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Effects of duckweed (*Spriodela polyrrhiza*) remediation on the composition of dissolved organic matter in effluent of scale pig farms

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ABSTRACT

The swine effluent studied was collected from scale pig farms, located in Yujiang County of Jiangxi Province, China, and duckweed (*Spriodela polyrrhiza*) was selected to dispose the effluent. The purpose of this study was to elucidate the effects of duckweed growth on the dissolved organic matter composition in swine effluent. Throughout the experiment period, the concentrations of organic matter were determined regularly, and the excitation–emission matrix (3DEEM) spectroscopy was used to characterize the fluorescence component. Compared with no-duckweed treatments (controls), the specific ultra-violet absorbance at 254 nm ($SUVA_{254}$) was increased by a final average of 34.4% as the phytoremediation using duckweed, and the removal rate of DOC was increased by a final average of 28.0%. In swine effluent, four fluorescence components were identified, including two protein-like (tryptophan, tyrosine) and two humic-like (fulvic acids, humic acids) components. For all treatments, the concentrations of protein-like components decreased by a final average of 69.0%. As the growth of duckweed, the concentrations of humic-like components were increased by a final average of 123.5% than controls. Significant and positive correlations were observed between $SUVA_{254}$ and humic-like components. Compared with the controls, the humification index (HIX) increased by a final average of 9.0% for duckweed treatments. Meanwhile, the duckweed growth leaded to a lower biological index (BIX) and a higher proportion of microbial-derived fulvic acids than controls. In conclusion, the duckweed remediation not only enhanced the removal rate of organic matter in swine effluent, but also increased the percent of humic substances.

Introduction

With the rapid development of pig industry, large amount of swine effluent discharges in a centralized place and causes great environmental contaminations as a high level of organic loading presented in sewage generally. Thus, it is very urgent and necessary to seek an effective method to resolve the intractable problem. As an eco-friendly and cost-effective method (Olguín et al., 2007; Muradov et al., 2014), phytoremediation has been used widely to renovate

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swine effluent due to the energy-saving and non-secondary pollution properties.

In general, ammonia nitrogen (NH_4^+-N) is the primary nitrogen form in swine effluent. As the prefer nitrogen source of duckweed growth, duckweed (*Spirodela polyrrhiza*) could endure in a medium with the concentration of NH_4^+-N as 240 mg/L (Cheng et al., 2002). As usual, duckweed has shown a high removal and recovery rate of nitrogen and phosphorus in swine effluent (Cheng and Stomp, 2009; Zhao et al., 2015), and the average removal rate of total nitrogen (TN) and total phosphorus (TP) is up to 96% and 89% (Mohedano et al., 2012). Meanwhile, Xu and Shen (2011) clarified that keeping the coverage of duckweed at 60% of water surface could achieve a higher removal rate of nutrient and a more duckweed biomass.

In aquatic systems, the refractory aromatic compounds could be degraded effectively as the synergistic effect of duckweed and microorganisms (Kristani et al., 2012), and the starch content of duckweed would also be enhanced to the level of grain coexisted with plant growth promoting bacteria (Appenroth et al., 2015). Cheng and Stomp (2009) studied that the content of starch combined in duckweed (*S. polyrrhiza*) was 45.8% biomass (dry weight basis) by culturing in anaerobic digested swine effluent. Thus, the application of duckweed is conducive to facilitate the circulation and transformation of organic matter in wastewater. At present, many studies concerning the duckweed remediation of swine effluent have been carried out, but mainly focus on the organic removal and recovery.

Dissolved organic matter (DOM) accounts the major proportion of nutrient in swine effluent (Leenheer and Rostad, 2004). To date, the environmental importance of DOM has been studied widely, including a key component in the biogeochemistry cycles of nutrients (Burrows et al., 2013; Li et al., 2016), a crucial factor for aquatic optical properties (Zhou et al., 2015), the ligand for binding metals (Shoji, 2008), and precursors of disinfection byproducts (Ritson et al., 2014). Therefore, it would provide a comprehensive understanding on the potential environmental risks of swine effluent by analyzing the DOM composition. Yet, the knowledge about the effects of phytoremediation on DOM composition is still limited now. In the present study, the duckweed (*S. polyrrhiza*) was introduced to treat swine effluent, and the purpose of this study was to elucidate the effects of duckweed growth on the DOM composition.

1. Materials and methods

1.1. Experimental design

In the present study, two scale pig farms were selected for study, namely Zhong-tong (ZT) and Wan-gu (WG) with an annual pig slaughter of 12,000 and 20,000 heads, respectively, located in Yujiang County (116°41′E–117°09′E, 28°04′N–28°37′N) of Jiangxi Province, China. In January 2015, a total of 100 L newly-generated swine effluent was collected from the sewage outlet for each pig farm. According to previous investigation, a mean of 2.0 kg solid manure, 3.3 kg urine, and 8.0 kg wastewater is produced by a fattening pig per day (Zhou et al., 2013).

A pot experiment was carried out in an artificial greenhouse, sited in the Ecological Experimental Station of Red Soil Academia Sinica. Duckweed (*S. polyrrhiza*) was collected from the paddy field in the Taihu Lake region of China, and sufficient duckweed fronds were achieved using a modified Steinberg nutrient in lab (Lu et al., 2014). After the thoroughly mixing, all the effluent was divided into several aliquots (10 L) hold with 15 L pre-rinsed buckets for each farm. The same number of *S. polyrrhiza* (20 fronds, approximately 236 mg) was threw in the swine effluent from WG and ZT farm, and defined as D(W) and D(Z) treatment, respectively. Meanwhile, these treatments were treated as the controls without duckweed, and defined as ND(W) and ND(Z) treatment, respectively. These capital cases (W and Z) represented the WG and ZT farm, respectively, and each treatment was triplicate.

