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Performance and mechanism of Carrousel oxidation ditch and water Spinach wetland combined process in treating water hyacinth (*Pontederia crassipes*) biogas slurry

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Abstract: Owing to its high concentrations of nitrogen and phosphorus, the slurry from water hyacinth (*Pontederia crassipes*) biogas production cannot be discharged directly without further treatment. To achieve the target of water recycling, a new strategy of combining a Carrousel oxidation ditch with a water spinach wetland was developed in this study for the harmless treatment of *Pontederia crassipes* biogas slurry. First, the water quality characteristics of the biogas slurry were measured. Then, comprehensive tests of the combined slurry treatment system were carried out to verify pollutant removal performance and mechanism. The results showed that the Carrousel oxidation ditch reduced the inlet pollutant load of the subsequent water spinach wetland. The chemical oxygen demand (COD), and ammonium nitrogen ($\text{NH}_4^+\text{-N}$), total nitrogen (TN), and total phosphorus (TP) contents of the average effluent from the combined system were less than 50 mg/L, 1.6 mg/L, 6 mg/L, and 0.5 mg/L, respectively, which means that all met urban sewage treatment standard of Level 1 Grade A (GB18918-2002). Gas chromatography – mass spectrometry analysis showed that the combined system had decreased various types of organic pollutants in the biogas slurry exponentially, efficiently removing alkane pollutants, aromatic hydrocarbons, and heterocyclic compounds. Scanning electron microscopy images revealed very large surface area of the water spinach roots in the wetland, which played important roles in enriching the microorganisms and trapping organic matter. Plant absorption, microbial degradation, and filtration were the primary ways in which the water spinach wetland purified the biogas slurry.

Introduction

The Tongyu River, an inland navigable river in Jiangsu Province, China. It is considered to be the “golden waterway” connecting the northern and southern parts of the province. It is also one of the sources of drinking water in Yancheng, the largest city in northern Jiangsu Province with an area of approximately 150,000 ha and an estimated population of 8.1 million (Wang et al. 2010). Highly polluting industries have gradually moved from the southern to the northern parts of this province, thus threatening the water quality of the Tongyu River. Polluted wastewaters can be derived from domestic sources in urban areas, industrial and agricultural sources, and aquaculture (Aleksandra et al. 2022, Ariffin et al. 2019). All of these have contributed to high concentrations of nitrogen and phosphorus in the Yancheng section of the Tongyu River. In recent years, the common water hyacinth (*Pontederia crassipes*) has spread quickly in this section of the river, with the infestation causing not only eco-environmental damage by blocking waterways but also disruption of the aquatic ecosystem equilibrium, including

a reduction in biodiversity (Carnaje et al. 2018, Das et al. 2016, Guragain et al. 2011). The salvage of *Pontederia crassipes* is considered one of the most efficient approaches to removing excess nitrogen and phosphorus from polluted waters (Sierra et al. 2022).

The strategies currently in use for *Pontederia crassipes* removal include biological, chemical, and physical methods and combinations thereof (Carlini et al. 2018, Zhang et al. 2016). The anaerobic fermentation of *Pontederia crassipes* for biogas production has enormous potential for producing biomass energy and thus contributing to meeting regional energy demands (Zhu et al. 2019). However, although the use of *Pontederia crassipes* as the feedstock for biogas production has a number of advantages, the anaerobically digested effluent has thus far not met environmental requirements owing to its high carbon, nitrogen, and phosphorus contents (Appels et al. 2011, Godin et al. 2013, Zhang et al. 2016). *Pontederia crassipes* biogas slurry emissions may cause water eutrophication, resulting in the excessive growth of algae and other aquatic organisms.

One possible solution is the use of an oxidation ditch, which is an improvement to the traditional activated sludge process and has been shown to remove organic matter, nitrogen, and phosphorus from wastewater effectively (Li et al. 2019). Constructed wetlands, which have proven to be efficient and economical means of environmental protection, have been widely used for the secondary treatment of domestic sewage, landfill leachate, aquaculture wastewater, and industrial wastewater (Bergier et al. 2016, Das et al. 2019). In this study, we developed a strategy of combining a Carrousel oxidation ditch with a water spinach wetland and assessed the efficiency of the system in purifying biogas slurry. The aims of this study were to determine the performance and underlying mechanism of this combined system in removing pollutants from *Pontederia crassipes* biogas slurry.

