Organics and nutrient removal from swine wastewater by constructed wetlands using ceramsite and magnetite as substrates

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ABSTRACT

The discharge of swine wastewater has posted a serious threat to water bodies in rural areas and been receiving increasing attention worldwide. This study investigated the treatment performances of synthetic wastewater and real swine wastewater in subsurface flow constructed wetland (SSFCW) with different substrates (gravel, ceramsite and magnetite). The results showed that all systems exhibited better removal performances and higher nitrous oxide (N_2O) emissions for synthetic wastewater than real swine wastewater. Magnetite-based CW was efficient for organic matter removal with chemical oxygen demand (COD) removal for 53.0–91.5 %. Ceramsite-based CW was effective in removing nutrients with 11.0–63.2 % for total nitrogen (TN) removal and 93.2–95.5 % for total phosphorus (TP) removal. Five dissolved organic matters (DOM) (UVA humic-like, UVC humic-like, tyrosine-like and tryptophan-like substances) were identified in swine wastewater before and after the treatment of CWs. The relative proportion of humic substance in all CWs fluctuated slightly which might result in the decrease of organics removal and the restriction of heterotrophic denitrification in CWs with swine wastewater. While the tyrosine-like and the tryptophan-like showed an opposite variation.

1. Introduction

Recently, the severe environmental problems have been caused in rural areas due to the discharge of swine wastewater resulting from the high expansion of breeding markets. Generally, the higher loading of pollutants in swine wastewater in which chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) could reach 5000–30000, 800–6000, and 100−1400 mg L^{-1}, is the main characteristic as well as the major obstacles when treating swine wastewater [1,2]. Typically, traditional wastewater treatment technologies such as flocculation, anaerobic digestion treatments [3], sequencing batch reactors (SBR) [4], anaerobic-aerobic-oxidation pond [5], might have limited capacity in eliminating nitrogen, phosphorus, and organic matters, which still needed to be further treated and recycled properly. Employing the secondary treatment after conventional treatments would be necessary to achieve a harmless disposal [6].

Constructed wetlands (CWs), as an environmental-friendly and economical technology, have been applied successfully to treat various types of wastewaters and purify secondary effluents [7]. However, the improvement of nitrogen removal by microbial addition and

micro-electrolysis required additional equipment and extra cost [8,9]. Over the last 10 years, intensive studies have been focused on pursuing cost-effective and efficient substrates in order to increase the treatment capacity [10]. Natural ores and synthetic substrates applied in CWs have the advantages of being cheap, readily available and having a good effect on nitrogen and phosphorus removal [11,12]. Some substrates including natural ores have unique physicochemical properties for efficient contaminant removal, for example, minerals with high content in Fe or Fe oxides have high phosphorus removal capacities [13]. It was reported that not only magnetite adsorbed stable and had little effect by temperature, but the leaching risk of heavy metals was too small to cause secondary pollution [14]. Some microorganisms with magnetotactic could utilize oxygen or nitrates as terminal electron acceptor for metabolism to removal pollutants [15]. The magnetotactic bacteria migrated in the direction of magnetism [16] which allowed microorganisms to accumulate on the surface of magnetite. Therefore, it could be implementable to use magnetite which had the characteristics of to accumulate microorganisms and absorb phosphorus-containing pollutants as a substrate for swine wastewater purification in CWs. Moreover, synthetic materials, such as novel ceramics, have been developed and applied in
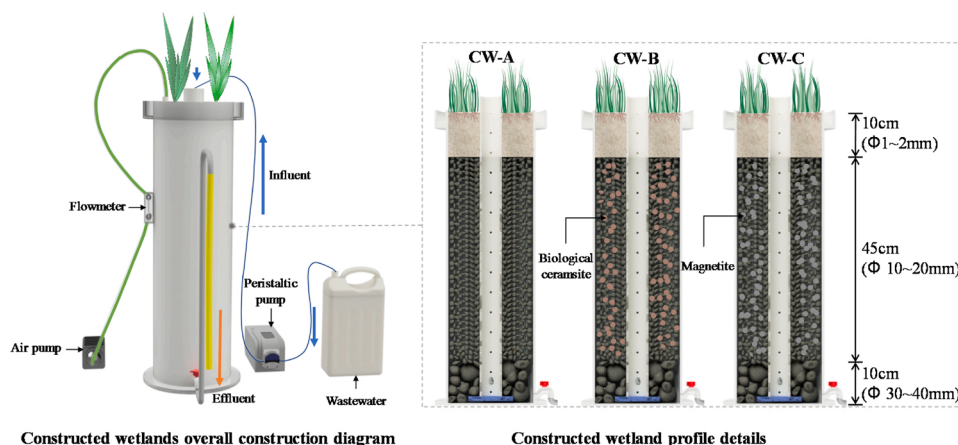

Fig. 1. The schematic diagram of experimental downflow constructed wetlands (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite).

CW wastewater treatments. For instance, the vesicles biological ceramics as CWs substrate achieved removal efficiencies up to 61 % for COD, and 56 % for ammonia-nitrogen (NH_4^+-N) with industrial wastewater due to the higher effective surface areas [17]. In addition, the catalytic ceramics could reduce or degrade many refractory contaminants in wastewater by the micro-electrolysis reactions. Wu et al. [18] found that the novel catalytic-ceramic-filler made from catalytic micro-electrolysis merged with absorption-biodegradation sludge could degrade organic matters more than 90 %. Whether it is natural ore or synthetic substrates as matrix, it has the own advantages for removing pollutants [11]. Few studies have been applied to characterize magnetite representing natural ore and catalytic ceramics substrate representing the synthetic substrates by a comprehensive approach. Therefore, it is possible to compare the decontamination characteristics of natural ore and synthetic substrates applied to CWs and provide a theoretical basis for practical application.

