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Effect of zinc ions on nutrient removal and growth of *Lemna aequinoctialis* from anaerobically digested swine wastewater

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ABSTRACT

The effect of Zn^{2+} on ammonium and phosphorous removal and duckweed growth was evaluated for treatment of anaerobically digested swine wastewater (ADSW) at various initial Zn^{2+} concentrations ranging from 1.0 to 15 mg/L. *Lemna aequinoctialis* taken from a local pond was selected for the treatment, and its fresh weight and contents of proteins, photosynthetic pigments, and vitamin E were examined. Results showed that the optimal Zn^{2+} concentration was 5.0 mg/L for NH_3-N and TP removal, the duckweed growth, and the accumulation of proteins in the duckweed. A maximum content of photosynthetic pigments increased with the increase of initial Zn^{2+} concentration, and it arrived earlier for a higher concentration of Zn^{2+}. Vitamin E content in the duckweed reached 4.5 mg/kg at 15 mg/L Zn^{2+} in 12-day cultivation, which showed the potential for producing and harvesting a high value-added product of vitamin E by culturing duckweed in ADSW.

1. Introduction

Anaerobically digested swine wastewater (ADSW) is characterized by its high concentration of ammonium and phosphorus (Vanotti et al., 2017; Luo et al., 2016). Heavy metals including zinc and copper have also been used as feed additives in many feed factories and livestock farms (Guo et al., 2013). Nevertheless, only 10–20% of heavy metals can be absorbed by livestock (Wu et al., 2017b; Suzuki et al., 2010), and most of them remain in ADSW. Heavy metals are difficult to be removed even by anaerobic digestion (Wu et al., 2017a,b; Wang and Chen, 2006). Thus, swine wastewater is also characterized by various heavy metals, especially Cu and Zn (Daverey et al., 2014). Furthermore, heavy metals can inhibit various microorganisms and plants, which pose serious challenges to traditional treatment methods including microbiological treatment and phytoremediation for ADSW.

Phytoremediation, a biological technology on the basis of the application of plants to extract, sequester or detoxify pollutants, is widely used on nutrient removal from ADSW (Ye et al., 2016; Sooknah and Wilkie, 2004). Nutrients including ammonium and phosphorus from ADSW are absorbed and degraded by aquatic plant and rhizosphere microorganisms (Tandon et al., 2013; Wen et al., 2016). After harvest, nutrients are removed from ADSW and are recovered as biomass. Compared with other physical and chemical treatment methods, the use of aquatic plants for phytoremediation of ADSW is considered to be cost-effective due to its high potential for removing and recovering nutrients, easy harvesting of plants and the high productivity (Tandon et al., 2013; Lee et al., 2002). In addition to low installation and operation costs, this technology can utilize the harvested plants as the value-added byproducts such as biofuels (Cheng and Stomp, 2009).

Duckweed has paid close attention for phytoremediation on nutrient removal from ADSW due to its following advantages: (i) resistance to pollution; (ii) fast growth; (iii) high biomass production; (iv) synergistic assimilation by microbial communities attaching to the plant; (v) easy removal from water; and (vi) wide distribution in natural aquatic ecosystems (Mkandawire and Dudel, 2007). Compared with other aquatic plants, duckweed is a free floating plant with high value-added products including proteins, starches, vitamin E and so on (Cheng and Stomp, 2009). Thus, duckweed has become an established alternative solution to recycling of nutrients from ADSW. Cheng et al. (2002) reached a maximum duckweed growth of 31.92 g/m^2 day when

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Spirodela punctala was cultivated in synthetic ADSW, and removal rates of both NH_3-N and PO_4-P were higher than 99.2% during 16-day cultivation. Xu and Deshusses (2015) cultivated *Spirodela punctala* by using diluted pig effluent in a pilot-scale culture pond. After the harvest, the annual yield of starches and ethanol by duckweed could reach 9.42×10^3 kg/ha and 6.42×10^3 L/ha, respectively, during 10-day batch cultivation. Therefore, the grown duckweed, owing to its excellent productivity of energy substances, could realize resource utilization for wastewater (Cui and Cheng, 2015; Pant et al., 2012). However, there are not any enough lignin in duckweed (Ge et al., 2012), so the duckweed is sensitive to heavy metal pollution in an aquatic ecosystem, such as in an ADSW treatment process where there was usually a high concentration of Zn^{2+} ranging from 1.5 to 30 mg/L (Daverey et al., 2014).

Zinc is a common heavy metal that serves as an essential micronutrient for plants (Rouff and Juarez, 2014). When available, it is easily absorbed by plants, and plays a critical role in plant growth. It is present in various enzyme systems, is fundamentally constructive to the synthesis of proteins, vitamin E and carbohydrates, and promotes RNA synthesis for protein production while also participating in DNA protection (Rouff and Juarez, 2014). However, high concentrations of Zn^{2+} will result in toxic effects on plants due to the destruction of the dynamic balance between the reactive oxygen species (ROS) and ROS scavenging system, which is necessary for plant growth (Cakmak, 2000). Low concentrations of Zn^{2+} can cause deficiency and affect the activities of rhizosphere microorganisms (Huang et al., 2008). It's worth noting that these effects are not the same in all rhizosphere microorganisms and can vary considerably among different types of plants (Rouff and Juarez, 2014).

