

Nitrogen and phosphorus removal and physiological response in aquatic plants under aeration conditions

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ABSTRACT: To analyze the variation of physiological responses between *Nymphaea tetragona* Georgi. and *Pontederia cordata* L. and the water qualities under aeration conditions, the selected plants were cultivated in 12 purifying-tanks (aeration, non aeration), to treat heavily polluted river water. The characteristics of both plants were investigated, which included contents of chlorophyll and soluble protein, activities of peroxidase and catalase, accumulations of nitrogen and phosphorus, densities of tillers and roots, lengths of roots, culms and leaves, biomass of roots and shoots. The water qualities were analyzed correspondingly. Results indicated that aeration affected morphological and physiological characteristics of the plants and the water qualities and effects became more significant on *N. tetragona* than *P. cordata*. Biomass and length of roots, culms and leaves under the non aeration conditions exceeded that under the aeration conditions. Aeration contributed to the activities increase of peroxidase and catalase of the roots and the contents decrease of chlorophyll and soluble protein of the leaves. Nitrogen and phosphorus contents of the roots, culms and leaves increased under the non aeration conditions. Aeration resulted in tillers and roots densities of *N. tetragona* decreased, while they increased for *P. cordata*. Total phosphorus and soluble phosphorus removals decreased 8.42 % and 8.05 % in the tank with *N. tetragona* under the aeration conditions. In the tank with *P. cordata*, total nitrogen and NH_4^+ -N removals increased 14.44 % and 16.06 % under the aeration conditions. This work provided valuable data for optimizing the plants allocation in the ecological restoration project of the polluted water.

Keywords: Morphological and physiological characteristic; *Nymphaea tetragona* Georgi.; *Pontederia cordata* L.

INTRODUCTION

Increasing of industry and urban population leads to most urban rivers throughout China have been heavily polluted, especially the urban stagnant flow channels.

Dissolved oxygen concentrations of the stagnant flow channels almost turn to zero and there live few aquatic animals and plants direct to the poor self-purification of the channels. Most of all, the typical reason of this phenomenon is lack of dissolved oxygen. Dissolved oxygen concentration directly affected the decomposition and degradation of the organic materials of the wastewater, even the self-purification of the water (Bodelier *et al.*, 1996; Kowalchuk *et al.*, 1998; Kirk and Kronzucker, 2005). Therefore, the degradation of pollutants in stagnant water can be considerably improved by recovering dissolved oxygen. Artificial aeration was just an

essential approach for the dissolved oxygen recover of the heavily polluted stagnant water and even the ecological restoration of the polluted stagnant flow channels. Numerous studies have recently been reported (Scholz and Lee, 2005; Ouellet-Plamondon *et al.* 2006; Akpor *et al.*, 2008; Banu *et al.*, 2008). Aeration technique meant that atmosphere or pure O_2 was artificially pumped into polluted water and the dissolved oxygen was reinforced to increase and recover and the activities of the aerobic microbes in the water were returned and even enhanced. Thus, the organic materials were purified and the water quality was improved (Subhasis *et al.*, 1999; Soltanali and Haghani, 2008; Madukasi *et al.*, 2010). Engineering practices also demonstrated that aeration technique was effective to help treating polluted water (Scholz and Lee, 2005; Ouellet-Plamondon *et al.* 2006; Guo *et al.*, 2008; Thakre *et al.*, 2009; Igbiosa and Okoh, 2009).

Floating-beds form natural phytodepuration

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systems and artificially constructed floating-beds are currently used extensively for the purposes of treating domestic, agricultural and industrial effluent (Davies *et al.* 2005; Kovacic *et al.* 2006; Nahlik and Mitsch, 2006; Dhote and Dixit, 2009). An important part of the purification process is the oxidation of ammonium compounds to nitrite and nitrate (Kirk and Kronzucker, 2005); for this reason, the efflux of oxygen from the plant's roots promotes the growth of aerobic nitrifying bacteria (Bodelier *et al.* 1996; Kowalchuk *et al.* 1998). Therefore, aeration is suitable to enhance nitrogen removals. The plant-periphyton complex also plays an important biofilter role in the adsorption and retention of cations (Judith and Peddrick, 2004).