Dissolved organic matter (DOM), including humic acid, fulvic acid, several hydrophilic organic acids, amino acids, etc. [19,20], was consisted of a complicated mixture of molecules with a relatively broad range of molecular weight-bearing both polar and non-polar sites. Previous studies proposed that the labile fraction of DOM directly regulated gross nitrogen cycle rates, as it was the initial substrate for the nitrogen cycling pathway [21,22]. Considering large amount of DOM in swine wastewater originated from feed residues and pig metabolic products might play a vital role in the nitrogen cycle as an active component in CWs treatment [23]. Hence, studying the composition and migration of DOM can reflect the degree of denitrification and the relationship between nitrogen cycle and organic matter degradation. Excitation-emission matrix (EEM) fluorescence spectroscopy is a widely used method that has been used to investigate the performance of fluorogenic components that originated from the terrestrial, autochthonous, and anthropogenic origin. The combination of parallel factor (PARAFAC) analysis and EEM is a powerful tool in the assessment of DOM dynamics, which could solve the restriction for assessing the DOM components because that the locations of individual peaks are sensitive to physical chemical conditions. Therefore, EEM-PARAFAC used to analyze the DOM components in swine wastewater and the relative changes before and after the CWs could reflect the degree of degradation of organic matter and the progress of denitrification, which was used as a reference for the actual operation of the CWs.

The purposes of this study were (1) to evaluate comparatively the removal performance in CWs employed with biological ceramics and magnetite for treating synthetic wastewater and real swine wastewaters; (2) to characterize the potential emission of nitrous oxide (N_2O) from the CWs with different substrates; and (3) to explore the dynamic transformation of DOM in water by EEM-PARAFAC for clarifying removal mechanisms of organics and nutrients.

2. Material and methods

2.1. Materials

Magnetite and catalytic biological ceramic were selected as typical natural materials and synthetic materials respectively and used as substrates in CWs in this study. The magnetite was obtained from Haozhou, Anhui province, China. The main components of the magnetite were FeO and Fe_3O_4 that accounted for 31.0 % and 69.0 %, and the particle average size was $10-20$ mm. Catalytic biological ceramic was prepared from clay, scrap iron, and copper sulfate solution with low bulk density and grain density with a diameter of $10-20$ mm, which could prevent short-circuiting of wastewater and blocking of the constructed wetlands. Clay and scrap iron were dried in stove at 105 °C for 4.0 h, crushed in a ball mill and subsequently sieved. After mixing, 10 wt% of copper sulfate solution was added to pelletize, then the raw pellets were sieved and dried. Finally, the pel-lets were sintered at 850 °C for 30 min in an electric tube rotary furnace. More details about the biological ceramic can be found in previous study [18].

2.2. Experimental system setup

Three types of lab-scale CW systems designed in subsurface flow styles, including gravel-based constructed set as a control (CW-A), biological ceramic-based CW (CW-B) and magnetite-based CW (CW-C), were setup in this study. Each system was made from PVC plastic pipes with a height of 65 cm and 20 cm in diameter, (Fig. 1). There were three layers in the CWs from the top down. In the first layer, 10 cm of the fine sand layer ($\Phi = 1-2$ mm) was set up for plant (Iris pseudacorus) to grow. In the next layer, the matrix in CW-A was gravel ($\Phi = 10-20$ mm), CW-B and CW-C were mixtures of biological ceramic ($\Phi = 10-20$ mm) and gravel ($\Phi = 10-20$ mm) (1:1 of volume ratio), magnetite ($\Phi = 10-20$ mm) and gravel ($\Phi = 10-20$ mm) (1:1 of volume ratio), respectively. The last part was supporting layer with gravel coarse gravel ($\Phi = 30-40$ mm). In the center of CWs, a PVC perforated tube (70 cm high and 3 cm in diameter) was inserted in order to facilitate the measurement of dissolved oxygen (DO) inside of the system and at the bottom of which an aeration stone connected to an air pump was placed for oxygen supplement. All CWs were then flooded with synthetic wastewater and acclimated for inoculating microorganisms. At the beginning of experimental studies, synthetic wastewater was inoculated with 1 % crude sewage obtained from the secondary sedimentation tank in Yang ling sewage. After batch wise operation to achieve sufficient growth in the CWs, continuous feeding was started, and microorganisms were acclimatized to the feed solution until steady-state conditions were reached. Mixed liquor suspended solids (MLSS) concentration was used as the primary indicator of steady-state conditions. when nearly the
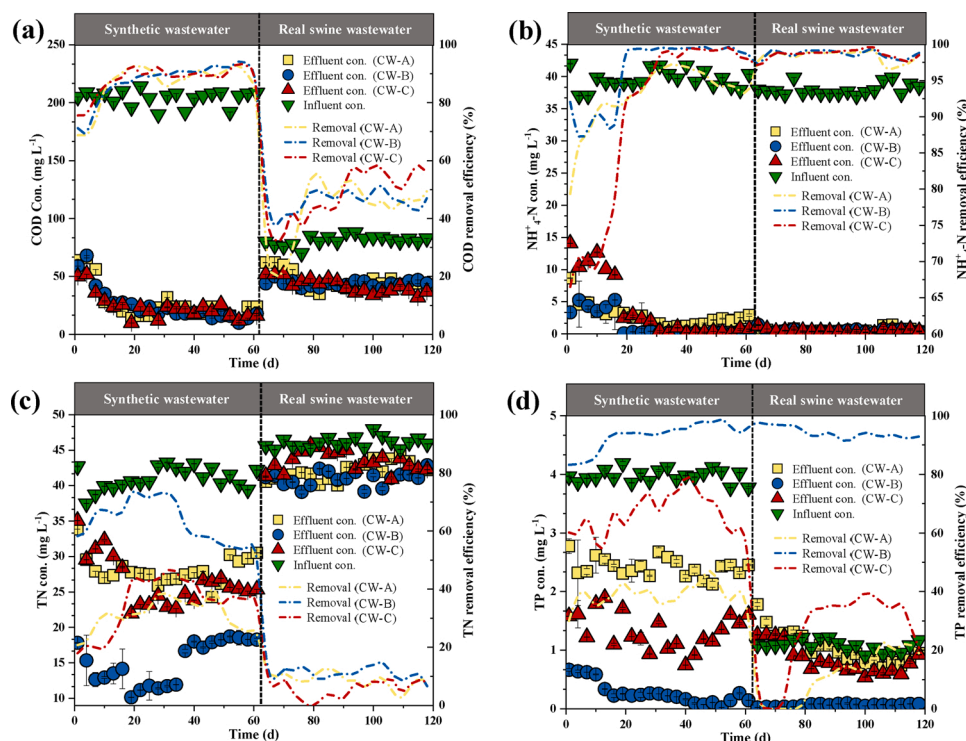
Fig. 2. The influent and effluent concentrations and removal efficiencies of COD (a), NH4+-N (b), TN (c) and TP (d) in different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) during the whole experimental period.