Materials and methods

Water quality of the influent

Raw wastewater was collected from an anaerobic reactor treating *Pontederia crassipes* in the Tongyu River. The main water quality indicators are shown in Table 1. The 5-day ratio of biological oxygen demand to chemical oxygen demand (COD) of the biogas slurry was approximately 0.5, which indicates its biochemical treatability and capacity for aerobic treatment.

Experimental setup

The experiment was conducted from April to June 2022 in the municipal laboratory of Yancheng Institute of Technology. The system (Fig. 1) was composed of a storage tank, a Carrousel oxidation ditch, a desilter, and a water spinach wetland. The precipitated biogas slurry was transported to the Carrousel oxidation ditch using an electric centrifugal pump. The effluent then flowed to the desilter, with a hydraulic retention time of 10 h. The water in the sedimentation tank was then pumped into the water spinach wetland, the effluent of which subsequently drained into a nearby water body.

The Carrousel oxidation ditch was a four-channel circular ditch, with a total working volume of 150 L. Each channel was 0.1 m wide, with a water depth of 0.5 m. The straight portion of each channel was 0.9 m in length. The radius of the large semicircular portion was 0.2 m and that of the small semicircular portion was 0.1 m. The dissolved oxygen (DO) level, which was detected online using a DO meter (YSI-58, YSI Inc., Yellow Springs, OH, USA), was regulated through manual adjustment of the aerator rate. The Carrousel oxidation ditch was initially seeded with sewage sludge collected from municipal wastewater treatment plants. The ratio of mixed liquor volatile suspended solids (MLVSS) to mixed liquor suspended solids (MLSS) in the inoculated sewage sludge was 0.75. After the inoculation, the sewage sludge concentration in the Carrousel oxidation ditch was 29.5 g MLVSS/L.

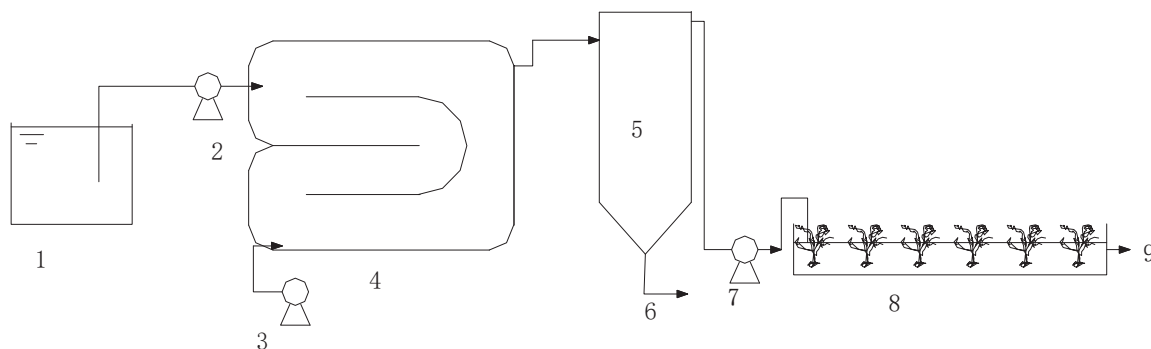
The wetland was constructed using concrete bricks to form a shallow pool of 15 m × 1.5 m × 0.3 m (length × width × depth). A channel was set in the front of the pool for distributing water and another one was created in the back of the pool for gathering the effluent water. The bottom slope of the pool was 1% and the water depth was maintained at 0.1 m. The hydraulic loading rate was 0.25 m³/(m²·d). In this experiment, water spinach purchased from local farmers was chosen as the aquatic plant and was the only species planted in the wetland pool itself.

Analytical methods

The water quality was analyzed on-site immediately after collection of the water sample. The COD, total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH₄⁺-N), MLVSS, and MLSS were measured in accordance with standard methods (China 2004). Water spinach root samples were collected from the pool after stable operation of the wetland and examined using scanning electron microscopy (SEM, model JSM 5600LV, Shimazu, Tokyo, Japan) (Yu 2017). Refractory organics were analyzed and determined using gas chromatography – mass spectrometry (GC-MS) (Wu 2013).