However, practical experiences demonstrated that when polluted stagnant water was aerated, except for the water quality improvement (Scholz and Lee, 2005; Ouellet-Plamondon *et al.*, 2006), pressurized (convective) gas flow from the aerator affected the morphological and physiological characteristics of the aquatic plants under the certain conditions (such as, too high of the gas flow rate and too long of the aeration time), for the pressurized (convective) gas flow from the aerator most likely affected the exposed roots of the aquatic plants, especially the tender ones. Most of the literature studies on aquatic plant treatment of wastewater report purification efficiencies, but not the correlation between the purification and the physiological response of the aquatic plant. There are no reports about the effects of aeration on the morphological and physiological characteristics of the aquatic plants till now. However, knowledge about that aspect is of most importance to effectively utilize the combined technique of aquatic plants and artificial aeration to treat and restore the heavily polluted stagnant channels.

Physiological status of the aquatic plant was essential for the nitrogen and phosphorus absorption and removal of polluted water (Li *et al.*, 2007). Enzymes activities of the plant tissues were associated with the physiological status of the plants. Adversity led to the superoxide radicals increased in the plant cells and caused the oxidation stress (Jabs, 1999; El-Diwani *et al.*, 2009). There was an antioxidant enzyme system in the cells of the plant. Catalase and peroxidase were major components of the system and they had a synergy effect in scavenging and preventing active oxygen radicals (Kaufmann *et al.*, 2002). Especially, peroxidase existed extensively in the

cells, taking part in physiological activities and sensitive to adverse conditions (Tan *et al.*, 2006). Hence, peroxidase was significantly associated with the plants resistance to adverse conditions (Li *et al.*, 2000; Yousaf and Sarwar, 2008).

Both *Pontederia cordata* and *Nymphaea tetragona* have ornamental value, along with good purification ability, but are not extensively used in the environmental engineering till now. In this study, *P. cordata* (Fig. 1) and *N. tetragona* (Fig. 2) were employed to treat heavily polluted stagnant water from Gong-ye channel by field test. Effects of aeration on the morphological and physiological characteristics including peroxidase and catalase activities of the roots, chlorophyll and soluble protein contents of the leaves of the plants and the nitrogen and phosphorus removals of polluted water were analyzed.

The objective of this study is to investigate effects of artificial aeration on the physiological responses of



Fig.1: *P. cordata* under non aeration conditions on July 19th 2008



Fig. 2: *N. tetragona* under non aeration conditions on July 19th 2008

the aquatic plants and the variation of the water qualities to provide valuable data for employing aquatic plants to treat polluted stagnant water. Date and location of the research carried out throughout the study was On October 11st 2008 and Gong-ye channel, respectively.

MATERIALS AND METHODS

Device construction and plant culture

Gong-ye channel is heavily polluted and stagnant. It lies in Pu-tuo district of Tao-pu town, Shanghai City. October 11st 2008, the quality of the polluted channel water was as follows: ρ (COD_{cr}), 170.6–187.5 mg/L; ρ (BOD₅), 63.02–69.23 mg/L; ρ (NH₄⁺-N), 30.81–34.17 mg/L; ρ (total nitrogen), 51.35–53.39 mg/L; ρ (soluble phosphorus), 0.144–0.146 mg/L; ρ (total phosphorus), 0.228–0.240 mg/L; pH, 7.9–8.5.