same MLSS concentrations were measured during a period of three times the current sludge age, presence of steady-state conditions was accepted.

2.3. Operation of experimental systems

There were two experimental stages in this study, and all CWs were operated for 120 days (0–63 days for treating synthetic wastewater and 66–120 days for real swine wastewater). In the first stage, the influent was synthetic wastewater mainly containing sucrose, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, $FeSO_4$, and $CaCl_2$, according the discharge standard of pollutants for livestock and poultry breeding (GB18596-2001) in China (COD for 400 mg L^{-1}, NH_4^+-N for 80 mg L^{-1}, TP for 8 mg L^{-1}). Owning to the poor TN removal performance, the influent was diluted to the half to investigate the treatment performance. The concentrations of synthetic wastewater were COD for 200 mg L^{-1}, NH_4^+-N for 40 mg L^{-1}, and TP for 4 mg L^{-1}. The influent of digested swine wastewater at the second stage was also diluted to the same NH_4^+-N concentration of synthetic wastewater in the first stage. Swine wastewater was collected from the Yangling Pig Fram (Shanxi, China) and kept in a refrigerator at 4℃ before use. The wastewater was used as the influent for all CWs without any modifications such as pH adjustments or addition of nutrients or trace metals. Detailed characteristics of influent can be found in Supplementary Information. Intermittent aeration was applied for one hour at 0 h, 6 h, 12 h, and 18 h daily with an air-flow rate of 1.0 L min^{-1} in all systems [25]. The hydraulic retention time of CWs was 3 days with the hydraulic loading rate106 mm d^{-1}. The air temperature was 25 ± 1 ℃ during the experimental period.

2.4. Sampling and analysis

2.4.1. Water sampling and measurement

During the experimental period, the influent and effluent were sampled every three days [25]. The water samples in each CW were collected at 9:00 am and analyzed the concentrations of COD, NH_4^+-N, nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), TN, and TP based on

the standard methods [26]. DO concentrations were determined in situ using a DO meter (HQ40d, HACH, USA).

2.4.2. Gas sampling and measurement

To investigate the variations of N_2O emission fluxes from CW systems, a static-stationary chamber was used to collect gases samples during the experimental period. And the gas samples were collected in a 250 L polymethyl methacrylate closed chamber [27]. The gas samples were gathered at 9:00−10:00 am every 20 min, and then they were measured by gas chromatography (GC, Agilent Technologies 7890B) which was equipped with a flame ionization detector and a capillary column (HP-5MS, 30 m × 0.25 μm × 0.25 μm) within 24 h.

2.4.3. UV–vis and fluorescence analysis

When the influent was real swine wastewater, water samples of influent and effluent were collected in different CWs and filtered using a 0.45 μm membrane for measuring dissolved organic carbon (DOC) by a total organic carbon analyzer (TOC-L CPN, Shimadzu, Japan).

Before fluorescence spectrum test, all the filtered water samples were diluted with Milli-Q water below 10 mg L^{-1}. The diluted samples were analyzed by a fluorescence spectrometer (Shimadzu, RF-6000, Japan). The excitation wavelengths were 200−500 nm with 5.0 nm bandwidth and scanning intervals, and the emission wavelengths were 250−550 nm with 3.0 nm bandwidth and 2.0 nm scanning intervals. The scanning speed was fixed at 6000 nm min^{-1}, and the internal filter effects of all EEMs were corrected using absorbance measurements, and we eliminated most of the effects concerned with Raman scatter by subtracting the Milli-Q blank. The PARAFAC modeling was carried out in MATLAB 7.0 (Natick, MA, USA) using the DOM Fluor v.1.7 Toolbox. EEM-PARAFAC contains all the tools needed for identifying outlier samples, performing split-half analysis, and diagnosing residual errors. Fluorescent DOM components were identified by split-half analysis and consistency diagnostics. Several important optical indexes were used, including fluorescence index (FI) [28], humification index (HIX) [29], freshness index (β:α), and biological index (BIX) [30].

Table 1

Effluent concentrations and overall removal performances of different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) (Mean ± SD).