Prior to experiment, a diluted swine effluent (5%, *V/V*) was applied to the domestication of duckweeds for a week. Then, the domesticated duckweed was used to treat the original effluent. The experiment was conducted for four and a half months, and the effluent samples (50 mL) were collected at 0, 15, 45, 75, 105, and 135 days, respectively. The amount of water evaporation was supplemented with tap water for twice a week, and the duckweed was not harvested until the end of experiment. The concentrations of organic matter in the original effluent examined were summarized in Table 1.

1.2. Duckweed biomass and nutrient content

At the end of experiment, all duckweeds were harvested and determined the biomass after water drained off. Then, the fresh duckweeds were dried to a constant weight at 70°C, and determined the moisture content and dry weight (DW, g).

Table 1 – Concentration of organic matter in the original swine effluent.

Treatment	NH_4^+-N (mg/L)	DOC (mg/L)	COD (mg/L)	TDN (mg/L)	TDP (mg/L)
ND(W)	81.8 ± 4.2 a	130.1 ± 29.8 bc	350.2 ± 63.7 b	137.9 ± 36.1 ab	40.5 ± 2.4 a
D(W)	71.7 ± 2.8 b	107.0 ± 1.8 c	307.8 ± 18.4 b	125.0 ± 38.4 b	35.3 ± 1.8 b
ND(Z)	86.9 ± 5.9 a	249.3 ± 25.2 a	557.2 ± 112.6 a	184.8 ± 3.9 a	28.3 ± 2.3 c
D(Z)	81.8 ± 4.2 a	160.0 ± 19.0 bc	626.2 ± 66.3 a	126.9 ± 11.0 b	31.9 ± 2.8 bc

Standard deviation was measured with three replications. Different lowercases within the same column indicated significant at the level of $p < 0.05$. COD: chemical oxygen demand; TDN: total dissolved nitrogen, TDP: total dissolved phosphorus.

According to Eq. (1), the relative growth rate (RGR, g/(m^2·day)) was calculated for each duckweed treatment. Five-gram dried duckweed was grinded (30–50 mesh), and analyzed the content of total nitrogen (TN) and total phosphorus (TP). The content of crude protein (CP) was calculated according to Eq. (2), and a recommended factor ε (6.25) was applied in the study (Mohedano et al., 2012).

$$RGR = (DW_t – DW_0)/m \cdot t \quad (1)$$

where, m (m^2) is the area of bucket, and t (day) is experimental period.

$$CP = TN \cdot \varepsilon \quad (2)$$

where, CP (g crude protein/100 g dry matter) is the content of crude protein, TN (g total nitrogen/100 g dry matter) is the content of total nitrogen, and ε is the factor.

1.3. *Fluorescence measurement and parallel factor analysis (PARAFAC)*

Prior to fluorescence measurement, all effluent samples were filtered through 0.45 μm filters (Millipore, USA), and the filtrates were stored in pre-combusted (550°C, 6 hr) amber bottles. The fluorescence measurement was completed using a fluorescence spectrometer (Hitachi *F*-7000, Japan) with a 700-voltage xenon lamp. The excitation (Ex.)/emission (Em.) scanning ranges used was 200–450 nm and 250–600 nm, respectively, and readings were collected at 5-nm intervals for excitation and 1-nm intervals for emission using a scanning speed of 2400 nm/min. The band-passes of both excitation and emission were set 5 nm.

A Milli-Q water blank was subtracted to eliminate the water Raman scatter peaks from the excitation–emission matrices (EEMs). According to Wada et al. (2007), the fluorescence intensity was calibrated in quinine sulfate units (QSUs). Rayleigh scatter effects were removed by deleting all emission readings in the two regions (Em. wavelength ≤ Ex. wavelength + 5 nm, and ≥Ex. wavelength + 300 nm), and replaced with zeroes (Zhang et al., 2010). Without any assumptions on the number or the spectral shape of fluorophores, the complex mixture of DOM had been decomposed into individual fractions with PARAFAC model (Zhang et al., 2010). Based on the studies of Stedmon and Bro (2008), PARAFAC analysis was completed using MATLAB (R2009a, USA), with a split-half analysis employed to validate the identified components.

In brief, PARAFAC analysis decoded the data matrix into a set of trilinear terms and a residual array, and the modeling process terminated by minimizing the sum of square residuals (Stedmon et al., 2003). Meanwhile, the split-half analysis involved dividing the complete data set into two groups randomly, and then making a PARAFAC model for both groups independently. If the loadings from both models were the same, the number of components was validated as the uniqueness of PARAFAC model (Zhang et al., 2010).

