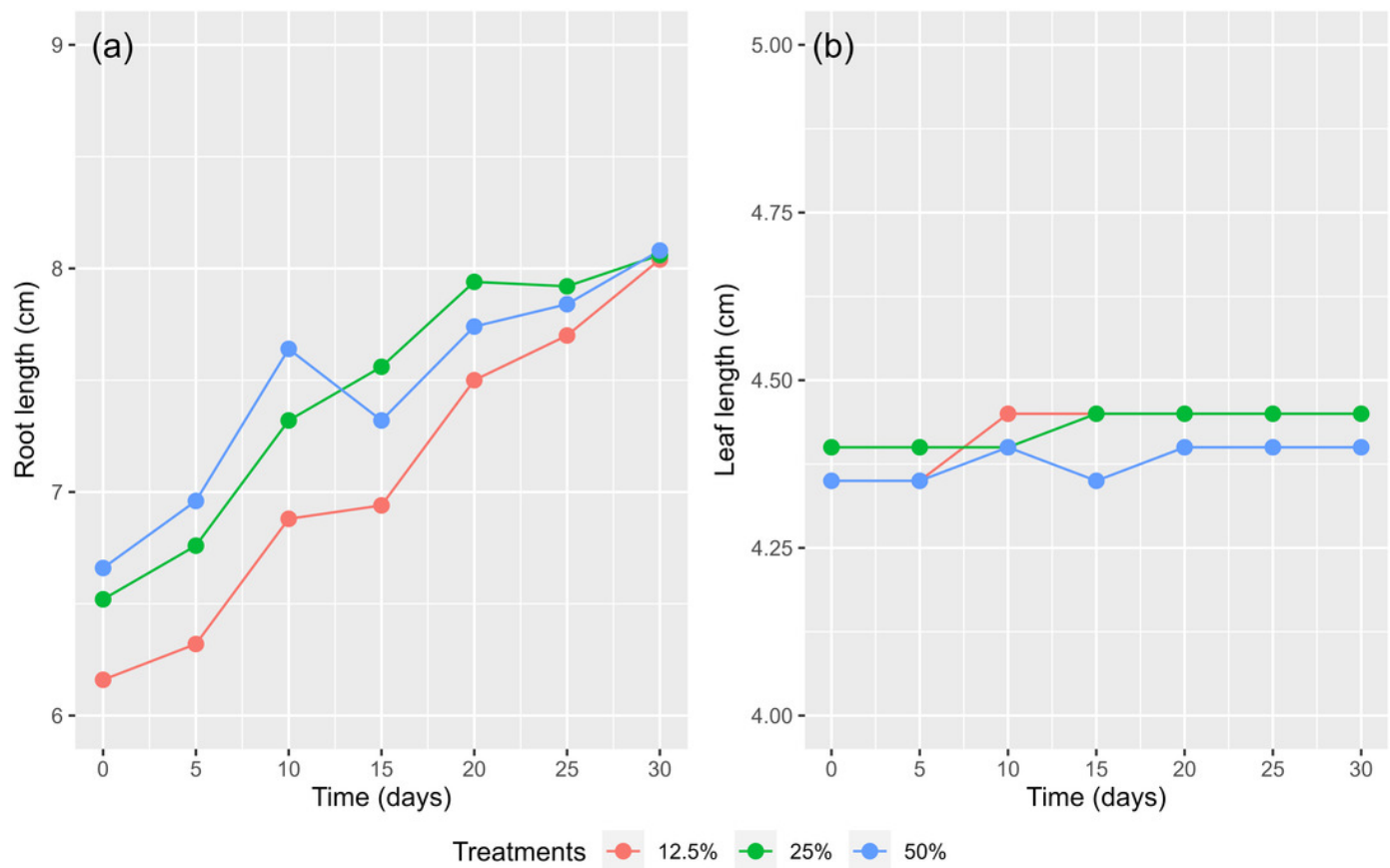
Figure 10

Pearson correlation of pollutant parameters