Parameters	Synthetic wastewater (20−60 d)			Swine wastewater (80−120 d)		
	CW-A	CW-B	CW-C	CW-A	CW-B	CW-C
COD (mg L^{-1})	20.79 ± 4.96	17.70 ± 3.51	19.43 ± 4.37	43.06 ± 3.05	43.67 ± 2.02	38.90 ± 4.40
RE (%)	89.80 ± 2.46	91.31 ± 1.73	91.49 ± 2.04	48.05 ± 3.91	47.32 ± 2.79	53.04 ± 5.79
NH_4^+-N (mg L^{-1})	1.96 ± 0.64	0.24 ± 0.14	0.97 ± 0.97	0.58 ± 0.33	0.51 ± 0.29	0.50 ± 0.26
RE (%)	95.04 ± 1.71	99.40 ± 0.35	97.54 ± 2.47	98.40 ± 0.90	98.59 ± 0.79	98.60 ± 0.74
NO_3^--N (mg L-1)	25.05 ± 1.61	10.40 ± 1.58	22.02 ± 1.17	37.07 ± 1.65	36.21 ± 1.06	38.74 ± 1.99
NO_2^--N (mg L-1)	0.28 ± 0.25	3.14 ± 0.98	0.58 ± 0.61	0.06 ± 0.04	0.22 ± 0.09	0.06 ± 0.03
TN (mg L^{-1})	27.72 ± 1.63	15.26 ± 3.26	24.58 ± 1.50	42.32 ± 1.30	41.19 ± 1.00	43.60 ± 1.33
RE (%)	33.12 ± 4.96	63.16 ± 8.10	40.72 ± 4.24	8.39 ± 3.15	10.98 ± 2.35	5.61 ± 3.59
TP (mg L^{-1})	2.38 ± 0.15	0.18 ± 0.08	1.22 ± 0.27	0.91 ± 0.14	0.07 ± 0.01	0.71 ± 0.11
RE (%)	40.49 ± 4.03	95.46 ± 1.92	69.43 ± 6.58	14.98 ± 6.25	93.15 ± 1.18	33.68 ± 5.10
DO (mg L^{-1})	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.01	0.04 ± 0.02	0.07 ± 0.02	0.06 ± 0.02
pH	7.56 ± 0.22	6.56 ± 0.32	7.72 ± 0.16	7.54 ± 0.11	6.54 ± 0.43	7.69 ± 0.10

RE: Removal efficiencies.

UV–vis absorption was measured on a SHIMADZU UV-2450 UV–vis Spectrophotometer (Shimadzu, Japan) in a 1 cm quartz cuvette and UV–vis spectra were recorded from 200 to 800 with the slit width of 1 nm. Several important UV–vis spectral parameters were used in this study, including $SUVA_{254}$ [31] and S_R [32].

3. Results and discussion

3.1. Overall treatment performance

3.1.1. Organics removal

During the experiment period, the COD removals in CWs were lower for real swine wastewater than that for synthetic wastewater (Fig. 2a). In 1–63 days, the average influent COD concentration was 192.79 mg L^{-1} in synthetic wastewater, and COD removal efficiencies of CW-A, CW-B, and CW-C were 89.8 %, 91.3.0 %, and 91.5 %, respectively (Fig. 2a). In this period, the distinctions of the COD removals were not apparent among the three CWs, and the COD removal of each system presented an upward trend at the beginning and then kept steady. This result indicated that the gravel, magnetite, and biological ceramic substrates in CWs were effective in removing the simple organic matter. Compared to CWs with a similar COD influent concentration [33], the COD removals varied from 55 % to 82 %, which were higher in this study. In the second stage with the influent COD concentration of 81.69 mg L^{-1} in swine wastewater, the removal of all CWs had become worse with the removal efficiency of 44.3–53.0 % (Fig. 2a). However, more reactions of iron oxidation and reduction might occur in magnetite-based CWs [34], which resulted in the higher COD removal efficiency in CW-C than in CW-A. Furthermore, the possible reason for better removal efficiency of synthetic wastewater might be that the organic matter in synthetic wastewater was characterized by high biodegradability, such as glucose and sucrose. The biodegradability of real wastewater was partial in practical condition and the hydrolysis and biodegradation of suspended solids was approximately 80 % under anaerobic conditions [35]. However, organic matter in synthetic wastewater could be assumed 100 % biodegradable. Hence, the swine wastewater contained more complex

organic matter, which could be difficult for microorganisms to utilize and degrade [20]. Poach et al. [36] previously reported a similar COD removal efficiency of 44 % for swine wastewater, which demonstrated that organic matters from swine wastewater could not be completely degraded by CWs.

3.1.2. Nitrogen removal

Swine wastewater has been considered as one of the major sources of nitrogen pollution discharged into environment. In this study, the NH_4^+-N influent concentrations were 39.46 mg L^{-1} for synthetic wastewater, 38.04 mg L^{-1} for real swine wastewater. NH_4^+-N removal efficiencies were more than 95 % and 98 % for each CW, respectively (Table 1). As shown in Fig. 2b, in 1–30 days, NH_4^+-N removal exhibited a rising trend due to unstable operation and then maintained stable in 30–63 days. After the influent was changed into real swine wastewater, the NH_4^+-N removal could still achieve high efficiency.