Table 1. Composition of the biogas slurry of *Pontederia crassipes*

COD (mg/L)	BOD ₅ (mg/L)	TP (mg/L)	TN (mg/L)	NH ₄ ⁺ -N (mg/L)	protein (mg/L)	cellulose (mg/L)	pH
175–430	99.1–212.6	5.5–7.5	42–86	30–68	95–167	101.3–204.8	



1 – tank, 2 – electrical centrifugal pump, 3 – aeration pump, 4 – carrousel oxidation ditch, 5 – sedimentation tank, 6 – mud discharging pipe, 7 – electrical centrifugal pump, 8 – aquatic vegetables wetland, 9 – the effluent.

Fig. 1. Schematic diagram of the experimental setup

Results and discussion

Effects of the combined system on carbon oxygen demand reduction

Figure 2 shows the reduction in COD achieved by the combined system. The COD of the initial biogas slurry ranged from 175 to 430 mg/L, whereas the levels in the effluent were 100 mg/L and 50 mg/L after processing by the Carrousel oxidation ditch and water spinach wetland, respectively. Organic substrates were biodegraded mainly in the Carrousel oxidation ditch, likely because the strong turbulence at the top of the ditch accelerated mixing of the slurry with the activated sludge, allowing denitrifying bacteria in the anoxic zone to use the organic content as a carbon source (Jin et al. 2015). This resulted in a two-fold advantage: eliminating the need for an external carbon source and reducing the energy requirement for the aerobic degradation of organic matter (Xia et al. 2004, Yin et al. 2022). The ability to increase the DO concentration in the aerobic zone by controlling the aeration intensity promoted the removal of organic matter from the biogas slurry (Zhang et al. 2012).

The effluent containing minimally degraded organics was then treated in the water spinach wetland. The COD of the average effluent from the combined treatment processes was below 50 mg/L, thus meeting the standards of urban sewage treatment in China (GB18918-2002). The average efficiency of COD removal by the combined system was 85%. The COD removal effect was strengthened in the water spinach wetland, which provided a good microenvironment for the growth of relevant microorganisms (Liu et al. 2020). A large surface area of the water spinach roots in the constructed wetland enabled the enrichment of microorganisms and trapping of organic matter, the latter of which was removed via mechanical filtration by the roots as well as adsorption and fixation on the biofilm surfaces.

Effects of the combined system on total nitrogen and ammonium nitrogen removal

The average degrees of efficiency of the combined processes in removing TN and $\text{NH}_4^+\text{-N}$ from the *Pontederia crassipes*

biogas slurry are shown in Figs. 3 and 4, respectively. The concentration of $\text{NH}_4^+\text{-N}$ was 30–68 mg/L in the influent applied to the oxidation ditch, whereas it was less than 5 mg/L in the effluent, corresponding to the removal efficiency of 90%. The 42–86 mg/L TN concentration range in the initial biogas slurry was reduced to 33–70 mg/L after processing in the oxidation ditch. After processing in the water spinach wetland, the effluent $\text{NH}_4^+\text{-N}$ and TN concentrations were less than 1.6 mg/L and 6 mg/L, respectively. The $\text{NH}_4^+\text{-N}$ and TN removal rates at the end of the combined processes were 98% and 90%, respectively, complying with Level 1 Grade A of the national standard (GB18918-2002).

The ammoniation and nitrification rates were high in the oxidation ditch, resulting in large amounts of organic nitrogen, ammonia nitrogen, and nitrite nitrogen being produced through decomposition of the biogas slurry. The DO concentration in the anoxic zone of the oxidation ditch ranged from 0.8 to 1.0 mg/L. This high concentration largely hindered the denitrification process, resulting in the effluent not reaching the emission standards of urban sewage treatment (GB18918-2002).

The effluent from the Carrousel oxidation ditch was further treated by the water spinach wetland to remove ammonia nitrogen and nitrite nitrogen, thus reducing the TN concentration in the biogas slurry. Water purification by the wetland was achieved via plant nutrient uptake and microbial degradation and filtration processes, including denitrification. The nitrogen content of the influent biogas slurry was mostly (80%) nitrite nitrogen and nitrate nitrogen, both of which were removed via denitrification by the microbial community in the water spinach wetland (Jan 2010).