A test apparatus consisting of 14 purifying-tanks and one balance water tank were used as the experimental system. The device (Fig. 3) was composed of one balance water tank (upper dimension, L 1500 mm × B 1000 mm; downside dimension, L 1450 mm × B 950 mm; High, 600 mm) and 14 plastic purifying-tanks (upper dimension, L 1240 mm × B 620 mm; downside dimension, L 1150 mm × B 550 mm; High, 760 mm). The balance water tank had an overflow tube, to balance the water level and made every one of 14 tanks receive same influent. The polluted water was pumped into the balance water tank from the polluted river directly, through the fixed effluent tube into the purifying-tanks. The effluent tube on the purifying-tank monitored the water level in the tank at 0.60 m high. Each purifying-tank was separated by a baffle through the center and left about 20 cm space to the tank bottom to avoid causing water circuit flow. Each tank had a sediment exit on the bottom to discharge sediment mud every day. Continuous water flow was kept in each tank. Hydraulic retention time was 8 h. The type of ACO-004 electromagnetic atmosphere pump (60 L/min) from Zhejiang Sensen Shiye Limited Company was employed to aerate six tanks continuously from February 2008 to October 2008. Gas flow rate in each of six tanks was 30 L/min. Seedlings of *P. cordata* (high 10 cm) and *N. tetragona* (high 10 cm) were collected from Shanghai Zelong Biology Engineering Limited Company. The seedlings were water cultured in purifying-tanks

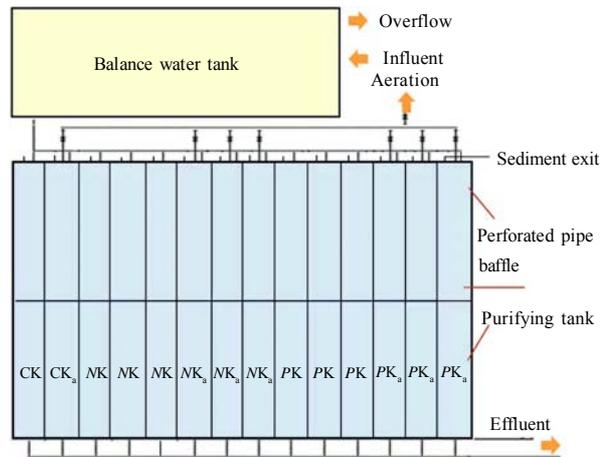


Fig. 3: Sketch of the plant purifying-tanks

with the same planting density of 13.0 /m² water area and two additional tanks without plant was as control. Treatment time was February -October 2008. Fig. 3 showed sketch of the purifying-tanks.

Pk_a and Pk represented aeration and non aeration purifying-tank for *P. cordata*; Nk_a and Nk represented aeration and non aeration purifying-tank for *N. tetragona*; CK_a and CK represented aeration and non aeration control

Sampling and analysis

On October 2008, 21 °C-29 °C, sunny, seven plants (each plant had two or more leaf slices) were selected at random from the healthy plants in every purifying-tank and measured the roots (taproot) lengths, culms lengths and leaves (upper leaf) lengths were measured and measured standard deviations (SD) were calculated. Meanwhile, healthy leaves (0.40 g) and roots (0.40 g) were collected from each one of the selected plants for use. Corresponding water analysis of the effluent from each of 14 tanks was done using the standard methods (APHA, 1998).

Chlorophyll content of the leaf of the aquatic plant

Method reported by Hegedüs et al. (2001) was employed to analyze chlorophyll content and it was improved as follows: 0.05 g leaf was obtained and 5 mL of 80 % acetone solution was added into, soaking 24 h and the absorbance of the soaked solution was measured with spectrophotometer (HITACHI U-1100). Its unit was mg/g. In this way, the chlorophyll content of the leaf of the aquatic plant was analyzed.

Enzyme solution preparation

Fresh root (0.30 g) and leaf (0.30 g) were prepared and refined in the freezing phosphate solution (pH = 7.8), centrifuged at 13000 r/min and in 4 °C for 30 min. The supernatant was collected for the steps below.

Soluble protein content and enzyme activities measure

Soluble protein content of the leaf of the aquatic plant was measured with coomassie brilliant blue (Wessel and Flügge, 1984). Standard curve reported using bovine serum albumin and the unit was mg/g. Catalase activity measure of the root of the aquatic plant was determined with UV-spectromete (Rao *et al.* 1996; Aktar *et al.*, 2009). D 240 value declined 0.1, represented one activity unit. Peroxidase activity measure of the root of the aquatic plant was performed with guaiacol (Zhang, 1990). Light intensity variation in per min represented the enzyme activity. D 460 value increased 0.1, represented one activity unit and the unit was U/(g·min). In this way, the soluble protein content of the leaf of the aquatic plant and the catalase and peroxidase activity of the root of the aquatic plant were analyzed.