However, there were differences in TN removal performance with CWs treated for synthetic wastewater and swine wastewater. When the TN influent concentration was 40.92 mg L^{-1} with synthetic wastewater, the removals were 33.1 %, 63.2 %, and 40.7 % for CW-A, CW-B, and CW-C, respectively (Fig. 2c). The TN removals were lower in CW-A and CW-C than previous study in which achieved 46.3–52.5 % with the same C/N ratio [33]. The dynamic transformations of nitrogen showed that the nitrogen forms of effluent were dominated by NO_3–N in CW-A and CW-C (Fig. S1). This phenomenon might be demonstrated that inhibited denitrification led to NO_3–N accumulation in a short time. However, the TN removal in CW-B was higher, that might be the catalytic ceramic created a reducing environment inside. Different from the nitrogen compositions of CW-A and CW-C in the effluent, that of CW-B was mainly NO_2–N at the beginning of this experiment, and this was because absorption and coagulation effects from iron (hydro) oxides and iron corrosion. As the weakening of reducibility inside the CW system, the primary nitrogen composition of effluent in CW-B was NO_3–N. The specific mechanisms for electrochemical reduction were still unresolved in many cases and many involved direct electron exchanges between nitrate and Fe° that were reduced to ferrous or ferric ion, or production of hydrogen gas (H_2) [18,37]. Because swine wastewater could include organic nitrogen came from microbial metabolites and feed residues, the TN concentration was slightly higher than that of synthetic wastewater. When the influent was swine wastewater, the TN removal efficiencies were reduced to 8.4 %, 11.0 %, and 5.6 % respectively for CW-A, CW-B, and CW-C (Table 1), besides the effluent nitrogen compositions were almost NO_3–N in all CWs. It indicated that the denitrification process was severely impacted when the influent was swine wastewater. However, Gonzalo et al. [35] observed that nitrification process is more affected by the change in wastewater type than the denitrification process. Compared to the synthetic wastewater, the C/N of real swine wastewater decreased from 4 to 1.78. According to Torrijos et al. [38], low C/N explained the unviability of the denitrification process and thus the low TN removal. On the one hand, this was because the concentration of organic matter in swine wastewater was lower than that of in synthetic wastewater, and on the other hand, the refractory organic compounds in swine wastewater could reduce the available carbon sources for denitrifying bacteria. In practical operation, the operation mode of CWs could be adjusted to improve the removal performance of swine wastewater. For example, two-step operation promoted the denitrifying bacteria enriched [39], or step-feeding of untreated wastewater could enhance denitrification process [38].

3.1.3. Phosphorus removal

Absorption of phosphorus on medium was a major removal mechanism for phosphorus in CWs [40]. In this study, the TP concentrations of synthetic wastewater and real swine wastewater were 3.97 mg L^{-1} and 1.08 mg L^{-1}, respectively. When the influent was synthetic wastewater, the TP removal efficiencies were 40.5 %, 95.5 %, and 69.4 % respectively for CW-A, CW-B, and CW-C (Table 1). As shown in Fig. 2(d), the
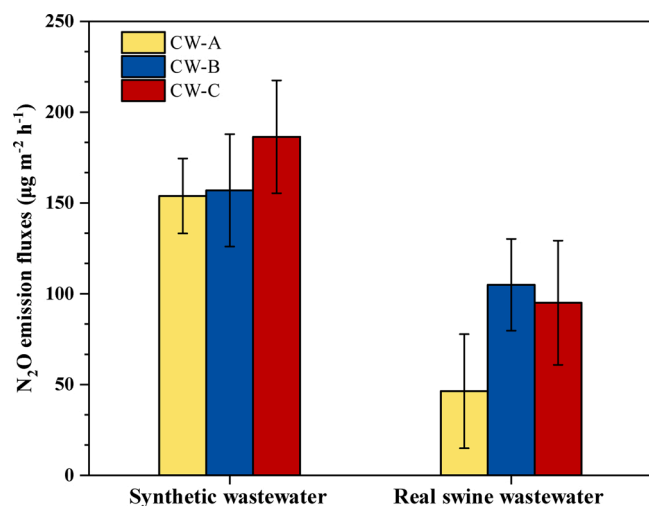
Fig. 3. Average N₂O emission fluxes in different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) with synthetic and real swine wastewaters.

Fig. 4. The influent and effluent concentrations of DOC in different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) with real swine wastewater.

TP removal in CW-B and CW-C was higher than CW-A, it indicated that the catalytic biological ceramic and magnetite had good adsorption properties for phosphorus. This might be absorption and coagulation during iron corrosion. The specific mechanism was the reaction of phosphate ions with the metallic cation such as Fe formed amorphous or poorly crystalline solids under the aeration condition [41]. In addition, for a direct chemical reaction, phosphorus can co-precipitate ferric oxyhydroxide [42]. However, as a kind of natural iron ore, the adsorption properties of magnetite was lower than that of ceramic. The differences in phosphorus removal between CW-B and CW-C were possibly due to different iron contents and specific surface area of the materials. When the influent was real swine wastewater, the TP effluent concentration was 0.06 mg L^{-1} (Table 1) in CW-B and it still could maintain stable performance. Nevertheless, CW-A and CW-C had kinds of degrees of desorption (Fig. 2d). Because the phosphorus concentration of real swine wastewater was lower than that of synthetic wastewater, CW-A and CW-C released several phosphorus that previously absorbed on the substrates. Although, CW-A and CW-C gradually recovered the removal of phosphorus, the removal performances were lower compared to the influent was synthetic wastewater. The removal efficiencies were finally stable at 15 % and 30 % respectively for CW-A and CW-C (Fig. 2d).