1.4. *Fluorescence index*

Initially, the humification index (HIX) was introduced by Zsolnay et al. (1999), and used to estimate the maturation degree of DOM in soil. The value of HIX was in the range of 0–1, increasing with the aromatic degree of DOM generally (Huguet et al., 2009). In the present study, HIX was defined as the ratio of the fluorescence intensities from 435 to 480 nm ($\Sigma FI_{435–480\ nm}$) to the sum of ($\Sigma FI_{300–345\ nm}$) and ($\Sigma FI_{435–480\ nm}$), both excited in 255 nm, as 5-nm intervals was used in the fluorescence measurement (Ohno, 2002; Zhang et al., 2010).

$$HIX = \Sigma FI_{435–480\ nm}/(\Sigma FI_{300–345\ nm} + \Sigma FI_{435–480\ nm}), \quad (3)$$
$$\lambda_{Ex} = 255\ nm$$

The biological index (BIX) was used widely to indicate the freshness degree of DOM, which was correlated closely to autochthonous biological activities (Huguet et al., 2009). In general, BIX was defined as the ratio between the fluorescence intensity at 380 nm and the maximum fluorescence intensities at the range of 420–435 nm, both excited in 310 nm. High values of BIX (>1) corresponded to predominantly biological origin of DOM, and low values (0.6–0.7) corresponded to lower autochthonous production (Zhang et al., 2010).

$$BIX = FI_{380\ nm}/FI_{max(420–435\ nm)}, \lambda_{Ex} = 310\ nm \quad (4)$$

Fluorescence index (FI_{370}) was often applied to distinguish the sources of isolated fulvic acids in aquatic systems, and defined as the ratio of the fluorescence intensity at 450 nm (FI_{450}) to the fluorescence intensity at 500 nm (FI_{500}), both excited at 370 nm (McKnight et al., 2001). High values of FI_{370} (≥1.9) corresponded to the microbial derived fulvic acids (FAs), and low values (≤1.4) for terrestrial-derived FA (Zhang et al., 2010).

$$FI_{370} = FI_{450\ nm}/FI_{500\ nm}, \lambda_{Ex} = 370\ nm \quad (5)$$

1.5. *Chemical measurement and statistical analyses*

The contents of TN and TP in duckweed were determined using alkaline potassium persulfate oxidation-UV spectrophotometric method and vanadium molybdate yellow colorimetric method, respectively, after H_2SO_4–H_2O_2 digestion (Zhu et al., 2011). The pH and electrical conductivity (EC) in swine effluent were measured using a pH meter and EC meter (Mettler Toledo, USA), respectively. The concentration of chemical oxygen demand (COD), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) in swine effluent were analyzed according to standard methods (Clesceri et al., 1998). Ultra-violet absorbance at 254 nm (UV_{254}) was measured using UV–visible spectrophotometer (Eppendorf, Germany), and dissolved organic carbon (DOC) was determined using total organic carbon (TOC) analyzer (Multi N/C 3100, Germany). The concentration of NH_4^+-N was measured using a flow analyzer (BRAN + LUEBBE, Germany). Difference of environmental parameters over experiment days was evaluated with t-test, with a p-value of 0.05 used to determine significance. Regression analyses were applied to examine the correlations between variables using a p-value of 0.05.

2. Results and discussion

2.1. *Duckweed biomass and the contents of TN and TP*

In the study, the moisture contents of duckweed in D(W) and D(Z) treatments were 89% and 90.4%, respectively, and the RGR of dry

Table 2 – Duckweed biomass accumulation and nutrient contents.

Treatment	Moisture content (%)	RGR (g/(m^2·day), DW)	TN (g/kg, DW)	TP (g/kg, DW)
D(W)	89.0 ± 0.0 a	1.91 ± 0.24 a	29.3 ± 0.7 b	15.3 ± 0.8 a
D(Z)	90.4 ± 0.4 a	1.89 ± 0.19 a	33.2 ± 2.1 a	15.1 ± 1.0 a

RGR: relative growth rate; DW: dry weight; TN: total nitrogen; TP: total phosphorus.
Different lowercases within the same column indicated significant at the level of $p < 0.05$. Standard errors were measured with three replicates.

weights (DW) were (1.91 ± 0.24) and (1.89 ± 0.19) g/(m^2·day), respectively. The TN contents of duckweed in D(W) and D(Z) treatments were (29.3 ± 0.7) and (33.2 ± 2.1) g/kg, respectively, and TP contents were (15.3 ± 0.8) and (15.1 ± 1.0) g/kg, respectively (Table 2). According to Eq. (2), the contents of crude protein (CP) calculated in D(W) and D(Z) treatments were 18.3% and 20.8%, respectively.

Compared with other macrophytes (e.g., *Pistia stratiotes*), duckweed had higher moisture contents generally, which were positively linked with the ambient temperature (Zhao et al., 2015). To some degree, the lower percent of lignin in cell wall might account for the higher moisture content of duckweed. The accumulation of duckweed biomass in our study was significant lower than the previous study (Edwards et al., 1992), which might be linked with a high density of duckweed. In our study, non-harvest of duckweed was carried out until the end of experiment, and a high density would limit the accumulation of duckweed biomass generally.

In general, the content of protein in duckweed ranged from 16% to 41.7% (Verma and Suthar, 2015), which correlated to the N concentration in waters (Mohedano et al., 2012). A significant difference of TN content was found between D(W) and D(Z) treatments ($p < 0.01$, *t*-test), and the D(Z) treatment had a higher TN and CP content than D(W) due to the higher concentration of NH_4^+-N of the former. As the preferred nitrogen source for duckweed growth, a high level of NH_4^+-N concentration was conducive to the accumulation of crude protein (CP) in duckweeds (Mohedano et al., 2012; Zhao et al., 2015). In addition, a high TP content of duckweed was observed in our study as similar to Zhao et al. (2015), although no significant difference of TP content existed between D(W) and D(Z) treatments. Thus, the nutrient content of duckweed links significantly with the initial N and P concentrations in medium (Cheng et al., 2002).