Nitrogen and phosphorus contents of the plant tissues and the biomass measure

Nitrogen contents in the samples of plant tissues including the roots, culms and leaves were determined by the Dumas combustion method using an automated CN analyser (LECOCHN-1000, LECO Company, St Joseph, MI, USA). Phosphorus contents in the corresponding plant tissues were determined by colourimetry (HITACHI U-1100 spectrophotometer) using the vanado-molybdate method after digesting material in a mixture of concentrated nitric and perchloric acids (Vogel and Bassett, 1978). The biomass (dry weight) of the plant tissues, including the roots and the shoots, was measured and the means of individual plant was obtained.

Analytical methods

Morphological and physiological indexes of seven plants of each purifying-tank were measured, and then the means of each index was obtained based on individual purifying-tank. And so did for the physical and chemical characteristics of the polluted water. In this way, three replicates (means) of each index were obtained and then the statistic analysis was conducted.

All data analysis was performed using independent-samples *t* test of the SPSS 15.0 software.

RESULTS AND DISCUSSION*Effects of aeration on morphological characteristics of the aquatic plants*

Under the conditions of long time continuous aeration, all the morphological characteristics of both plants were changed (Table 1).

Table 1 indicated that aeration affected morphological characteristics of the aquatic plants. Under the aeration conditions, the lengths of roots, culms and leaves decreased, respectively. Aeration was associated with the plants propagation. For *N. tetragona*, the tillers number in the non aeration tank was approximately 2.58 times of the aeration tank, due to the effects of water flow from the aeration on the roots growth. However, for *P. cordata*, the tillers in the aeration tank was about 1.60 times of the non aeration tank, because *P. cordata* under the non aeration conditions developed better than under the aeration conditions, sheltering the sunlight to be shared by the shootings, leading to the influence on the photosynthesis of the shootings and the growth of the tillers. However, the plants under the aeration conditions became comparatively dwarf, which was suitable for the shootings to catch sunlight and develop. Root density in the aeration tank was about 1.67 times of the non aeration tank, owing to the significant increase of the tillers.

Effects of aeration on physiological characteristics of the aquatic plants

Under the conditions of long period continuous aeration, all the physiological characteristics of both plants were changed (Table 2).

Table 2 indicated that aeration affected physiological characteristics of the aquatic plants. Under the aeration conditions, POD and CAT activities increased, respectively. Under two conditions, for *P. cordata*, CAT activities and the Chl (Chla, Chlb) contents varied significantly, while POD activities and SP contents did not. For *N. tetragona*, POD and CAT activities, SP, Chla and Chlb contents all varied significantly, respectively.

Aeration affected biomass and N and P contents of the plants tissues. Table 2 indicated that aeration influenced the biomass and the N and P contents of the plants tissues. In particular, the N and P contents

Table1: Effects of aeration on the morphological characteristics of the aquatic plants

Means	N -	N +	P -	P +
Root length (cm)	42.36 ± 4.13 a	21.39 ± 2.46 b	29.36 ± 2.13 a	16.69 ± 1.46 b
Culm length (cm)	35.27 ± 3.51 a	30.97 ± 2.40 b	57.27 ± 3.51 a	38.97 ± 2.40 b
Leaf length (cm)	7.67 ± 0.83 a	4.34 ± 0.51 b	16.67 ± 1.33 a	14.34 ± 1.25 b
Tiller density (tillers/m ²)	152 ± 10.0 a	59 ± 5.00 b	285 ± 18.0 a	455 ± 40.0 b
Root density (roots/m ²)	619 ± 38.0 a	221 ± 14.0 b	1312 ± 105 a	2194 ± 167 b

Mean ± S.D. (n = 3), Means in the same line followed by the same letter (a, b) are not significantly different at P-value <0.05 based on *t* test. *N* and *P* were *N. tetragona* and *P. cordata*, + and - were aeration and non aeration conditions; the same table below.