Therefore, the relatively high concentrations of Zn^{2+} in ADSW can affect the growth of duckweed and consequently affect nutrient removal. Recently, the effect of zinc ions on microbial activity for swine wastewater treatment has been studied (Zhou et al., 2015; Lotti et al., 2012). However, few reports are available on the effect of zinc ions on duckweed for ADSW treatment.

In this study, the effects of different initial concentrations of Zn^{2+} on nutrient removal and growth of *Lemna aequinoctialis*, taken from a local pond, were investigated in synthetic ADSW, and fresh weights and contents of photosynthetic pigments, proteins and vitamin E in *Lemna aequinoctialis* were also evaluated. These data and results are supposed to be referred in selection, design and operation of phytoremediation processes for treatment and recycling of ADSW contaminated by Zn^{2+} using *Lemna aequinoctialis*.

2. Materials and methods

2.1. Duckweed cultivation conditions

The duckweed used in this study were taken in August 10th 2016 from a local pond (28°10′55″ N, 112°56′18″ E), Hunan, China. The duckweed selected were incubated with the pond water (COD = 221 ± 20 mg/L, NH_3-N = 16.6 ± 2.5 mg/L, TP = 4.64 ± 0.8 mg/L) in 2.5 L polypropylene buckets. The buckets were placed in a light growth chamber at 22 °C under a light intensity of 60 ± 10 μmol m^{-2} s^{-1} by fluorescent lights and light/dark cycles (L:D) of 16:8 h to proceed the amplification culture. The pond water was replaced once every 5 days.

2.2. DNA extraction, PCR amplification and sequencing

The DNA of duckweed was extracted by using the Plant Genomic DNA Rapid Extraction kit (Bio Teke Corporation, Beijing, China) according to the manufacturer's instructions and protocols. The Polymeric Chain Reaction (PCR) universal eukaryotic primer 1 (F: 5′-CGTACTGTACTTTTATGTTTACGAG-3′ as the forward primer) and primer 2 (R: 5′-ATCCGGTCCATCTAGAAATATTGGTTC-3′ as the reverse primer) were used to amplify the primer 1 to 2 fragments of the ribosome with template DNA originating from the duckweed isolated by using the PCR protocol described by Les et al. (2002). The PCR reaction mixture contained 1 μL of DNA template, 3 μL of primer 1, 3 μL of primer 2, 2 μL of dNTP, 3 μL of buffer, 0.2 μL of DNA polymerase and 17.8 μL of H_2O in a volume of 30 μL. PCR thermal program included an initial denaturing step for 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C with a final extension of 10 min at 72 °C, then 12 °C hold. The PCR products were purified by using the Gel PCR Clean-Up System (Applied Biosystems, Foster, CA, USA) and the sequencing fragments were analyzed on 3730XL DNA Sequencer (Applied Biosystems, Foster, CA, USA). Sequence similarity searches were carried out by the BLAST server of the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/). The phylogenetic tree was constructed using the Neighbor-Joining method, as implemented within the MEGA7.0 program package.

Table 1
Ionic concentrations of synthetic ADSW.

Ionic concentrations (mg/L)	Value
COD	220 ± 10.5
NH_3-N	80.2 ± 4.6
PO_4-P	15.6 ± 1.2
NO_3-N	98.3 ± 4.1
Ca^{2+}	120 ± 5.6
Mg^{2+}	24.5 ± 2.3
K^+	97.5 ± 3.4
Na^+	175.5 ± 2.4
Cl^-	285 ± 4.5
SO_4^{2-}	123 ± 5.7
Fe-EDTA	39.9 ± 2.5
Minor elements	2.54 ± 0.2

2.3. Synthetic ADSW preparation

10 L of synthetic ADSW were prepared by mixing different salts in distilled water to obtain the ionic concentrations observed in Table 1. 0.1 mol/L HCl was added in the solution until pH reached 6.4 ± 0.5. The salts used for synthetic effluent preparation included NH_4Cl, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, $FeSO4{\cdot}7H_2O$, Na_2EDTA, H_3BO_3, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $Na_2MoO_4{\cdot}2H_2O$, and $CuSO_4{\cdot}5H_2O$. Then the synthetic solution was sterilized in an autoclave for 30 min at 121 °C before the test.