3.2. Potential of N₂O emission

To evaluate the characteristics of N₂O emission from CWs with different substrates, the release of N₂O was regularly tracked and observed in this experiment. The average fluxes of N₂O from CWs in different periods are shown in Fig. 3. During the whole period, the N₂O average fluxes were 94.05 µg m^{-2} h^{-1}-186.40 µg m^{-2} h^{-1} in different CWs. When the influent was synthetic wastewater, N₂O emission fluxes from CW-A, CW-B, and CW-C were 153.82 µg m^{-2} h^{-1}, 156.94 µg m^{-2} h^{-1}, and 186.40 µg m^{-2} h^{-1}. The possible reason for the higher emission flux from CW-C had been referred to in the previous study: under Fe^{3+}-reducing conditions, magnetite could remove NO₂- and reduce a portion of it abiotically to NO and subsequently to N₂O by a heterogeneous electron transfer process when NO₂- was available [37]. Microbial denitrification involved the reduction of NO₃- to NO₂-, which sequentially reduced further to nitric oxide (NO), nitrous oxide (N₂O), and eventually to N₂ [43]. And this process was usually influenced by nitrate availability, carbon availability, temperature, and pH. When the influent was swine wastewater, the denitrification was restricted due to few available carbon source, and the N₂O emission flux from CW-A,
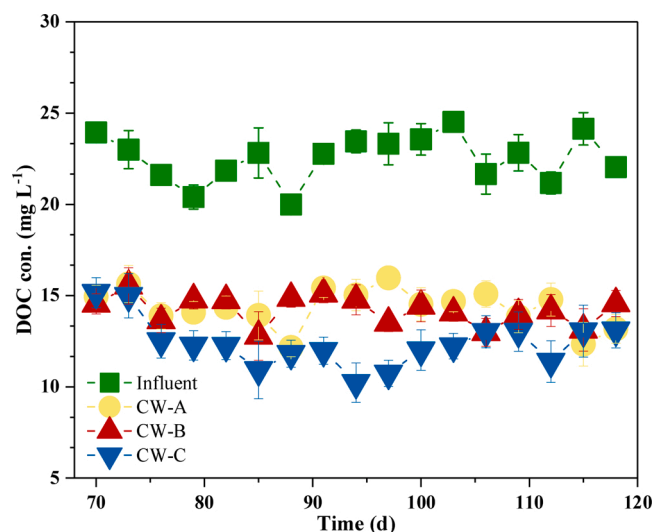
CW-B, CW-C decreased to 46.36 µg m^{-2} h^{-1}, 104.92 µg m^{-2} h^{-1}, and 95.04 µg m^{-2} h^{-1}. The emission fluxes of N₂O from CW-B and CW-C were higher compared with CW-A. According to Mander et al. [44], we calculated the emission factor (EF) of N₂O in Table S2. Compared to different influent, the EFs of CWS with synthetic wastewater were higher than that of real swine wastewater. Microbial denitrification was usually assumed to be mediated by organic carbon oxidation. However, there were some refractory organics in swine wastewater which were difficult to degrade. Compare to different substrates, the EFs of CW-B were highest, followed by CW-C, and CW-A had the lowest value. Some studies indicate that nitrate reduction and ferrous oxidation had the potential to interact with each other in anoxic environment when nitrate occurred in the ferric iron reduction zone [43]. In this study, the catalytic biological ceramic in CW-B contained iron and CW-C contained magnetite, so there was a possibility of creating a reducing environment in ferrous iron which made higher N₂O emission fluxes.

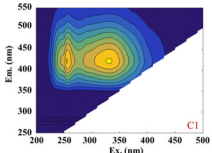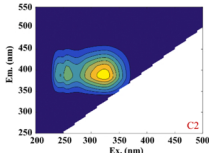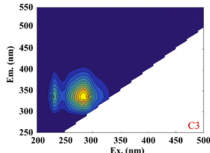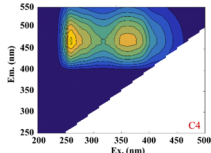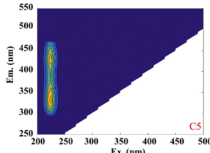### 3.3. Removal and characteristics of DOM

3.3.1. DOC removal

Long-term monitoring the DOC concentration, DOM composition, and maximum fluorescence intensity of influent and effluent in each system was conducted to further evaluate the migration and conversion of organic matter in swine wastewater and explore the reasons for the low nitrogen removal of CWs. DOC concentration was generally used to represent to the content of DOM [45]. Fig. 4 shows the variations of DOC concentrations when CWs purified swine wastewater. Consistent with the COD removal efficiency, the distinctions of the DOC removals were not apparent among the three CWs. CWs could only remove some simple and active organic components, and it was tough to completely decompose organic pollutants in swine wastewater in a short time. Low bioavailability of carbon sources in swine wastewater had restricted the denitrification process [20,24]. Therefore, in practical applications, it is possible to provide appropriate anaerobic condition to facilitate denitrification, or properly extend the hydraulic retention time to decompose refractory organic matter into available carbon sources to solve the problem of low nitrogen removal in CWs treated swine wastewater.
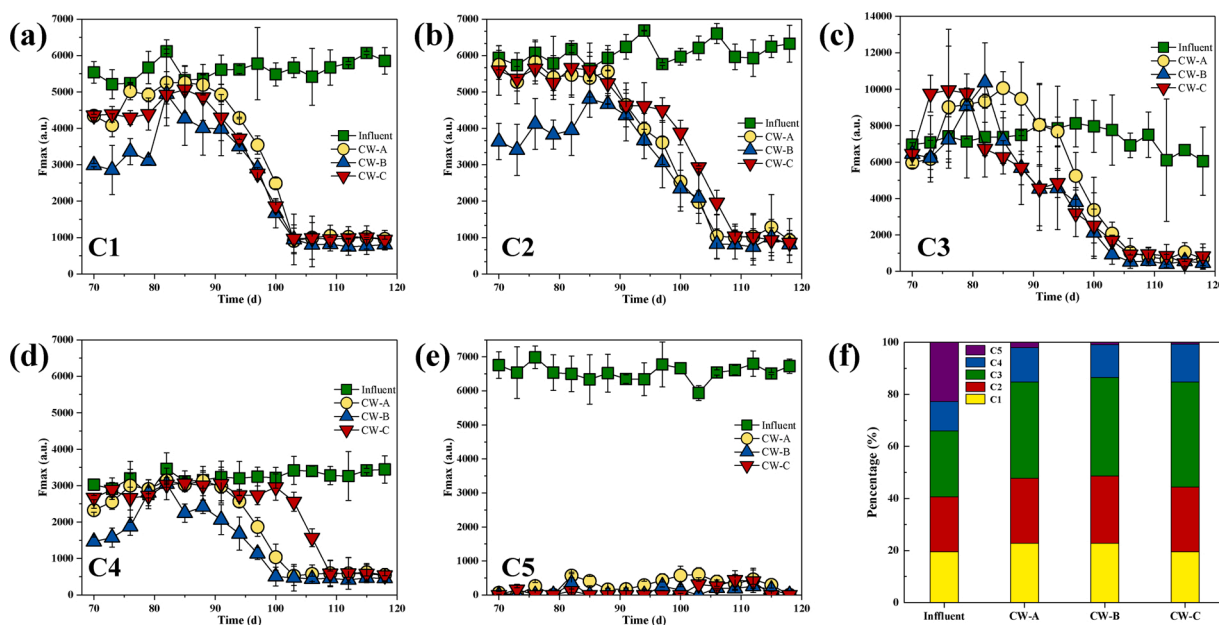
3.3.2. DOM components removal