Figure 11

Pearson correlation of water lettuce biomass
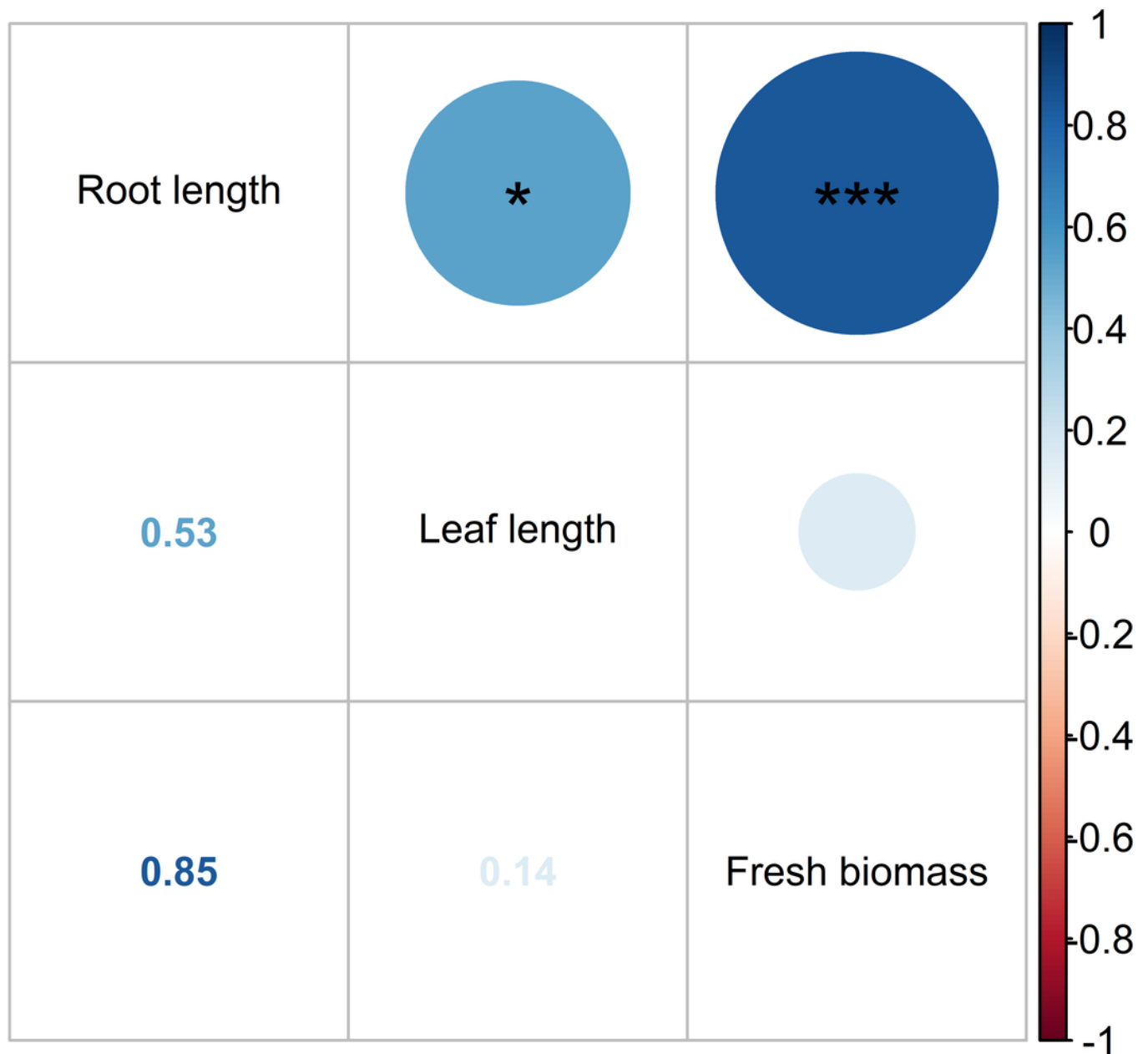
Figure 12