2.2. Effects of duckweed growth on the environmental parameters in swine effluent

Initially, the pH of WG and ZT farm was (8.35 ± 0.18) and (7.98 ± 0.08), and EC was (0.97 ± 0.02) and (1.15 ± 0.02) mS/cm, respectively (Fig. 1a, b). The specific UV absorbance at 254 ($SUVA_{254}$) was defined as the ratio of UV_{254} to the DOC concentration, and the mean of $SUVA_{254}$ in duckweed treatments (1.72 ± 0.09) was higher than controls (1.28 ± 0.06)

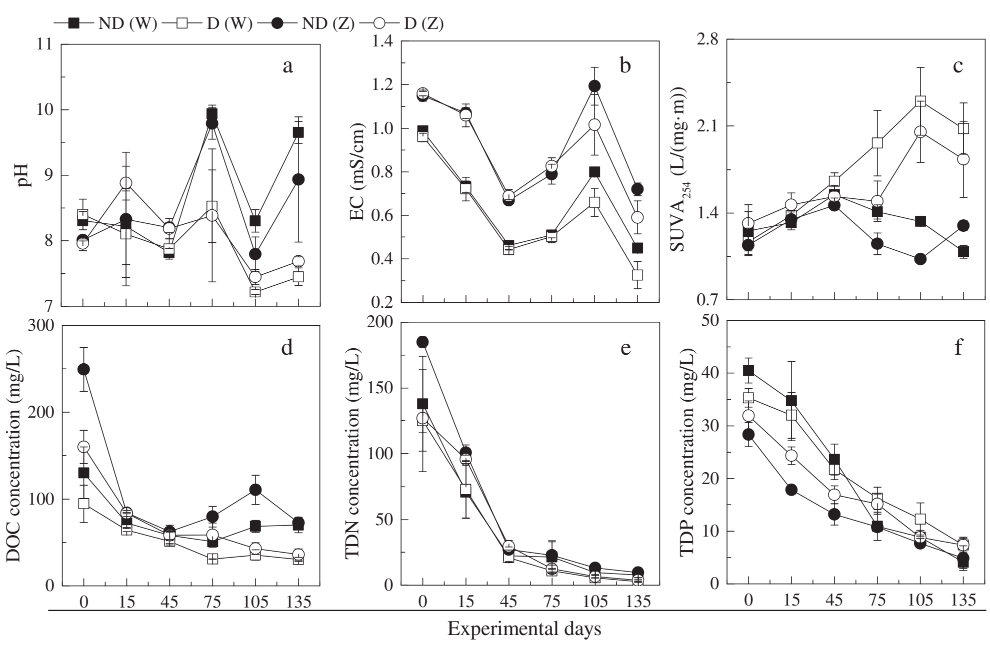

Fig. 1 – Variations of environmental parameters in swine effluent. EC: electrical conductivity, $SUVA_{254}$: the ratio of ultra-violet absorbance at 254 nm to DOC concentration, DOC: dissolved organic carbon, TDN: total dissolved nitrogen, ND: no-duckweed treatment, D: duckweed treatment, W: WG farm, Z: ZT farm. Standard errors were measured with three replicates.

(Fig. 1c, $p < 0.01$). By the end of experiment, the DOC concentration in swine effluents of ND(W), D(W), ND(Z), and D(Z) treatments deceased by a mean of 46.3%, 67.9%, 70.9%, and 77.5%, respectively (Fig. 1d), and these corresponding values of TDN were 94.5%, 97.8%, 94.8%, and 97.1%, respectively (Fig. 1e). Meanwhile, the removal rates of TDP in ND(W), D(W), ND(Z), and D(Z) treatments were 89.9%, 79.2%, 82.9, and 77.0%, respectively (Fig. 1f). Except for TDP, the removal rate of both DOC and TDN was enhanced obviously as the presence of duckweed compared with controls.

The swine effluent was often characterized by alkalescence and high conductivity as a high content of organic matter (Fridrich et al., 2014). In general, the oxygen produced by photosynthesis would be transported into wastewater close to duckweed roots, and a local oxygen-rich habitat favored the formation of nitrate, resulting in a low pH value (Zhou et al., 2015). Compared with controls, duckweed growth leaded to a significant reduction of pH and EC by a mean of 18.6% and 21.7%, respectively ($p < 0.01$, *t*-test), eventually. The index of $SUVA_{254}$ was often used as an indicator for the chemical composition of DOC, and high values of $SUVA_{254}$ corresponded to DOC with a high aromatic degree (Weishaar et al., 2003).

In aquatic systems, DOC often played an important role in the biogeochemistry cycling of nutrients. Through the entire experiment, the DOC concentration decreased obviously. Compared with controls, the removal of DOC was enhanced by a final average of 28.0% as the duckweed growth, which might be linked with the biomass accumulation and starch production (Cheng and Stomp, 2009). To some degree, a more suitable habitat could be constructed due to the presence of duckweed, which might favor a high level of microbial activities and contribute to the further decomposition of organic matter in swine effluent. For instance, the solar UV could be reflected as the shelter by *S. polyrrhiza*, and the content of dissolved oxygen would be increased in microsite level. Meanwhile, after 45 days of experiment, a slight increase of DOC was found for controls. In general, the photochemical degradation leaded to the decomposition of refractory DOM (Whitehead et al., 2000), which was contributed to the increase of DOC. Meanwhile, a high growth rate of duckweed biomass could be achieved as a warmer temperature, which was conducive to the further reduction of organic matter in swine effluent.