The root exudate of plants includes mainly sugars, amino acids, organic acids, and plant mucus, which are low-molecular-weight organic matter. These substrates are necessary for microbial denitrification, being utilized by the denitrifying bacteria (Vymazal 2007). The rapid growth of the water spinach plant demonstrated the ability of its root system to enhance microbial growth and utilize organic matter (Wu et al. 2013).

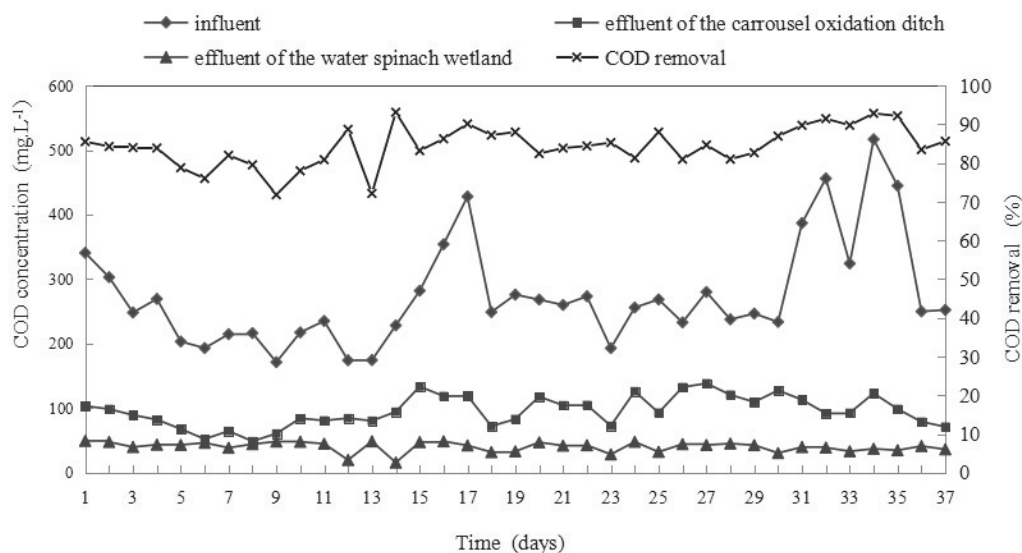


Fig. 2. Performance of the combined system in reducing the chemical oxygen demand in *Pontederia crassipes* biogas slurry

Effects of the combined system on total phosphorus removal

The average degrees of efficiency of the Carrousel oxidation ditch and water spinach wetland in removing TP from *Pontederia crassipes* biogas slurry are shown in Fig. 5. The TP concentration of the influent was 5.5–7.5 mg/L, whereas that of the effluent of the Carrousel oxidation ditch was 4–5 mg/L, corresponding to a removal efficiency of 20–30%. Following treatment by the wetland, the TP concentration of the effluent was less than 0.5 mg/L, which met the Level 1 Grade A standard for urban sewage treatment in China (GB18918-2002). The TP removal efficiency of the combined system was 95%, with the wetland having the higher activity in this regard.

The water spinach wetland removed TP mainly through adsorption, biological metabolism, and absorption. The plant roots and microbes worked synergistically in removing TP from the biogas slurry via physical, chemical, and biological

processes (Li et al. 2022). Inorganic phosphorus taken up by the roots is converted to plant organic components, such as ATP, DNA, and RNA, which can be removed through harvesting of the water spinach plants. An analysis of the harvested plants confirmed a high rate of TP removal, with 20% occurring via plant adsorption. The oxygen distribution resulting from the terraced wetland design created alternating oxic and anoxic conditions that benefited the growth of polyphosphate-accumulating microorganisms, which are crucial for biological phosphorus removal (Li et al. 2010).