2.4. Batch experiments

Batch experiments were conducted in 500 mL (6.4 cm × 6.4 cm × 12.5 cm) beaker in a light growth chamber. Each beaker contained 140 mL (3.4 cm deep in the beaker) of synthetic ADSW. It was initially seeded with the same amount (0.3 g fresh weight) of duckweed to cover the 80% surface area of 6.4 cm × 6.4 cm. Prior to culture initiation, the healthy duckweed had been selected and cleaned by distilled water. The beakers were then placed into a 22 °C light growth chamber with 16 h photoperiod and a photosynthetic photon flux density of 60 ± 10 μmol m^{-2} s^{-1} provided by wide spectrum fluorescent tubes. Destructive sampling was used to monitor the nutrient level and duckweed growth by taking a whole beaker as a sample for analysis. Triplicate samples and a control sample were taken every 4–6 d in each batch test. During experiments, pH decreased as the duckweed grew due to nitrification (Xu and Shen, 2011), so 0.1 mol/L NaOH solution was added to keep pH around 6.4 ± 0.5.

To investigate the effect of Zn^{2+} on nutrient removal and growth of duckweed from ADSW, different volumes of 4.424 g/L $ZnSO_4{\cdot}7H_2O$ solution was added to simulate different Zn^{2+} concentrations (1.0, 5.0, 10, 15 mg/L), which were used as the initial media in the batch tests. Avoid influencing the test caused by the deficiency of the ammonium or

phosphorus, the test of the effect of Zn^{2+} on nutrient removal was first conducted in 66 days to select the suitable cultivation time for the test of the effect of Zn^{2+} on growth of duckweed.

2.5. Analytical methods

The pH was monitored to evaluate duckweed growth with pH meter (PHS-3C). Standard Methods for Water and Wastewater Monitoring and Analysis (SEPAC, 2002) were used for NH_3-N (GB 7479-87), TP (GB 11893-89), and Zn (GB 7475-1987) analysis. The fresh weight of duckweed was measured using a balance immediately after the duckweed harvested was placed on paper towels for 5 min (Yang et al., 2010). The duckweed photosynthetic pigment (Chlorophyll a and b, and carotenoids) contents were analyzed using the method developed by Wellburn (1994). The duckweed protein contents were analyzed using Total protein quantitative assay kit (Coomassie brilliant blue method). To determine the vitamin E contents of duckweed, the some postharvest duckweed were freeze-dried at a freeze dryer for overnight. Then following the saponification extraction, vitamin E contents of the dried sample were determined using HPLC (GB/T 12388-1990).

2.6. Statistical analysis

The results were expressed as mean ± SE (standard error) of three replicates. One-way ANOVA was performed to compare all data. In both cases, significant differences between the analyses variables were considered when $P < .05$. All analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Characteristics and molecular identification of duckweed

The frond sizes of the duckweed species varied from less than one to several millimeters, with roots elongating to no more than several centimeters in length. The gene sequence amplified from this species was 868 bp in length, and showed similarities with other known sequences from green algae based on the BLAST results, and the homology reached 99% compared with *Lemna aequinoctialis matK* (AY034190.1). The phylogenetic analysis indicated that this species had a close relationship with *Lemna aequinoctialis* (Fig. 1).

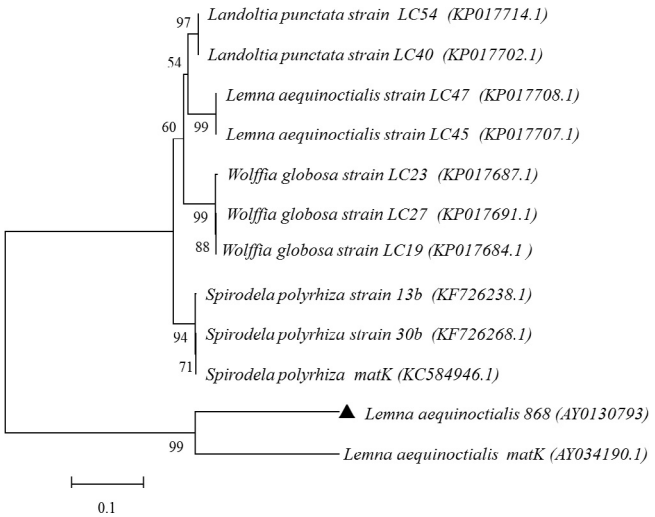

Fig. 1. Phylogenetic tree of *Lemna aequinoctialis* on the basis of partial primer 1 sequences (MEGA7.0). Numbers in the parentheses are accession numbers of each sequences in GenBank. Numbers at the nodes indicate bootstrap values (expressed as a %) with 1000 replicates. The scale bar measures the distance between species.