Biogas production from polyethylene digester
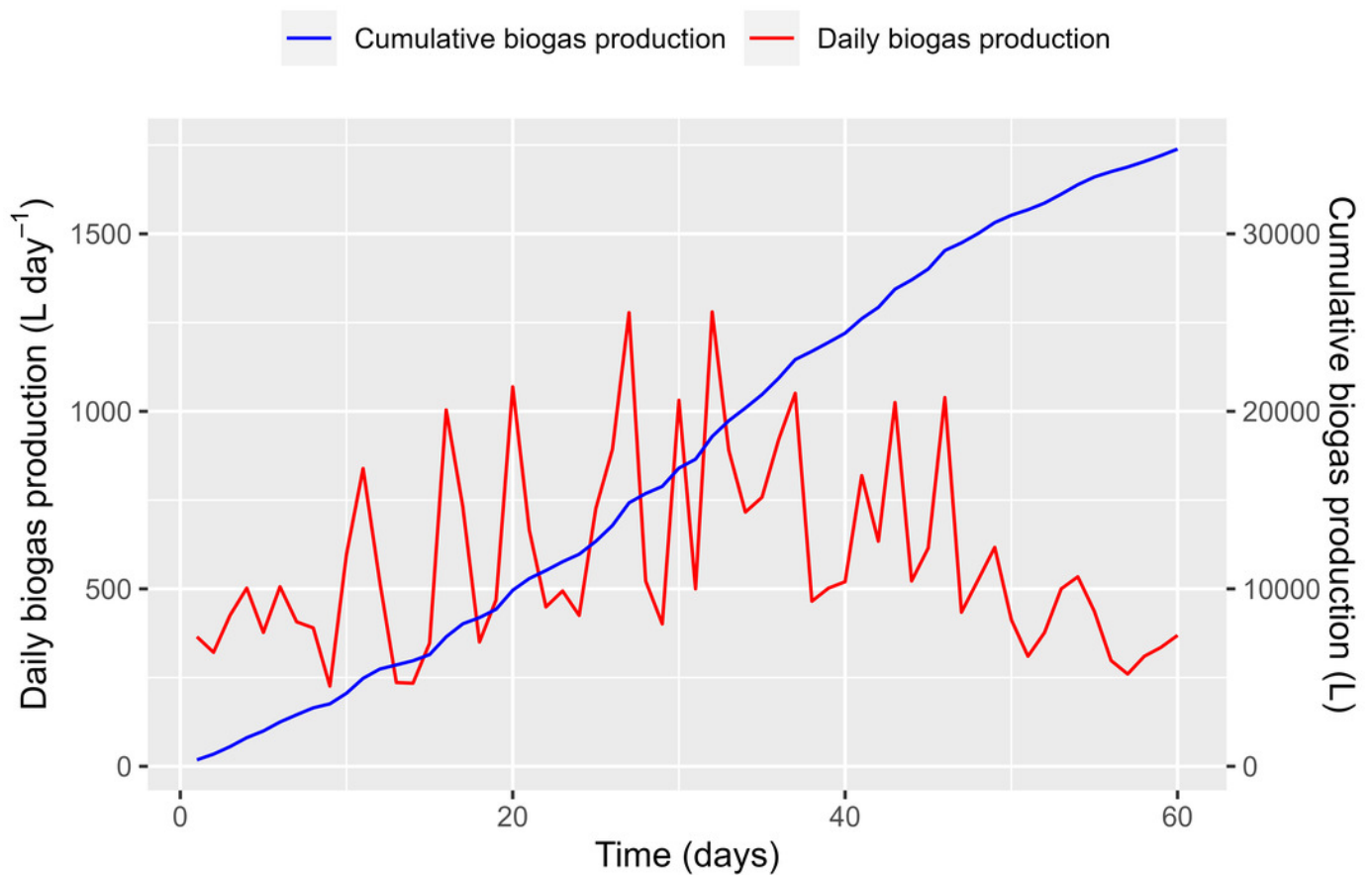
Figure 13

Biogas composition from testing polyethylene digester