The high removal rate of TDN might be linked with the high percent of NH_4^+-N in swine effluent. Zhou et al. (2010) studied that the nitrogen removal might be mostly depended on the microbial activities or associated chemical reactions. The ammonium removal was limited (18%) due to the uptake by duckweed plants, and more than 82% of nitrogen was influenced by other factors (Steen et al., 1998). In addition, the phosphorus removal was linked closely with the sedimentation or biological absorption, and the plant uptake was usually low in wetlands (Vymazal, 2007). In the present study, an approximate linear reduction of TDP was observed for all treatments, and decreased by a final mean of 82.2%, with a lower TDP concentration found in controls eventually. El Halouani et al. (1993) studied that the removal rate of P in wastewater by the process of precipitation as Ca–P was 75% when pH was over 7.6. As aforementioned, the values of pH in controls were higher than duckweed treatments, which might be contributed to the higher TDP removal rate of the former.

2.3. PARAFAC modeling of fluorescence components in swine effluent

The two common fluorescence components found in aquatic systems were protein fractions and humic materials (Coble, 1996). In this study, four fluorescence components were identified in swine effluent using three-dimensional excitation–emission matrix (3DEEM) spectroscopy and PARAFAC. Fig. 2 shows the EEM spectra and loading of each component. Contour plots presented spectral shapes of excitation and emission of derived components (Fig. 2a–d), and line plots represented the split-half validation for each identified component (Fig. 2e–h).

Component 1 (C1) presented two fluorescence peaks, with the major and minor peaks at excitation/emission wavelength of 225/350 nm and 275/350 nm, respectively (Fig. 2a, e). The spectral features were identical to the tryptophan-like fluorescence reported by Coble (1996), and the primary sources were both autochthonous products and bio-available substrates (Yamashita and Tanoue, 2003; Zhang et al., 2010). Component 2 (C2) also showed two fluorescent peaks, with the major and minor peaks located at 200/300 and 275/300 nm, respectively (Fig. 2b, f), which were similar to the previously reported tyrosine-like fluorescence in various aquatic systems (Stedmon and Bro, 2005; Hudson et al., 2007). Component 3 (C3) displayed two peaks, located at 225/405 and 315/405 nm, respectively (Fig. 2c, g). These fluorescence characteristics resembled the fulvic-like C2 observed by Zhang et al. (2010), which also existed prevalently in polluted waters (Guo et al., 2012; Zhou et al., 2015). Component 4 (C4) presented peaks at Ex/Em wavelength of 260/465 nm and 350/465 nm, respectively (Fig. 2d, h). These peaks were similar to the terrestrial-derived humic-like acid found in brown-water streams (Fasching et al., 2014), and also presented widely in wastewater (Stedmon and Bro, 2005).

2.4. Effects of duckweed growth on the relative concentration of DOM component

The maximum fluorescence intensities (FI_{max}) of DOM components were assumed to be proportional to its real concentration (Guo et al., 2012). According to the study of Baker (2002), the ratio of FI_{max} against DOC concentration was applied to represent the relative concentration of DOM in our study. Fig. 3 shows the concentration (FI_{max}/DOC) of individual component in swine effluent. More detail information concerning the values of FI_{max} was shown as Fig. S1. The mean concentrations of C1, C2, C3, and C4 in the original swine effluent of WG farm were 1.74 ± 0.03, 4.35 ± 0.18, 0.72 ± 0.09, and 0.33 ± 0.02, respectively; these corresponding values of ZT farm were 1.96 ± 0.29, 1.72 ± 0.13, 0.57 ± 0.04, and 0.35 ± 0.02, respectively. Thus, the protein-like components were the primary fluorophores in swine effluent initially.

For all treatments, the concentrations of C1 and C2 significantly decreased by a final average of 74.1% and 63.9% respectively, indicating the biodegradation of organic matter in swine effluent. A positive correlation was observed between C1 and C2 ($p < 0.001$, data not shown), and both of which were related to the concentration of amino acids (Yamashita and Tanoue, 2003). In general, the tryptophan-like C1 had a more pronounced peak than tyrosine-like C2, owing to a higher