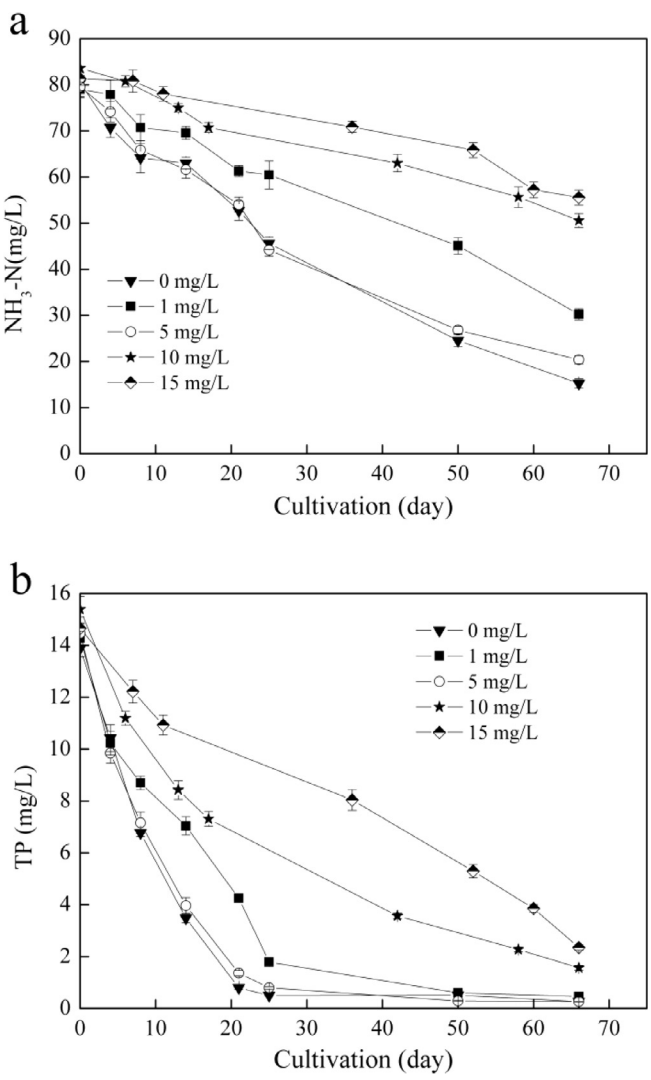

Fig. 2. Nutrient removal by *Lemna aequinoctialis* at various concentrations of Zn^{2+} within 66 days. (a) NH_3-N; (b) TP.

3.2. Effect of Zn^{2+} concentration on nutrient removal by Lemna aequinoctialis

Al-Nozaily et al. (2000) have demonstrated two mechanisms for nutrient recovery by duckweed, which were both the direct assimilation of duckweed and the biodegradation of rhizosphere microorganisms to synergistically purify the wastewater.

The removal rate of NH_3-N and TP under different initial concentrations of Zn^{2+} in synthetic wastewater was studied. As seen in Fig. 2, the concentrations of NH_3-N and TP generally decreased with time. Judging from the results of control, the removal rate of NH_3-N and TP were 84%, 98%, respectively when the test came to the end. It was suggested that *Lemna aequinoctialis* could efficiently remove NH_3-N and TP from ADSW, which was in agreement with the results of a study conducted by Cheng et al. (2002).

Heavy metal's damage to plants was a complex process. In general, the ROS were among the earliest responses following heavy metal's stress in plant cells. ROS, such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical (HO·), consisted of radical and non-radical oxygen (Vera-Estrella et al., 1994). Other studies (Asada and Takahashi, 1987; Vera-Estrella et al., 1994) have clearly demonstrated that a small amount of ROS were benefit for plant cells as the active signal of defense reaction of cells, but a large amount of ROS accumulation could damage to the plant cells mainly through destructing

ROS scavenging systems, and then the peroxidation damage occurred in plant cells (Cakmak, 2000; Wang and Chen, 2009).

Compared with the control (0.0 mg/L of Zn^{2+}), the removal rate of NH_3-N at various initial Zn^{2+} concentrations ranging 1.0–15 mg/L was inhibited significantly ($P < .05$) except for that at 5.0 mg/L of Zn^{2+} (Fig. 2a), which showed that the optimal Zn^{2+} concentration for removal of NH_3-N by *Lemna aequinoctialis* was 5.0 mg/L in synthetic ADSW. The higher (> 10 mg/L) or lower (1.0 mg/L) Zn^{2+} concentrations would inhibit removal of NH_3-N by *Lemna aequinoctialis*. When the Zn^{2+} concentration was too low, there was not enough Zn^{2+} to trigger the responsible ROS. But it could lead to intolerable toxicity to rhizosphere microorganisms including the nitrification and denitrification bacteria (Alaerts et al., 1996), which was mainly responsible for the inhibition of NH_3-N removal (Pant and Adholeya, 2009). When the Zn^{2+} concentration was about 5.0 mg/L, it affected positively on *Lemna aequinoctialis* in producing a little ROS. Vera-Estrella et al. (1994) proposed that the defense reaction of cells would be started by a small amount of ROS as the second messenger, the ROS scavenging system functioned to produce antioxidant enzyme and root exudates. The antioxidant enzyme would protect the cells of *Lemna aequinoctialis* from harm by Zn^{2+}. The root exudates including sugar and amino acids (Stottmeister et al., 2003) could provide sufficient nutrients for the damaged rhizosphere bacterial communities to recover and enhance the activity of rhizosphere microorganisms on removal of NH_3-N (Chen et al., 2016; Cheng et al., 2016). When the Zn^{2+} concentration was too high (> 10 mg/L), it could not only severely influence the survival of rhizosphere microorganisms, but also produce a large amount of ROS which was beyond the defensive capacity of *Lemna aequinoctialis* cell itself (Vera-Estrella et al., 1994). Therefore, the cells appeared the peroxidation damage which significantly inhibited the NH_3-N removal at high Zn^{2+} concentrations.