After the treatment of CWs for swine wastewater, DOM appeared the change of the composition and content. The EEMs locations, representative EEMs, and fluorescence compounds identified by PARAFAC

Table 2
EEMs locations, representative EEMs, and fluorescence compounds identified by PARAFAC analysis in CWs with real swine wastewater.

Components	C1	C2	C3	C4	C5
EEMs location	Ex: 350 (225) nm Em: 457 nm	Ex: 325 nm Em: 390 nm	Ex: 285 nm Em: 334 nm	Ex: 375 (265) nm Em: 460 nm	Ex: 225 nm Em: 418 (346) nm
Fluorescence compounds	UVA Humic-like	UVA Humic-like	Tyrosine-like	UVC Humic-like	Tryptophan-like
EEMs					
Reference	[28,46,47]	[28,46,47]	[48]	[28,46,47]	[44]

Fig. 5. Variations of fluorescence intensity of five components and relative distributions in DOM in different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) with real swine wastewater.

analysis are shown in Table 2. Fig. 5 gives the maximum fluorescence intensities (F_{max}) of five components in water samples. Due to the different fluorescence quantum yields of different substances, the fluorescence intensity could not be used to relate the component concentration directly, but it could be used to conduct a comparative analysis of each component separately in different CWs [40]. Component 1 exhibited its maximum at 350 (225) nm/457 nm (Ex/Em) which was distinguished as UVA humic-like. This component was a common aromatic amino acid with a larger molecular hydrophobic structure [28,46, 47]. It was found in DOM derived from plant and animal manure with an apparent molecular weight greater than 1000 Da [41]. The F_{max} of C1 showed a trend of increasing at the beginning and then decreasing to a relatively low level in all CWs (Fig. 5a). Meanwhile, the F_{max} of C1 in CW-B was the lowest in the initial stage. Component 2 exhibited its maximum at 325/390 nm (Ex/Em). This component was usually found in EEM of UVA humic substance with a smaller molecular size. In previous studies, a similar component was found in wastewater, wetland, and agricultural environment [28,46,47]. For 70–110 days, the F_{max} of C2 decreased with time, from 110 days, the F_{max} of C2 in CW-A and CW-C remained stable (Fig. 5b). However, the F_{max} of C2 in CW-B remained stable in 70–85 days. Due to the better adsorption ability of biological ceramic to macromolecules, C1 and C2 were removal faster in CW-B. The maximum fluorescence intensity of C3 was at 285/334 nm (Ex/Em), which was regarded as a tyrosine like substance [48]. It had

been detected in contaminated natural water and wastewater treatment plants [40] and was usually associated with easy biodegradation substance, like proteins or amino acid. The F_{max} of C3 in all CWs increased in the early stage (0−15d) (Fig. 5c), and it decreased to a low level in the end. Modern farming industries can achieve digestibility and yield by adding amino acids to pig feed, so excessive amino acids caused organic nitrogen pollution [49]. The protein of macromolecules could be decomposed into small size of amino acids by microorganisms which resulted in the F_{max} of tyrosine in all CWs was greater than that of in influent at the early stage. Furthermore, with hydrophilic structure, small molecules of amino acids could be solved easily, some amino acid degraded by aerobic bacteria to CO_2 and H_2O [40]. And as tyrosine substance was decomposed, that affects the weakness of its fluorescence intensity. C4 was composed of two excitation maxima at 265 and 375 nm, with one emission peak centered at 460 nm (Table 2). This component was usually found in EEM of humic substance and was a typical UVC humic-like with large molecular weight. It has been previously observed in both wetlands and forests [28,46,47]. Also, C4 was likely to be associated with microbial origins because the peak was related to soluble microbial byproduct-like materials. The F_{max} of C4 presented a decreasing trend which indicated the degradation of it in CWs (Fig. 5d). However, the difference was that the F_{max} of CW-A and CW-B decreased to a relatively low level after 100 days, while F_{max} of CW-C tended to be stable after 110 days. It was reported that the charge
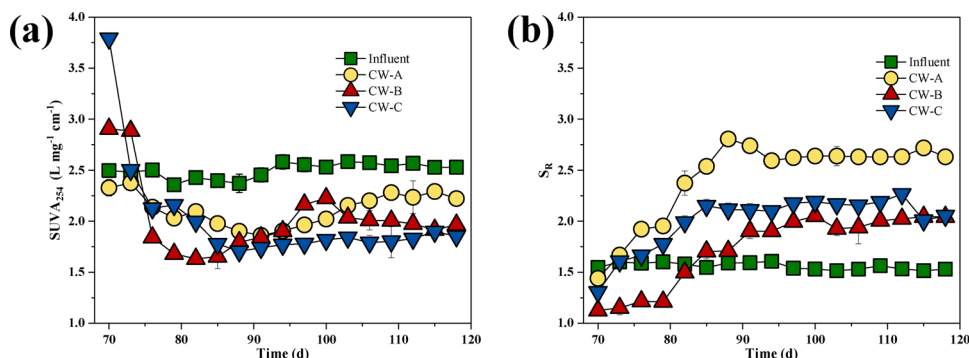
Fig. 6. Variations of SUVA$_{254}$ (a) and S$_R$ (b) in different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) with real swine wastewater.