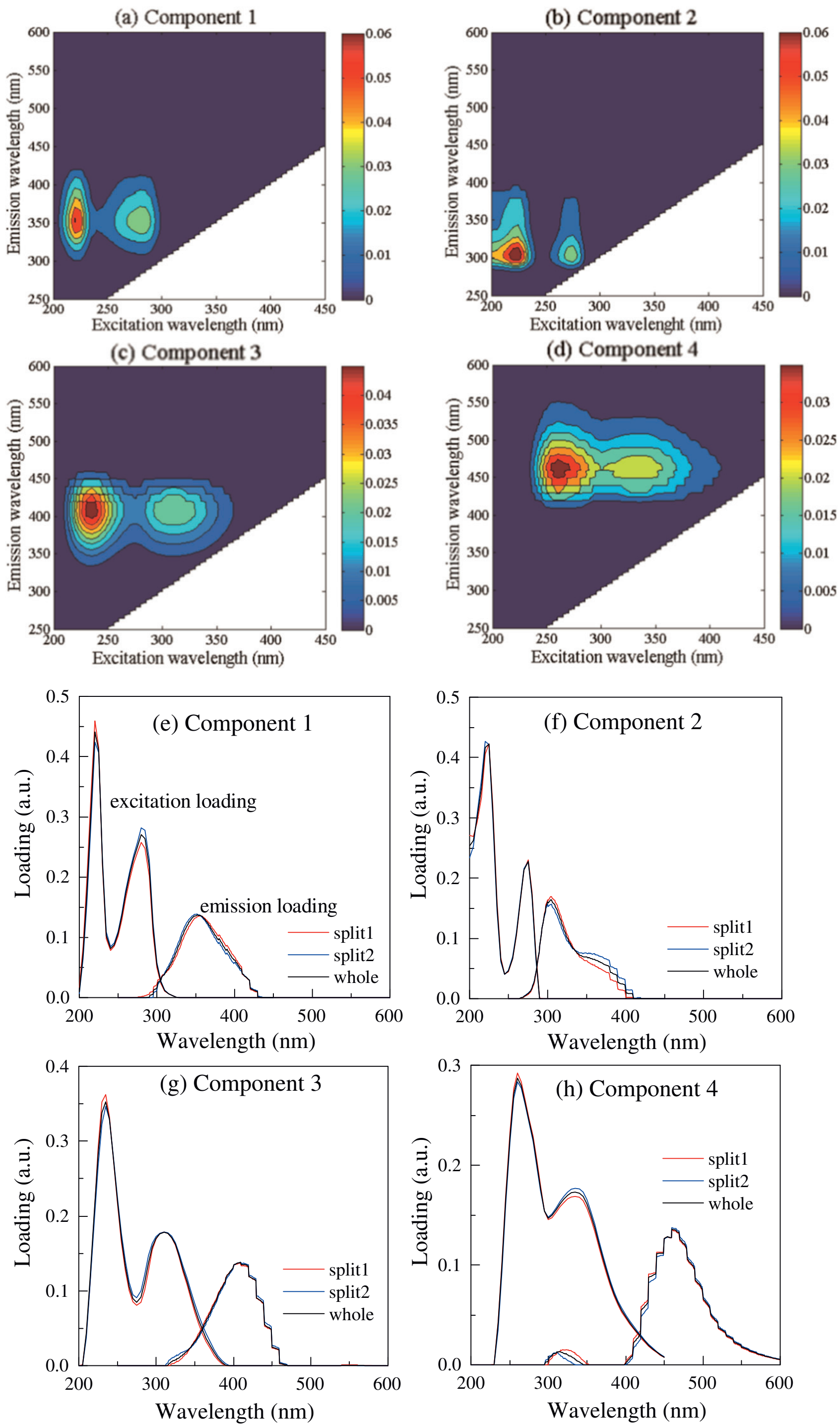

Fig. 2 – PARAFAC model output showing fluorescence signatures of four components identified in swine effluent. Contour plots present spectral shapes of excitation and emission of individual component (a–d). Line plots represent the split-half validation for each identified component (e–h). Excitation (left) and emission (right) loading spectra were estimated from two random halves of data set (Split1-red lines, Split2-blue lines) and the complete data set (black lines). Component 1: tryptophan-like component, Component 2: tyrosine-like component, Component 3: fulvic-like component, Component 4: humic-like component. PARAFAC: parallel factor analysis.

quantum yield of the former (Determann et al., 1998). Compared with controls, a high concentration of C1 and C2 was observed for duckweed treatments eventually, which might be linked with the biodegradation of duckweed residuals. A kinetics study showed that duckweed had a high-affinity transporter for the uptake of amino acids (Borstlap et al., 1986), and the duckweed growth led to the accumulation of free amino-acids and humic substances in aquatic systems (Thomas and Eaton, 1996).