Similar experimental results were obtained on the removal of TP over time in different initial Zn^{2+} concentrations (Fig. 2b). Therefore, the optimal Zn^{2+} concentration for nutrient removal by *Lemna aequinoctialis* was about 5.0 mg/L, and the higher (> 10 mg/L) or lower (1.0 mg/L) Zn^{2+} concentrations would inhibit the nutrient removal by *Lemna aequinoctialis* from synthetic ADSW.

3.3. Effect of Zn^{2+} concentration on assimilation of Zn^{2+} by Lemna aequinoctialis

In order to investigate the effect of Zn^{2+} concentration on assimilation of Zn^{2+} by *Lemna aequinoctialis*, the changes of Zn^{2+} concentrations under various initial Zn^{2+} concentrations in the simulated wastewater were examined (Fig. 3). Regardless of cultivation time, no obvious change was observed under the initial Zn^{2+} concentrations of 1.0–5.0 mg/L, indicating the few absorption or assimilation of Zn^{2+} by *Lemna aequinoctialis* and the good tolerability of *Lemna aequinoctialis* to the low concentrations of Zn^{2+}. However, the Zn^{2+} concentration decreased under the initial Zn^{2+} concentrations of 10–15 mg/L. The possible reason was that some of *Lemna aequinoctialis* cells were dead due to the peroxidation damage at the high concentrations of Zn^{2+}. Due to the peroxidation damage, the structure of cell nucleolus was destroyed including the disintegration of nucleolus and the rupture of nuclear membrane (Lombi et al., 2001). Then, chromatins and nucleic acids entered the ADSW following the rupture of cell membranes, which could be indirectly demonstrated by the faster drop of pH at the high Zn^{2+} concentrations, and their active substances could easily adsorb the Zn^{2+} through the complexation or chelation (Xing et al., 2007), which could lead to the decline of Zn^{2+} concentrations in the simulated wastewater.

3.4. Growth characteristics of Lemna aequinoctialis and biomass production

The growth characteristics of *Lemna aequinoctialis* under various Zn^{2+} concentration levels within 25 days were studied. It could be seen from Fig. 4 that there was no obvious lag phase, which was not consistent with the report by Cheng et al. (2002). It was probably because there were more microbial communities in pond water where *Lemna aequinoctialis* was incubated, while there were few microorganisms in the SAM medium where *Spirodela punctala* was incubated (Cheng et al., 2002). Thus, *Lemna aequinoctialis* incubated with the pond water could adapt more quickly, and consequently the lag phase was not obvious. In the control, this *Lemna aequinoctialis* grew well in synthetic ADSW. This result illustrated that *Lemna aequinoctialis* taken from water bodies or nature environment presented good performance in ADSW (Xu et al., 2011; Cheng and Stomp, 2009). Similar results were also observed in the cultivation of duckweed by Xu and Shen (2011).

It could be observed from Fig. 4 that the fresh weight of *Lemna aequinoctialis* at 5.0 mg/L Zn^{2+} was the highest, correspondingly up to 1.78 g, and no obvious inhibition was found when the time came was to 25 days. On the contrary, the fresh weight deceased to 1.09 g, 0.95 g and 0.83 g under the Zn^{2+} concentration of 1, 10 and 15 mg/L, exhibiting the corresponding inhibitive ratio of 29.0%, 38.9% and 46.6%, respectively. The results also suggested that the optimal Zn^{2+} concentration for *Lemna aequinoctialis* growth was about 5.0 mg/L in synthetic ADSW, and the higher (> 10 mg/L) or lower (1.0 mg/L) Zn^{2+} concentrations would inhibit *Lemna aequinoctialis* growth. The inhibition mechanism of Zn^{2+} on *Lemna aequinoctialis* growth could be the

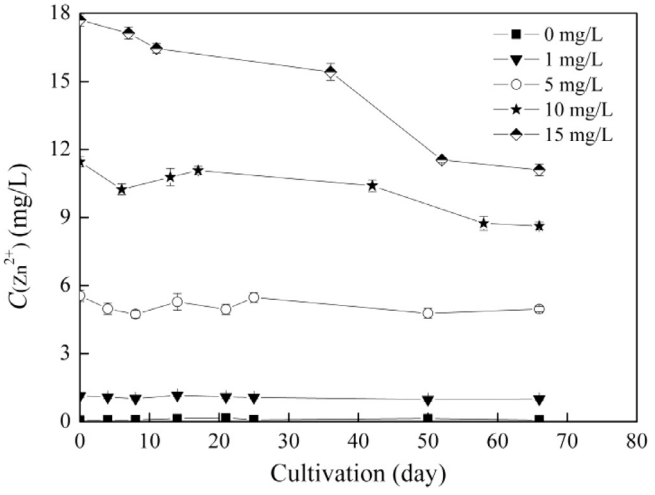

Fig. 3. The assimilation of Zn^{2+} by *Lemna aequinoctialis* at various initial concentrations of Zn^{2+} within 66 days.