Table: 2 Effects of aeration on the physiological characteristics of the aquatic plants

Means	N -	N +	P -	P +
POD activity [U/(g·min)]	0.18 ± 0.03 a	0.27 ± 0.09 b	0.07 ± 0.020 a	0.10 ± 0.03 a
CAT activity [mg/(g·min)]	0.63 ± 0.20 a	0.93 ± 0.51 b	0.54 ± 0.090 a	0.72 ± 0.16 b
SP content (mg/g)	41.58 ± 9.75 a	32.93 ± 5.69 b	86.6 ± 18.34 a	70.85 ± 7.07 a
Chla content (mg/g)	1.57 ± 0.11 a	1.25 ± 0.09 b	1.85 ± 0.070 a	1.53 ± 0.19 b
Chlb content (mg/g)	0.49 ± 0.02 a	0.37 ± 0.02 b	0.55 ± 0.020 a	0.43 ± 0.08 b
Nitrogen content (g/kg):				
Root	16.83 ± 1.75 a	11.95 ± 1.12 b	17.91 ± 1.820 a	12.87 ± 1.23 b
Culm	23.12 ± 1.97 a	16.25 ± 1.35 b	24.24 ± 1.990 a	17.67 ± 1.32 b
Leaf	26.13 ± 2.01 a	18.51 ± 1.52 b	28.01 ± 2.800 a	19.34 ± 1.56 b
Phosphorus content (g/kg)				
Root	2.41 ± 0.27 a	1.40 ± 0.19 b	2.45 ± 0.290 a	1.42 ± 0.20 b
Culm	3.18 ± 0.27 a	1.92 ± 0.16 b	3.35 ± 0.300 a	2.03 ± 0.25 b
Leaf	3.57 ± 0.31 a	2.18 ± 0.26 b	3.79 ± 0.370 a	2.33 ± 0.27 b
Water content rate (%)				
Root	92.5 ± 1.60 a	94.0 ± 2.20 a	91.2 ± 1.70 a	93.4 ± 2.20 a
Culm	93.2 ± 1.80 a	95.6 ± 2.30 a	92.3 ± 1.80 a	94.5 ± 2.30 a
Leaf	93.6 ± 1.90 a	96.5 ± 2.40 a	92.9 ± 2.10 a	95.1 ± 2.50 a
Biomass (g/plant)				
Root	23.67 ± 1.05 a	19.66 ± 0.74 b	20.55 ± 0.67 a	17.06 ± 0.61 a
Shoot	2.92 ± 0.10 a	2.47 ± 0.08 b	9.39 ± 0.34 a	7.94 ± 0.30 b

Mean ± S.D. (n = 3), Means in the same line followed by the same letter (a, b) are not significantly different at P-value <0.05 based on *t* test. *N* and *P* were *N. tetragona* and *P. cordata*, + and - were aeration and non aeration conditions

of the roots, culms and leaves decreased under the aeration conditions, respectively. It was because aeration affected the roots development, leading to the decrease of the N and P absorption of the roots, which directed to the decrease of the N and P accumulation of the culms and leaves tissues. Furthermore, under the non aeration conditions, the plants developed better, biomass of per plant was larger and the water content ratios of the plants tissues under two conditions did not vary significantly (Table 2), which indicated that the N and P absorption of per plant under the aeration conditions was weaker than under the non aeration conditions.

Aeration possibly affected the photosynthesis and transpiration of the aquatic plants. Chla and Chlb contents of the leaves of two plants under the aeration conditions were lower than under the non aeration conditions (Table 2), because the continuous water flow caused by the continuous pressurized (convective) gas flow from the aerator, influencing the

development of the exposed roots. Thus, the physiological status of the plants was affected, leading to the decline of the synthesis ability of the photosynthetic pigments of the leaves. However, Chl (Chla, Chlb) contents were obviously associated with the effective photosynthesis rates of the leaves. Therefore, aeration influenced the plants photosynthesis and the SP production.

Aeration possibly affected the transpiration of the plants. N and P contents of per unit of mass of the plant tissues including the root, culm and leaf under the aeration conditions were lower than under the non aeration conditions, the corresponding water content ratios did not vary significantly (Table 2) and the plants developed better under the non aeration conditions (Table 1), all that indicated that the soluble N and P absorption of the plants from the water in the aeration tank was weaker than in the non aeration tank in the same treatment period. Therefore, the transpiration absorption of the plants under the aeration conditions

was weaker than under the non aeration conditions. N and P were essential nutrients for the plants growth, thus, aeration affected the plants growth (Table 1).