The water spinach roots were observed using SEM (Fig. 6). The large surface area of the roots enabled the enrichment of microorganisms and trapping of organic matter, including phosphorus. Because of the ability of roots to trap sediment, elemental deposition was the main route of the phosphorus removal (Tuszynska et al. 2013). Furthermore, because the wetlands constructed in this study were built

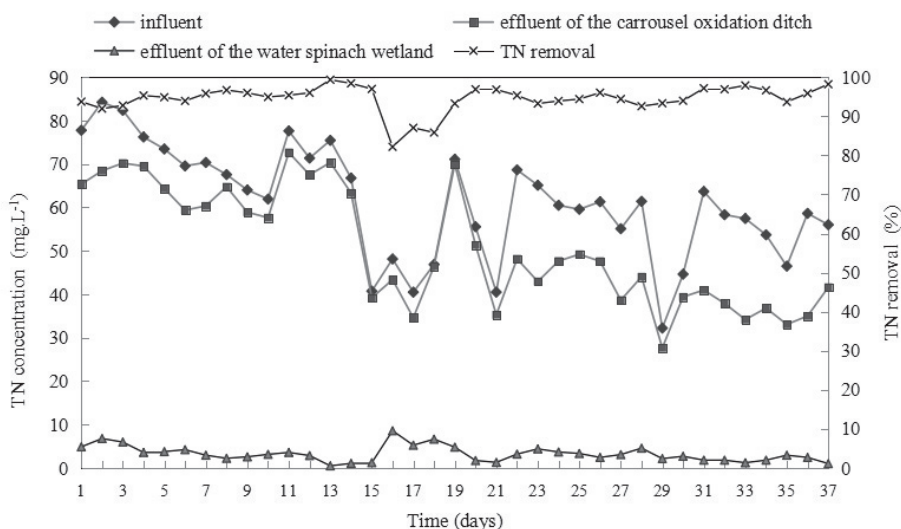


Fig. 3. Performance of the combined system in removing total nitrogen from *Pontederia crassipes* biogas slurry

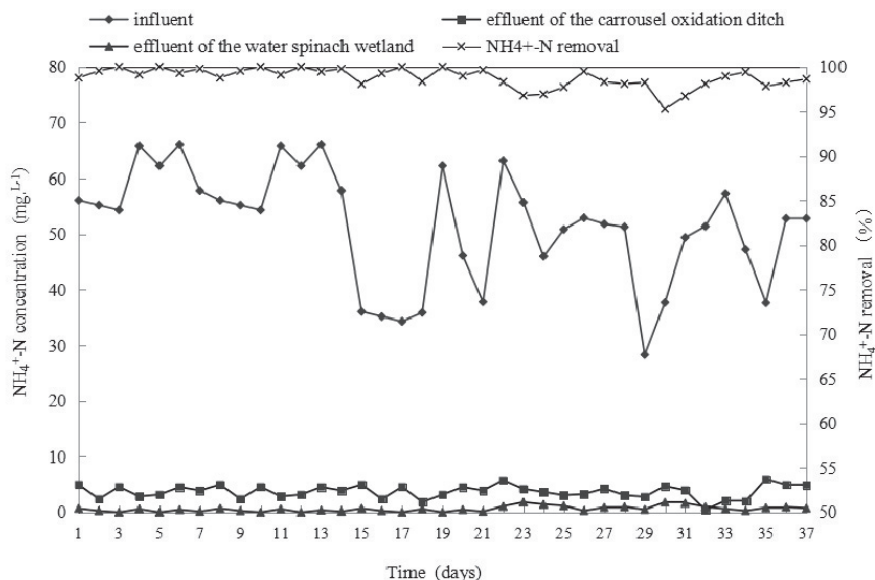


Fig. 4. Performance of the combined system in removing ammonium nitrogen from *Pontederia crassipes* biogas slurry

with concrete, the inorganic phosphorus in the biogas slurry reacted with calcium ions from the concrete, resulting in the precipitation of refractory phosphate (Patyal et al. 2021).

Effects of the combined system on the removal of refractory organic material

To evaluate the performance of the combined system in removing refractory pollutants, the organic compositions of the influent and effluent were analyzed using GC-MS (Table 2).