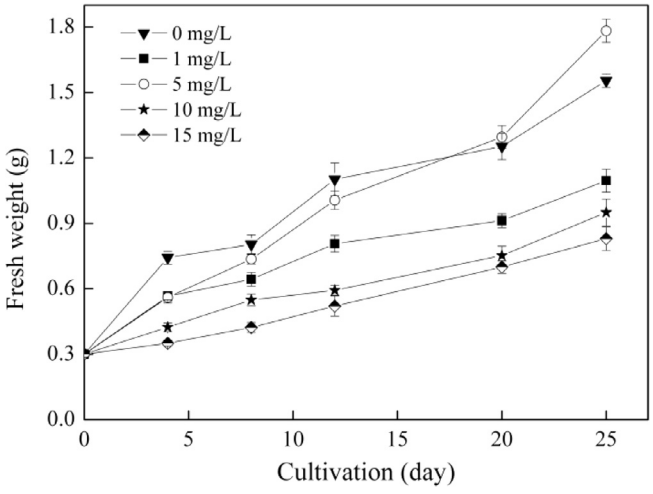

Fig. 4. Growth curves for *Lemna aequinoctialis* at various concentrations of Zn^{2+} within 25 days.

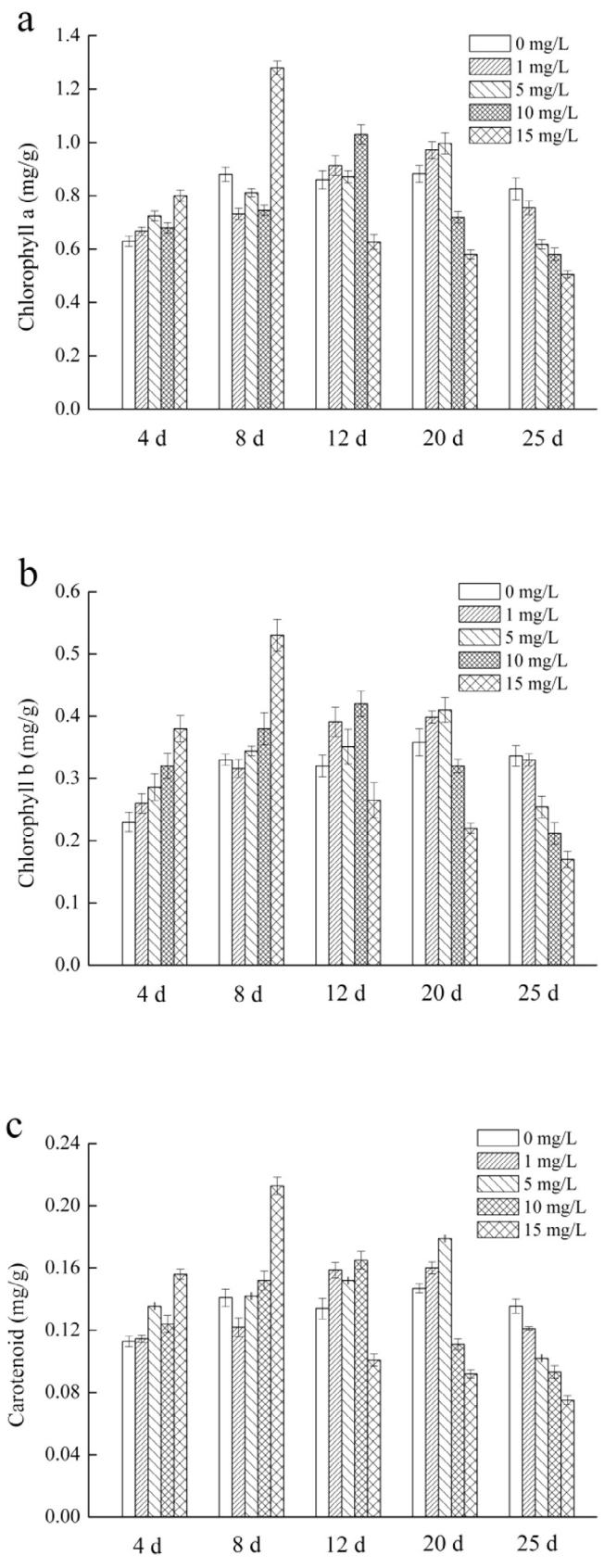

Fig. 5. Photosynthetic pigments in *Lemna aequinoctialis* at various concentrations of Zn^{2+} within 25 days. (a) chlorophyll a; (b) chlorophyll b; (c) carotenoids.

same with the functioning mechanism of Zn^{2+} on nutrient removal by *Lemna aequinoctialis* as stated above. Low concentrations of Zn^{2+} influenced rhizosphere microorganism, and high concentrations of Zn^{2+} damaged *Lemna aequinoctialis* cells, resulting in the *Lemna aequinoctialis* adversely growing.