Aeration also affected anti-enzyme activities of the plants. Enzyme activity was associated with the physiologic status of the plants. Adversity resulted in the super oxide radicals increased in the plant cells (Jabs, 1999). CAT and POD had synergy effect in scavenging and preventing the active oxygen radicals (Kaufmann *et al.* 2002; Martí *et al.* 2009). POD responded sensitively to the adverse conditions (Tan *et al.* 2006) and it was obviously associated with the plant resistance (Li *et al.* 2000).

Aquatic plant growth was usually determined by water quality, sediment mud quality and hydrological conditions. In this study, because it was a water culture experiment, there was no effect of the sediment mud on the plants growth. Continuous pressurized (convective) gas flow from the aerator changed the microcosmic hydrological conditions of the tanks. The pressurized (convective) gas flow most likely injured some exposed roots, especially the tender ones, affecting the plants growth. And the plants responded physiologically, such as the decline of Chla, Chlb and SP contents and the increase of POD and CAT activities (Table 2). The mechanism of this phenomenon was different to the reports of Li *et al.* (2007). They reported that while the aquatic plant was soaked in heavily polluted water for a long period, the POD activity of the plant roots increased, due to the absorption for numerous soluble pollutants from the water. Recently, other reports indicated (Lizskay *et al.* 2003; Hou *et al.* 2004) that POD played an important role healing the injury for the plant, distributing extensively in the callus and it was associated with the plant cell growth and the plant structure regeneration.

Effects of aeration on the polluted water qualities

Long period continuous aeration influenced the COD_{cr}, N and P removal rates of the polluted river water (Table 3).

Table 3 indicated that aeration affected the water purification. For the tank with *P. cordata*, DO concentrations, COD_{cr}, TN and NH₄⁺-N removals under the aeration conditions increased, respectively. While the removal ratios of TP and soluble P under two conditions did not vary significantly. For the tank with *N. tetragona*, DO increased 1.57 mg/L under the aeration conditions, while COD_{cr}, TN, NH₄⁺-N, TP and soluble P

removal ratios decreased under the non aeration conditions. Aquatic plants were dependent on transpiration to absorb and remove the soluble N and P of polluted water (Luo *et al.* 2006; Suthar and Singh, 2008). The decrease of soluble P removal under the aeration conditions was because the soluble P sedimentation and the plant transpiration were influenced by the aeration. In the aeration tank with *P. cordata*, higher DO concentration was suitable for heterotrophic microbes to remove COD_{cr}. Lower pH value in the aeration tank was resulted from the higher NH₄⁺-N removal. For the tank with *N. tetragona*, the pH value decrease under the non aeration conditions was due to the higher NH₄⁺-N removal. COD_{cr} removal increase under the non aeration conditions was due to the obviously larger area of the roots surface, leading to more heterotrophic microbes of the biofilm on the roots developed, causing the better degradation and decomposition of the organic pollutants. Additionally, good sedimentation in the non aeration tank also played a role in removing COD_{cr}.

Effects of aeration on the N removal of the polluted water

Aeration affected N removal of the polluted water by the aquatic plants. NH₄⁺-N of polluted water was the important source for the N require of the aquatic plant tissues (Fan and Li, 2005). Friday and Quarmby (1994) reported that when NH₄⁺-N existed along with NO₃⁻-N, NH₄⁺-N had advantage to be absorbed by the aquatic plants. The most important factor influencing N absorption was the root surface area, inner structure and water quality (Fan and Li, 2005). In this study, for *N. tetragona*, effects of aeration on the plant transpiration played a role in removing N. Most importantly, in the aeration tank, the shorter and the lower density of the plant roots resulted in the decline of microbes of the biofilm on the roots. Therefore, although there was higher DO in the aeration tank, the N removals between two types tanks did not vary significantly. In particular, although DO concentration in the aeration tank was about 2.73 times of the non aeration tank (Table 3), being suitable for aerobic microbes to degrade and decompose NH₄⁺-N (Bodelier *et al.* 1996; Kowalchuk *et al.* 1998; Kirk and Kronzucker, 2005; Scholz and Lee, 2005; Ouellet-Plamondon *et al.* 2006), aeration significantly affected the growth of the plants, leading to the obvious decline of the root length and the root density. The root length and the root density in the non aeration tank were about 1.98 times and 2.80 times