Despite the fact that the content of refractory organic material in the effluent had been reduced by anaerobic fermentation, a large proportion of it remained. Nearly 50 compounds were detected in the untreated *Pontederia crassipes* biogas slurry, including 23 types of alkanes and olefins, 10 types of aromatics, 6 types of carboxylic acids, and 16 types of esters (Table 2). Eight compounds were among those targeted as priority environmental pollutants in China (Ren et al. 2004). The Tongyu River pollution is caused by

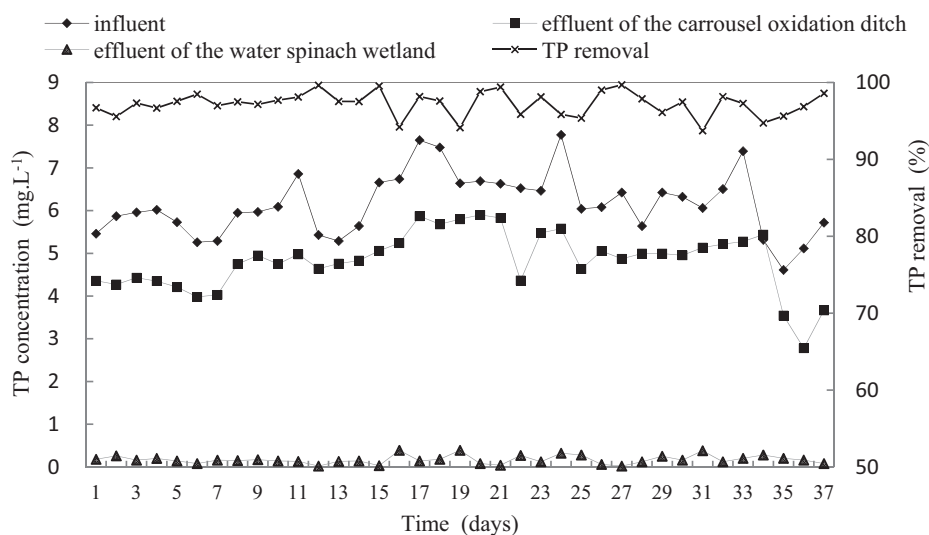


Fig. 5. Performance of the combined system in removing total phosphorus from *Pontederia crassipes* biogas slurry

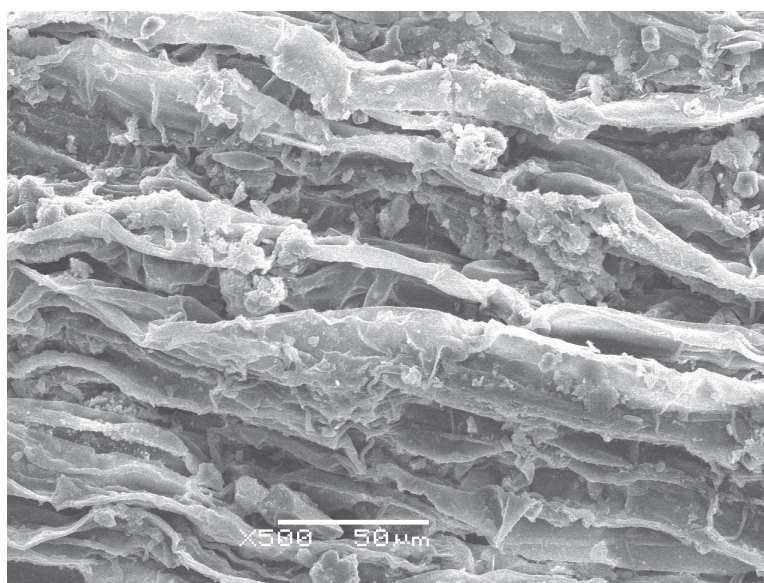


Fig. 6. Scanning electron microscopy images of water spinach roots

Table 2. Organic species in the untreated *Pontederia crassipes* biogas slurry

Number	Organic species	Type
1	alkanes	11
2	olefins	12
3	aromatics	10
4	carboxylic acid	6
5	esters	16

industrial wastewater, untreated sewage, and agricultural non-point pollution sources, which explains the complex pollutant constituents (including many refractory organic compounds) of the *Pontederia crassipes* biogas slurry.

As shown in Tables 2 and 3, the numbers of organic pollutants in the influent and effluent of the combined system were 48 and 15, respectively. The number of refractory organic pollutants in the effluent was significantly decreased after processing through both the Carrousel oxidation ditch and water spinach wetland. The GC-MS analysis verified that organic pollutants in the biogas slurry could be removed effectively by the combined system, which performed well in removing alkane pollutants, aromatic hydrocarbons, and heterocyclic compounds. The compounds removed may have been partially processed macromolecules or microbial metabolites.