Table 3
Fluorescence indices of the influent and effluent in different CWs (CW-A: control CW, CW-B: CW with biological ceramsite, CW-C: CW with magnetite) with real swine wastewater (Mean ± SD).

Parameters	Influent	CW-A	CW-B	CW-C
FI	2.07 ± 0.02	2.05 ± 0.01	2.11 ± 0.03	2.02 ± 0.02
HIX	2.56 ± 0.04	2.76 ± 0.57	2.63 ± 0.12	2.98 ± 0.30
β:α	0.86 ± 0.02	0.86 ± 0.03	0.93 ± 0.04	0.87 ± 0.03
BIX	0.92 ± 0.03	0.92 ± 0.03	1.00 ± 0.05	0.96 ± 0.16

of pure magnetite changed from positive to negative at pH near to 8, which was the isoelectric point of magnetite, large aggregates could form near this pH. At this time, the stable solution would be far away from magnetite [50], so the UVC humic-like in the swine wastewater was difficult to be absorbed by magnetite. C5 was centered at a maxima Ex/Em wavelength pair of 225 nm/418 (346) nm identified as a tryptophan-like substance [51]. The F$_{max}$ of C5 (Fig. 5e) in CWs indicated that tryptophan-like substance was easy to degrade. It had also been reported that the small molecules of amino acids might bind to the humic acid of large molecules, so it might cause the reduction of C5 in CWs.

The relative proportion of the maximum fluorescence intensities of the five components in CW-A, CW-B, CW-C and influent are exposed in Fig. 5f. Overall, the relative proportion of humic-like substance (C1, C2, and C4) did not change significantly, while the relative proportion of amino acid substance (C3 and C5) varied greatly. The relative proportion of C3 increased from 27.7 % in influent to 37.0 %, 37.9 %, and 40.4 % respectively for CW-A, CW-B, and CW-C. Also, the relative proportion of tryptophan-like substance (C5) declined from 22.8 % in influent to 2.1 %, 0.8 %, and 0.6 %, respectively for CW-A, CW-B, and CW-C. This was because that the by-products could produce by decomposition of macromolecular DOM, the other was microorganisms in the metabolic process would produce extracellular polymers including polysaccharide, the protein compound, a third might be the transformation of tryptophan-like substance. This also confirmed that due to its high bioavailability, small-molecule amino acid substances reduced the content of organic matter in the effluent of CWs. At the same time, the relative proportion of humic acid substances remains stable, which resulted in the decrease of COD removal and the low heterotrophic denitrification in CWs for treating swine wastewater.

3.3.3. Dynamics in DOM quality indexes

SUVA$_{254}$ and S$_R$, as the UV–vis spectral parameters, can be used as indicators for the further evaluation of DOM components (Fig. 6). SUVA$_{254}$ has been widely accepted as a surrogate for probing the degradation rate of DOM [48]. The value of SUVA $_{254}$ in CWs showed a decreasing and subsequently stabilizing trend, and it was lower than the influent. The change of SUVA$_{254}$ suggested that the chromophores in

DOM, which mostly consist of high molecular size aromatic rings, might be broken down into smaller size non-aromatic rings [52]. The value of S$_R$ showed the opposite trend. The results of UV–vis spectra were consistent with the F$_{max}$ of DOM components.

Besides, some optical indexes, including FI, HIX, β: α, and BIX, can reflect the origin and quality of DOM (Table 3). The FI values of influent and effluent were around 2. According to the previous study [53], it can be inferred that the DOM derived from extracellular release and leachate from bacteria and algae. HIX indicated the humic substance content or extent of humification [54]. Overall, the values of all CWs were higher than that of influent which meant the humification of swine wastewater was increased after the treatment of CWs.

4. Conclusion

The discharge of swine wastewater has posted a serious threat to water bodies in rural areas and been receiving increasing attention worldwide. This study investigated the treatment performances of synthetic wastewater and real swine wastewater in subsurface flow constructed wetland with different substrates (gravel, ceramsite and magnetite).The removal performances in CWs were better for treating synthetic wastewater than that for swine wastewater. Magnetite-based CW was efficient for organic matter removal with chemical oxygen demand (COD) removal for 53.0–91.5 %. Ceramsite-based CW was effective in removing nutrients with 11.0–63.2 % for total nitrogen (TN) removal and 93.2–95.5 % for total phosphorus (TP) removal. The relative proportions of DOM components (mainly humic-like substances and amino acid substances) in swine wastewater, the relative content of these components both decreased after the treatment of CWs, but the proportion of tyrosine like substance increased. The humic substance of low bioavailability resulted in the decrease of COD removal and the low heterotrophic denitrification in CWs for treating swine wastewater.

CRediT authorship contribution statement

Lu Dong: Investigation, Writing - original draft. **Zhiping Qi:** Investigation, Writing - original draft. **Mengqi Li:** Investigation, Writing - original draft. **Yan Zhang:** Data curation. **Yingrun Chen:** Formal analysis, Investigation. **Yuanfeng Qi:** Formal analysis, Investigation. **Haiming Wu:** Conceptualization, Supervision, Funding acquisition.

Declaration of Competing Interest

This manuscript has not been published before nor submitted to another journal for the consideration of publication. The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jece.2020.104739.

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