Table 3: Effects of aeration on the water purification by the aquatic plants

Means	N -	N +	P -	P +
TP removal rate (%)	30.43 ± 2.01 a	22.01 ± 0.890 b	34.35 ± 2.37 a	30.69 ± 2.11 a
Soluble P removal rate (%)	30.00 ± 2.16 a	21.95 ± 0.840 b	37.50 ± 2.18 a	34.31 ± 2.02 a
TN removal rate (%)	27.08 ± 1.65 a	23.51 ± 1.170 a	30.69 ± 1.49 a	45.13 ± 2.01 b
NH ₄ ⁺ -N removal rate (%)	29.63 ± 1.56 a	25.35 ± 1.590 a	33.28 ± 2.09 a	49.34 ± 3.07 b
COD _{cr} removal rate (%)	43.62 ± 2.83 a	39.57 ± 2.370 a	48.32 ± 2.74 a	60.65 ± 3.31 b
DO concentration (mg/L)	0.91 ± 0.04 a	2.48 ± 0.120 b	0.98 ± 0.05 a	2.56 ± 0.13 b
pH value	8.2 ± 0.10 a	8.3 ± 0.100 a	8.1 ± 0.10 a	7.7 ± 0.10 b
Water temperature (°C)	26.1 ± 0.10 a	25.3 ± 0.100 a	26.0 ± 0.10 a	25.1 ± 0.10 a

Mean ± S.D. (n = 3), Means in the same line followed by the same letter (a, b) are not significantly different at P-value < 0.05 based on *t* test. N and P were *N. tetragona* and *P. cordata*, + and - were aeration and non aeration conditions

of the aeration tank (Table 1), leading to the significant variation between the root surface areas of two tanks, which most possibly made the microbes of the biofilm on the roots vary significantly. In addition, aeration affected the N absorption of the plant roots.

For the tank with *P. cordata*, although N absorption of per plant declined under the aeration conditions, TN and NH₄⁺-N removals were obviously higher than under the non aeration conditions (Table 3), since the DO increased significantly in the aeration tank, which was suitable for anoxic and aerobic microbes (e. g. nitrite bacteria and nitrifying bacteria) of the biofilm on the roots to decompose NH₄⁺-N (Bodelier *et al.*, 1996; Kowalchuk *et al.*, 1998; Kirk and Kronzucker, 2005). Luo *et al.* (2006) reported that N absorption of the aquatic plant was only approximately 17% of the N removal of the polluted water, and the most N removal was critically performed by microbes (Bodelier *et al.* 1996; Kowalchuk *et al.* 1998; Kirk and Kronzucker, 2005). In particular, the root in the non aeration tank was longer than in the aeration tank, approximately 1.76 times (Table 1), while the root density of the aeration tank was approximately 1.67 times of the non aeration tank (Table 1). Thus, for *P. cordata*, the surface areas of the roots between two tanks most possibly did not vary significantly. However, DO in the aeration tank was approximately 2.61 times of the non aeration tank (Table 3), which was more suitable for aerobic microbes to degrade and decompose NH₄⁺-N (Bodelier *et al.*, 1996; Kowalchuk *et al.*, 1998; Kirk and Kronzucker, 2005), leading to the obvious increase of the N removal.