3.5. Effect of Zn^{2+} concentration on photosynthetic pigments

The decline of photosynthetic pigments was a mechanism to protect plants from oxidative damage under heavy metal stress in plants (Müller et al., 2001). It could lead to the some decrease in photosynthesis, and consequently limiting the excessive production of ROS to reduce the oxidative damage to plants (Han et al., 2002).

The contents of photosynthetic pigment (chlorophyll a, b and carotenoids) in various initial Zn^{2+} concentrations of synthetic ADSW for 25-day batch culture were studied. As seen in Fig. 5, the chlorophyll a, b and carotenoids content firstly increased and then stabilized in the control, suggesting that *Lemna aequinoctialis* grew well in the ADSW. The chlorophyll a, chlorophyll b and carotenoids contents firstly increased and then decreased sharply at 1.0–15 mg/L Zn^{2+}. In addition, the maximum value of photosynthetic pigment increased and its appearance time decreased with increased Zn^{2+} concentration. As a protective mechanism (Müller et al., 2001), the photosynthetic pigments began to largely synthesize when the *Lemna aequinoctialis* firstly exposed to the Zn^{2+} solution. Thus, the content of photosynthetic pigments at 1.0–15 mg/L Zn^{2+} were higher than in the control for the initial 4-day cultivation (Fig. 5). Moreover, the *Lemna aequinoctialis* needed to synthesize more photosynthetic pigments for coping with the increasing Zn^{2+} and alleviating the toxicity to plants (Uruç Parlak and Demirezen Yilmaz, 2012), which was the reason for the higher the initial Zn^{2+} concentration was, the higher the maximum value of photosynthetic pigments was. But with the extension of time, the center cations of chloroplast were gradually changed due to the inhibition of key enzyme activity by Zn^{2+} on synthesis of photosynthetic pigments (Miller and Cox, 1983). Therefore, there was a sharp decrease in photosynthetic pigments, and it occurred earlier for a higher concentration of Zn^{2+}. Similar result were obtained from the experiment where the toxicity of boron to *Lemna minor* L. and *Lemna gibba* L. was studied by Gür et al. (2016).

3.6. Effect of Zn^{2+} concentration on contents of proteins and vitamin E

Nutrients could be substantially removed as duckweed grew in wastewater, which was mainly attributed to the protein accumulation of duckweed (Gaur and Suthar, 2017).

The changes of protein contents in *Lemna aequinoctialis* under various initial Zn^{2+} concentrations were measured. As seen in Fig. 6a, the protein contents increased with the increase of cultivation time, which might be because the NH_3-N could be partly assimilated by *Lemna aequinoctialis* to synthesis proteins (Luo et al., 2016). It could be demonstrated by Fig. 2 where the NH_3-N concentrations decreased with the increase of time. Moreover, the protein content at 5.0 mg/L Zn^{2+} was the highest in the same cultivation time, suggesting that the optimal Zn^{2+} concentration for synthesis of proteins in *Lemna aequinoctialis* was about 5.0 mg/L. The reason might be that 5.0 mg/L Zn^{2+} could markedly stimulate the enzyme activity for protein synthesis, and the excessive ROS could be timely scavenged by ROS scavenging system (Asada and Takahashi, 1987). Therefore, the synthesis of proteins in *Lemna aequinoctialis* was promoted, thereby the protein content at 5.0 mg/L Zn^{2+} was higher than in the control. However, the protein contents under the Zn^{2+} concentrations of 1.0, 10, 15 mg/L were lower than in the control. Low concentrations of Zn^{2+} could inhibit the nutrient transport to duckweed by rhizosphere microorganisms, and high concentrations of Zn^{2+} could produce a large amount of ROS, resulting in the irreversible peroxidation damage to proteins (Vera-Estrella et al., 1994).