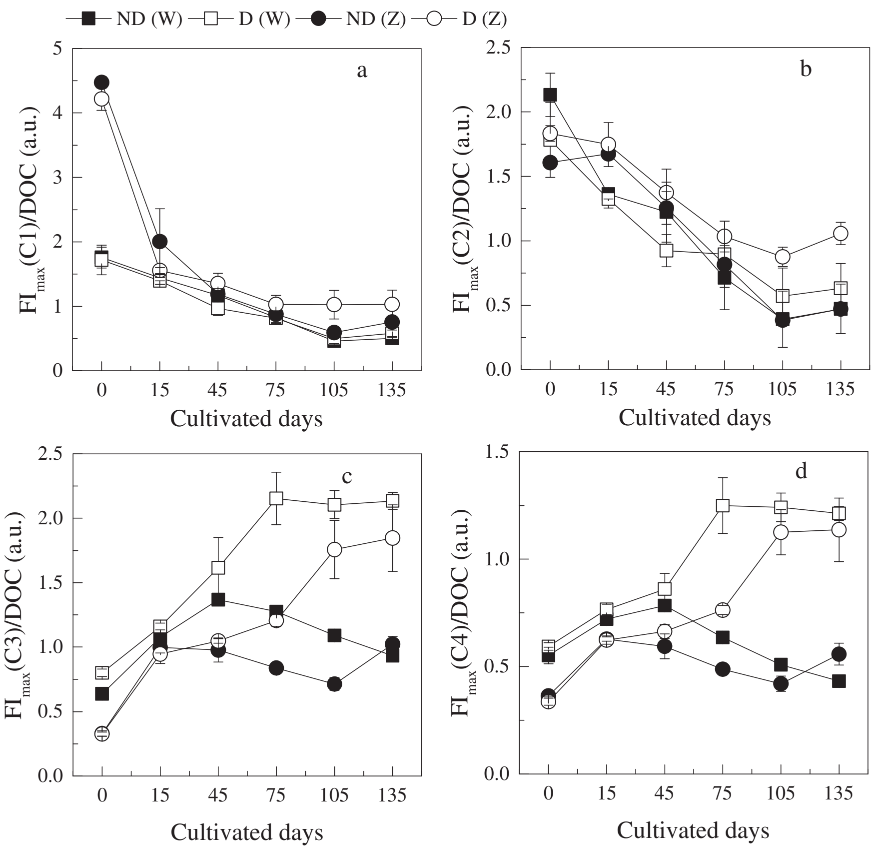

Fig. 3 – Variations of the concentration of DOM components identified in swine effluent, C1: tryptophan-like component, C2: tyrosine-like component, C3: fulvic-like component, C4: humic-like component. Standard errors were measured with three replications.

Although the surplus biomass of duckweed would lead to the release of DOC as the decomposition of duckweed residual, and the removal rate of DOC in effluent was enhanced as the phytoremediation using duckweed in our study.

Compared with controls, the concentrations of C3 and C4 in D(W) and D(Z) treatments increased by a final average of 104.7% and 142.3%, respectively. As usual, the humic compounds showed more resistant to microbial degradation than proteinous materials. Zhou et al. (2015) studied that the FI_{max} of protein-like components increased first and then decreased rapidly as the microbial degradation of organic matter, whereas the humic-likes increased gradually. As same with the protein-like components, a positive relationship was observed between C3 and C4 ($p < 0.001$, data not shown), indicating a similar variation or common source. In general, the fulvic-like C3 had higher fluorescence intensities than humic-like C4 as the lower molecular weight (MW) and aromatic degree of the former (Hudson et al., 2007). In aquatic systems, humic substances (HS) were produced either by biochemical degradation of plant residues or by polycondensation of relatively small organic compounds as microbial activities (Yamashita and Tanoue, 2003; Xiao et al., 2011). With respect to ND(W) or ND(Z) treatment, photochemical degradation might account for the further degradation of HS as the absence of duckweed coverage.

Two verified hypotheses had been advanced to elucidate the accumulation of HS as the duckweed growth. First, HS might be produced by degradation of biopolymers or by condensation of low MW compounds released from dying fronds; second, the HS were generated by healthy duckweeds to improve the biological fitness of the habitat (Thomas, 1990; Shoji, 2008). Shoji (2008) studied that a high percent of humic material could weaken the toxicity of several metals as the production of organ-metal complexes. Significant and positive correlations were found between $SUVA_{254}$ and humic-like components (Fig. 4), indicating that the high proportions of humic-like components weakened the DOC reactivity. In aquatic systems, the DOM with a high aromatic degree was more difficult to be consumed by microorganisms (Weishaar et al., 2003; Helms et al., 2008). Thus, the duckweed treatment is conducive to strengthen the percent of humic-like components and reduce the reactivity of DOM in swine effluent.

2.5. *Effects of duckweed growth on the values of fluorescence index*

Throughout the experiment period, the humification index (HIX) in swine effluents of ND(W), D(W), ND(Z), and D(Z) treatments ranged from 0.47–0.72, 0.51–0.83, 0.22–0.67, and 0.23–0.69, respectively (Fig. 5a). A significant difference was

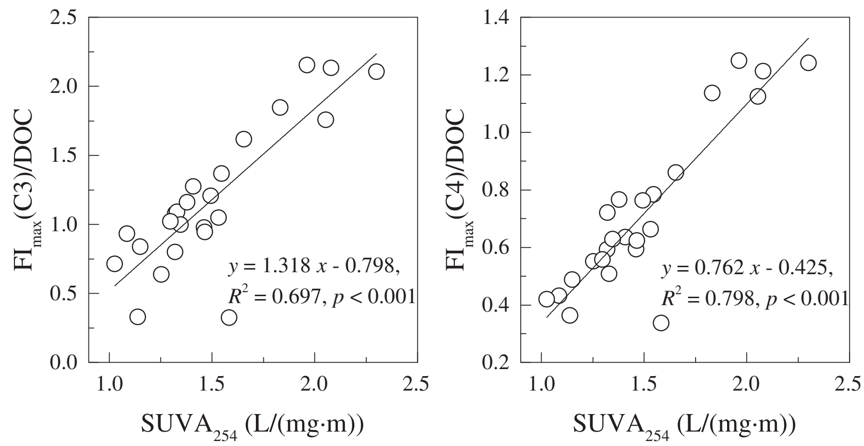

Fig. 4 – Correlations between $SUVA_{254}$ and the concentration of humic-like components. FI_{max}: the maximum fluorescence intensities; $SUVA_{254}$: specific ultra-violet absorbance at 254 nm.

observed between D(W) and ND(W) treatment eventually ($p < 0.01$, t-test). The ranges of BIX of ND(W), D(W), ND(Z), and D(Z) treatments were 0.77–1.00, 0.77–0.86, 0.65–0.94, and 0.68–0.89, respectively. The BIX of D(W) and D(Z) treatments increased rapidly at the first 45 days and then leveled off, whereas those of ND(W) and ND(Z) treatments slightly increased further. As a result, the values of BIX in controls were significantly higher than those of duckweed treatments ($p < 0.01$, t-test) (Fig. 5b). With respect to FI_{370}, the ranges of ND(W), D(W), ND(Z), and D(Z) treatments were 1.42–1.70, 1.54–1.76, 1.45–1.64, and 1.41–1.60, respectively, indicating a mixture source of microbial-derived and terrestrial-derived DOM (Fig. 5c).