The Carrousel oxidation ditch supported the growth of various microbial communities, as the structural characteristics of the reactor created aerobic, anoxic, and anaerobic zones (Xu 2017). This favorable environment promoted gene exchange and recombination, resulting in new pathways that enhanced the degradation of refractory organic compounds. Bacterial colloids were found on the biological membrane in the oxidation ditch. The high surface reactivity and large surface areas of these bacterial colloids in the reactor enabled their adsorption or enrichment of pollutants in the biogas slurry. Protozoans (e.g., rotifers and ciliates) in the reactor consumed the small sludge particles, thereby reducing the turbidity of the influent, and directly dissolved organic compounds in the reactor. Consequently, the oxidation ditch reduced the inlet pollutant load of the water spinach wetland.

After the degradation of macromolecular organic matter in the Carrousel oxidation ditch, other organic pollutants in the wastewater were degraded easily in the wetland. Plants in wetlands can assimilate, degrade, and metabolize organic matter directly. The strong root system of water spinach provides an ideal habitat for microorganisms, with the transfer

of oxygen from the roots increasing the microbial richness in the rhizosphere. Microbial co-metabolism is one of the most important degradation pathways of refractory organics in wetlands (Yang et al. 2022, Zhai et al. 2013).

Conclusions

To achieve the goal of water recycling by eliminating or reducing the pollutants in *Pontederia crassipes* biogas slurry, we proposed a combination of a Carrousel oxidation ditch and water spinach wetland. The efficiency and mechanism of pollutant removal by the combined system were explored through its nitrogen and phosphorus removal performance. Moreover, using GC-MS, the removal of organic pollutants was analyzed and the mechanisms were postulated. The main results are summarized below.

The combined treatment with a Carrousel oxidation ditch and water spinach wetland was effective in treating *Pontederia crassipes* biogas slurry. The maximum rates of COD, $\text{NH}_4^+\text{-N}$, TN, and TP reduction in the combined system were 85%, 98%, 90%, and 95% respectively. The two treatment processes in the system complemented each other, as the water spinach wetland made up for the incomplete treatment by the Carrousel oxidation ditch, while the latter reduced the inlet pollutant load of the wetland component. The concentrations of COD, $\text{NH}_4^+\text{-N}$, TN, and TP in the effluent of the wetland were less than 50 mg/L, 1.6 mg/L, 6 mg/L, and 0.5 mg/L, respectively, thus meeting the Level 1 Grade A standard for urban sewage treatment in China (GB18918-2002). Most of the TN and TP in the slurry were removed by the water spinach wetland. Water spinach was chosen as the aquatic plant, because it has a very large biomass and a high demand for nitrogen and phosphorus. These two elements are thus removed through harvesting of the plants.

According to the GC-MS analysis, the various types of organic pollutants in the biogas slurry were decreased

Table 3. Organic pollutants in the effluent of the combined system

Number	Organic pollutant	Matching degree (%)
1	3-hydroxy-4-methoxy cinnamic acid	68.24
2	2,6-(methyl propyl)-phenol	54.67
3	2-deoxy cytidine	70.76
4	Maleic acid	64.65
5	the benzene ring propylamine	63.65
6	4-hydroxyl coumarin	54.51
7	2-(n-butyl sulfonium)-thiazole	92.88
8	5-methyl cytidine	86.82
9	amino acid whey	75.56
10	4-(2-phenyl propyl) sulfonyl morpholine	63.33
11	1-(2-pyridyl)-2,2-dimethyl-2-piperidine	91.99
12	1-Bromo-iso-octane	72.67
13	N-2-(trifluoro methoxy phenyl) acetamide	73.93
14	dihydrocurcumin	77.35
15	hesperidin	75.63

exponentially after treatment by the combined system, with biochemical treatment resulting in the removal of aromatic hydrocarbons and alkane pollutants. SEM images showed that the water spinach roots had a large surface area, a factor that led to the enrichment of microorganisms and trapping of organic matter. Our study demonstrated the benefits of including a water spinach wetland in water purification, as it promoted effluent treatment via plant uptake, microbial degradation, and filtration of the pollutants. The synergistic degradation of organic material by the enriched microbes in the Carrousel oxidation ditch and wetland was the main reason for the high rate of organic pollutant removal by the system.

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