Effects of aeration on the P removal of the polluted water

P removal of the polluted water was affected by the aeration. Soluble P absorption by the roots from polluted water was determined by the biological character of the root and the water quality (Fan and Li,

2005). After soluble P was absorbed by the roots from water (Thiebaut and Muller, 2003), it was then transported to the shoots (Baldy *et al.*, 2007). For *N. tetragona*, tiller, biomass, root length, root density and the P content of the plant all obviously decreased under the aeration conditions (Tables 1 and 2). Compared to the non aeration conditions, the removal of TP and soluble P of the polluted water by the plant obviously decreased 8.42 % and 8.05 % (Table 3) under the aeration conditions. However, for the tank with *P. cordata*, P removals of the polluted water under two conditions did not vary significantly (Table 3). It was because although biomass, root length and the P content of per plant decreased, tillers and root density increased obviously under the aeration conditions.

In general, effects of aeration on the TN and TP removals of the water by *N. tetragona* were more significant than by *P. cordata*. Under the aeration conditions, TN and TP removals of the water by *N. tetragona* were 23.51% and 22.01% and they turned to 45.13 % and 30.69 % by *P. cordata*, respectively. Therefore, the TN and TP removals of the water by *P. cordata* were better than by *N. tetragona* under the aeration conditions.

Effects of the aeration on the morphological and physiological characteristics of *N. tetragona* and the water purification by the plant were more significant than *P. cordata*. In particular, aeration did not affect the POD activity and the SP content significantly for *P. cordata*, whereas they varied significantly for *N. tetragona*. Aeration contributed to the obvious decline of both the tillers and the roots density of *N. tetragona*, while they changed contrarily for *P. cordata*. Therefore, *P. cordata* comparatively developed better than *N. tetragona* under the same aeration conditions. Aeration resulted in the decline of COD_{cr}, TN and NH₄⁺-N removals and a somewhat



increase of pH value in the tank with *N. tetragona*, while COD_{cr}, TN and NH₄⁺-N removals in the aeration tank with *P. cordata* were higher than in the non aeration tank and directed to a somewhat decrease of the pH value in the aeration tank. Furthermore, TN and TP removals of the water by *P. cordata* were higher than by *N. tetragona* under the aeration conditions. Therefore, in the ecological restoration engineering of stagnant water, when different aquatic plants (such as *P. cordata* with good growth and water purification ability, *N. tetragona* with good ornamental value and less prominent ability of the water purification and poor develop character) were prepared to plant on the floating-beds, the layout of the planted sites and the aerators sites should be optimized carefully. Only in this way, effective water purification was able to be achieved and maintained continuously, along with the environment beautification.

Same analysis was performed on the other two days April 26th 2008 and July 19th 2008 and the similar results were obtained (data omitted). The results indicated that effects of aeration on the aquatic plants and the water purification were more significant on October 11st 2008 than on the other two days under the similar growth conditions as the former day for the plants, which was possibly due to the difference of the plants' physiological status at their different growth stages.

Finally, it can be concluded, both *N. tetragona* and *P. cordata* developed well and had good removals of N and P in heavily polluted stagnant water. While long period continuous aeration affected the physiological status of the aquatic plants and the N and P removals of the polluted water. The effects became more significant on *N. tetragona* than *P. cordata*. Change in the physiological response of the aquatic plants for the aeration generally corresponded to the variation of N and P removals of the polluted water.

Aeration contributed to the increase of POD and CAT activities of two plants and the decrease of SP, Chla, Chlb, N and P contents and biomass of the plants. Aeration resulted in the tillers and the roots densities of *N. tetragona* decreased, while they changed contrarily for *P. cordata*.

Under the aeration conditions, for the tank with *N. tetragona*, TP and soluble P removals of polluted water obviously decreased, and for the tank with *P. cordata*, TN and NH₄⁺-N removals of polluted water obviously increased. The TP and soluble P removals for the tank with *P. cordata* and the TN and

NH₄⁺-N removals for the tank with *N. tetragona*, did not vary significantly under two conditions, respectively. *P. cordata* was more suitable to develop and remove TN and TP of the polluted water than *N. tetragona* under the aeration conditions, and the removal rates reached 45.13 % and 30.69 %, respectively.

In conclusion, the effects of artificial aeration on the physiological characteristics of the aquatic plants were heavier at the final growth stages of the plants than at the other growth stages. Particularly, they were more significant on *N. tetragona* than on *P. cordata*. However, the change tendency of the water qualities differed to the variation of the physiological characteristics of the plants.

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