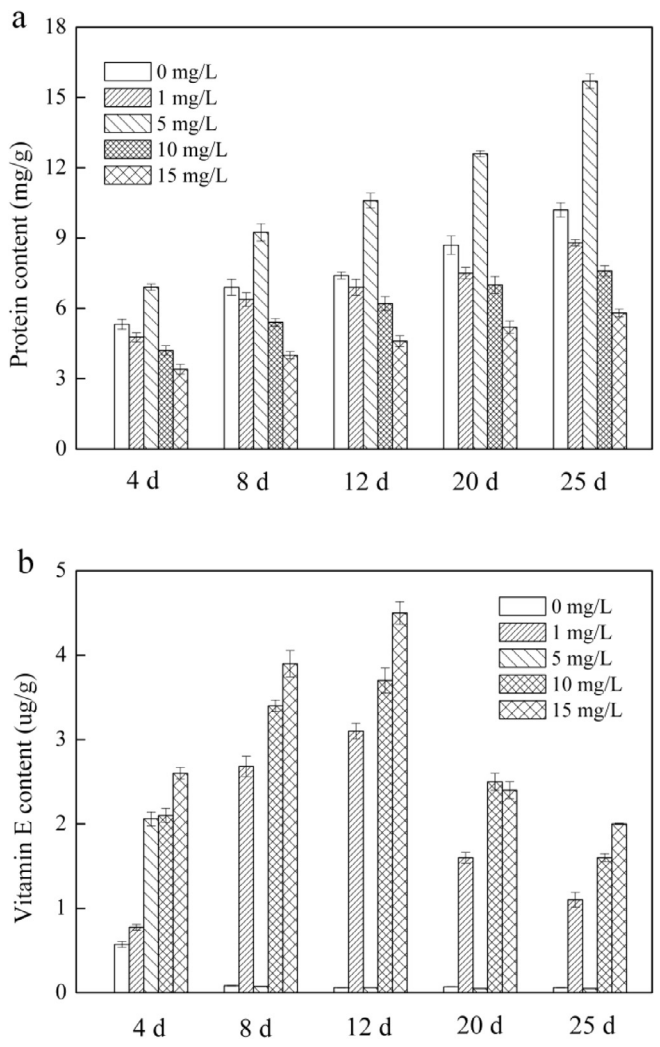

Fig. 6. Protein and vitamin E contents in *Lemna aequinoctialis* at various concentrations of Zn^{2+} within 25 days. (a) proteins; (b) vitamin E.

Vitamin E could be found in all plant tissues, which usually was present in the photosynthetic cells. It could directly restore ROS, while also taken part in the scavenging of ROS as the enzyme substrate (Asada and Takahashi, 1987). So, the vitamin E was a kind of non-enzymatic antioxidant (lipophilic antioxidant) in the anti-oxidation system of plants. Meanwhile, the vitamin E was proved to be an effective plant biomarker of pollution. For example, the low vitamin E contents showed that the plants was high tolerant to the pollution, while the relatively high vitamin E contents showed that the plants was under the stress of severe pollution (Grijalbo et al., 2016).

The changes of vitamin E contents in *Lemna aequinoctialis* under various initial Zn^{2+} concentrations were shown in Fig. 6b. In the initial 4 days, the contents of vitamin E increased with the increase of initial Zn^{2+} concentration, which could be explained by the corresponding incremental stress imposed by the contaminants including Zn^{2+} and high-strength nutrients when the *Lemna aequinoctialis* was firstly exposed to the synthetic ADSW (Collin et al., 2008). However, in the culture from 8 to 25 days, the vitamin E content at 5.0 mg/L Zn^{2+} fall to be close to zero, this value was almost the same with the value of control, suggesting that the *Lemna aequinoctialis* completely adapted to the synthetic ADSW at 5.0 mg/L Zn^{2+}, and this result was in good agreement with present result which was that the optimal Zn^{2+} concentration on *Lemna aequinoctialis* growth was 5.0 mg/L. In addition, the vitamin E contents at 1.0, 10, 15 mg/L Zn^{2+} still increased until 12 days, showing that the stress of contaminants on *Lemna aequinoctialis* still existed. Then, their contents fall to a certain value, illustrating that the *Lemna aequinoctialis* have gradually adapted to the simulate wastewater (Grijalbo et al., 2016), but the effect mechanism of lower or higher Zn^{2+} concentrations always exists, so the vitamin E contents at 1.0, 10, 15 mg/L Zn^{2+} didn't fall to zero. Moreover, it was observed that the vitamin E content maximized 4.5 mg/kg at 15 mg/L Zn^{2+} in 12-day cultivation (Fig. 6b). The high content of vitamin E was associated with high concentration of Zn^{2+}, suggesting that high concentration of Zn^{2+} could be considered as a promising method to increase vitamin E accumulation in the duckweed.

These results illustrated that it could achieve the recovery of nutrients as *Lemna aequinoctialis* could accumulate high content of proteins or vitamin E in ADSW contaminated by Zn^{2+}. While more investigations are needed both on improving the yield of proteins, vitamin E or other high value-added products in duckweeds for treatment of ADSW at full scale and on phytoremediation processes.

4. Conclusions

Lemna aequinoctialis collected from a local pond was cultivated in synthetic ADSW at various initial Zn^{2+} concentrations ranging from 1.0 to 15 mg/L. The optimal Zn^{2+} concentration was 5.0 mg/L for the removal of NH_3-N and TP, the *Lemna aequinoctialis* growth, and the synthesis of proteins. The maximum value of photosynthetic pigment increased and its appearance time decreased with increased Zn^{2+} concentration. Vitamin E content maximized 4.5 mg/kg at 15 mg/L of Zn^{2+} in 12-day cultivation, suggesting that ADSW with a high concentration of Zn^{2+} had potential to be recycled by producing high value-added vitamin E in the duckweed.

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