Compared with controls, the humification index (HIX) increased by a final mean of 9.0% for duckweed treatments. Initially, a high percent of protein-like components corresponded to a low humification degree of DOM, which was derived from autochthonous biological degradation of organic matter (Sierra et al., 2005; Hudson et al., 2007). Then, the aromatic degree of DOM would increase gradually as the microbial degradation (Guo et al., 2012). Grube et al. (2006) studied that the percentage of aromatic material was strengthened significantly during the fermentation of sewage sludge, while those of aliphatics and amides decreased gradually. In general, the duckweed growth provided an oxygen-rich environment for microbial activities (Zhao et al., 2015), and resulted in a high humification degree of DOM as the strengthened microbial degradation (Zhou et al., 2015).

The initial increase of BIX might be related to the production of lower MW substances. Furthermore, compared with duckweed treatment, a higher value of BIX could be achieved in control, corresponding to a higher level of DOM freshness. In general, the existing photochemical degradation of DOM would contribute to the production of low MW organic molecules, which had a great impact on biological processes (Zhang and Qin, 2007; Dalzell et al., 2009). Meanwhile, as aforementioned, the values of $SUVA_{254}$ in duckweed treatments were significant higher than controls, indicating a

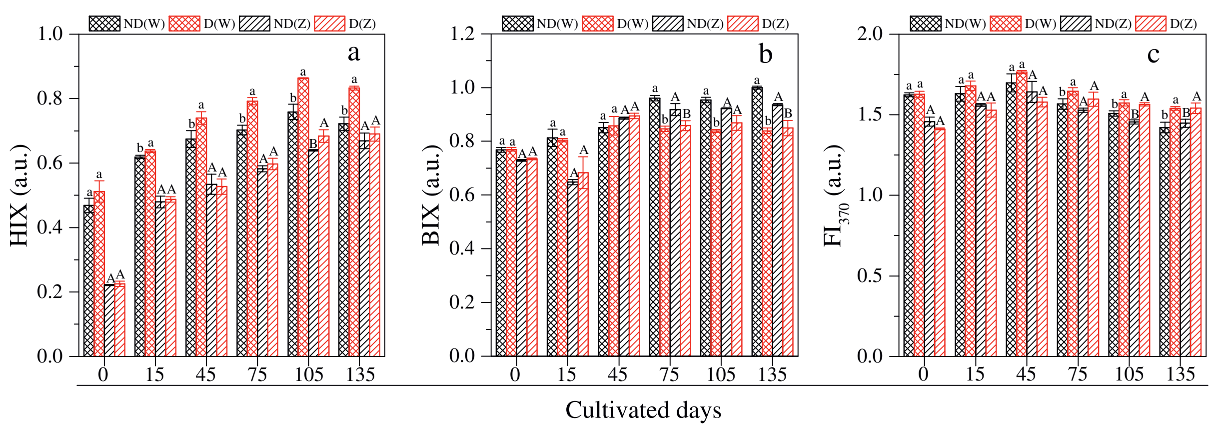

Fig. 5 – Variations of HIX (a), BIX (b), and FI_{370} (c) in swine effluent. HIX: humification index, BIX: biological index, FI_{370}: the index of sources of isolated fulvic acids. Standard errors were measured with three replicates. Different lower or upper cases indicated significance between duckweed treatment (N) and non-duckweed treatment (ND) at the level of $p < 0.05$ or $p < 0.01$, respectively. HIX: humification index; BIX: biological index.

strengthened humification degree of DOC of the former. Thus, to some degree, the activity of DOC could be reduced as the presence of duckweed.

In swine effluent, the source of DOM was a mixture of terrestrial-derived humic material and microbial-derived humic substances. Before the 45 days of experiment, the FI_{370} was correlated significantly with BIX ($p < 0.05$, data not shown), indicating that the biological products favored an increasing FI_{370}. But since then, FI_{370} decreased significantly, which might be linked with either the degradation of microbial-derived materials or the accumulation of terrestrial-derived fulvic acids. In general, the FI_{370} increased with the production of microbial-derived FA, and decreased with the formation of terrestrial-derived humic substances (McKnight et al., 2001; Zhang et al., 2010). Compared with controls, higher values of FI_{370} were observed in duckweed treatments eventually ($p < 0.01$, *t*-test), indicating a high proportion of microbial-derived humic substances as the duckweed growth. Thus, the remediation of duckweed on the swine effluent was conducive to the transformation of active DOC into the more stable organic compounds.

3. Conclusions

In the present study, duckweed (*S. polyrrhiza*) was used to renovate swine effluent due to its high growth rate and tolerance of organic nutrient. In general, the TN and TP contents of duckweed were linked with the nutrient concentration in swine effluent. Throughout the experiment period, the duckweed growth leaded to the reduction of DOC concentration, and weakened the DOC activity as increasing values of $SUVA_{254}$ were observed. With the increase in experimental days, the relative concentration of fulvic-like and humic-like component increased gradually with the growth of duckweed. The source of fulvic acids was a mixture of terrestrial-derived and microbial-derived organic materials in swine effluent, and the proportions of microbial-derived fulvic acids could be strengthened as the presence of duckweed. In conclusion, duckweed growth not only enhanced the removal rate of DOC in swine effluent, but also reduced the DOM reactivity.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.06.033.

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