
Enhancement of nitrate removal at the sediment-water interface by carbon addition plus vertical mixing

Xuechu Chen*^{a,b}, Shengbing He^a, Yueping Zhang^c, Xiaobo Huang^a, Yingying Huang^a,
Danyue Chen^a, Jianwu Tang^b, Xiaochen Huang^c

^a School of Environmental Science and Engineering, Shanghai Jiao Tong University, Dong Chuan Road 800, Shanghai, 200240, P. R. China.

^b Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

^c Shanghai East Sea Marine Engineering Survey & Design Institute, Shanghai 200137, P.R. China

Abstract

Wetlands and ponds are frequently used to remove nitrate from effluents or runoffs. However, the efficiency of this approach is limited. Based on the assumption that introducing vertical mixing to water column plus carbon addition would benefit the diffusion across the sediment–water interface, we conducted simulation experiments to identify a method for enhancing nitrate removal. The results suggested that the sediment-water interface has a great potential for nitrate removal, and the potential can be activated after several days of acclimation. Adding additional carbon plus mixing significantly increases the nitrate removal capacity, and the removal of total nitrogen (TN) and nitrate-nitrogen (NO_3^- -N) is well fitted to a first-order reaction model. Adding *Hydrilla verticillata* debris as a carbon source increased nitrate removal, whereas adding *Eichhornia crassipe* decreased it. Adding ethanol plus

*Corresponding author Tel: +86-21-34203734 FAX: +86-21-54740825 E-mail address: cxcsnow@163.com

mixing greatly improved the removal performance, with the removal rate of NO_3^- -N and TN reaching 15.0-16.5 $\text{g m}^{-2} \text{d}^{-1}$. The feasibility of this enhancement method was further confirmed with a wetland microcosm, and the NO_3^- -N removal rate maintained at 10.0-12.0 $\text{g m}^{-2} \text{d}^{-1}$ at a hydraulic loading rate of 0.5 m d^{-1} .

Keyword

Nitrate removal, Sediment-water interface, Carbon addition, Vertical mixing

1. Introduction

Surface runoffs in some valleys are under a high load of nitrate from sewage plants and agricultural non-point sources. These runoffs ultimately flow into lakes or estuaries and are likely to cause the occurrence of harmful algal blooms (Kroeger et al., 2006; Galloway et al., 2008; Suddick et al., 2013). In many countries, such as the United States and China, in order to remove nitrate from effluents or runoffs, wetlands, riparian buffer strips, and ponds have been restored or artificially constructed at the interface between cultivated land and receiving waters. However, the efficiency of these ecological approaches is limited, and a large amount of land is usually required to achieve satisfactory removal (Jorgensen et al., 1994; Reilly et al., 1999; Coveney et al., 2002). Therefore, developing measures to improve nitrate removal is of increasing interest.

Denitrification is a microbial-mediated process that uses nitrate as an electron acceptor and organic carbon as an electron donor to obtain energy for growth and maintenance. In this stepwise process, nitrite, nitric oxide and nitrous oxide are produced as intermediate products and nitrogen (N_2) as the gaseous end product. The availability of carbon and nitrate, oxygen concentration, pH and temperature are main parameters influencing denitrification. In wetlands, plant productivity, either from biomass or

root release can be used as the internal carbon source to fuel denitrification. Previous studies suggested that supplying additional carbon is an alternative measure for improving nitrate removal in wetlands or ponds. It can be achieved by optimizing the physical habitat for maximum plant productivity or harvesting the plants but leaving the residuals in place (Lin et al., 2002; Bastviken et al., 2005; Wen et al., 2010). However, the improvement in nitrate removal by such internal carbon sources is limited and is usually below $1 \text{ g m}^{-2} \text{ d}^{-1}$. When an external carbon source such as acetate is added, the nitrate removal capacity would increase to its maximum, about $4 \text{ g m}^{-2} \text{ d}^{-1}$. (Ingersoll and Baker, 1998; Vidal et al., 2002; Pungrasmi et al., 2013).

Unlike the biological denitrification process, which involves controlling requisite anoxic conditions in a bioreactor, wetland denitrification occurs in anoxic zones of the sediment–water interface or in anoxic microsites of a biofilm attached to plant tissue or substrata (Pfenning and McMahon, 1997; Bruesewitz et al., 2011; Yu et al., 2012; Finlay et al., 2013). The sediment-water interface is a shallow, oxic-anoxic layer that separates a mixture of solid sediment and interstitial water from an overlying body of water (Lerman, 1978; Sweerts et al., 1991). Recent studies on natural wetlands and ponds have indicated that the nitrate and carbon across the water-sediment interface would be consumed more quickly than with an internal vertical diffusive supply, and as a result, limiting the nitrate removal (Reinhardt et al., 2006; Wilson et al., 2013). Accordingly, when supplying additional carbon to wetlands or ponds, the diffusive carbon and nitrate supply across sediment –water interface might become a limiting factor instead of carbon source itself. Therefore, we hypothesize that introducing vertical mixing to water column plus carbon addition would benefit the diffusion across sediment–water interface, and thus further enhance the nitrite removal capacity to an extremely high level.

To our knowledge, although previous studies investigated various kinds of carbon sources that can

enhance nitrate removal in wetlands or ponds, rare studies focus on regulating mass diffusing across sediment – water interface. This study focuses on nitrate removal at the sediment-water interface of wetlands or pond systems with the following objectives: to comprehensively investigate the nitrate removal potential at sediment–water interface; to determine the mechanism for the increase of nitrate removal by carbon addition plus vertical mixing; to identify a feasible method for engineering application.

2. Materials and Methods

2.1 Batch test

The experiment was conducted in growth chambers with the temperature maintained at 25-26 °C. The reactor for the batch tests was a small glass cylinder with a diameter of 15 cm and a height of 25 cm. The surface sediment samples (0-10 cm) were collected from a lake located at the Shanghai Jiao Tong University, Minhang campus, by using Ekman-type grab sampler (15.2cm × 15.2cm). After removing the gravel and plant and animal residues, the sediment samples were mixed and transferred to the cylinders, maintaining a layer of 5 cm. Then, 15 cm of lake water was injected into each cylinder, and stabilized for 24 h before the tests were initiated. There was a slight variation (<5%) in the average concentration of total nitrogen (TN), nitrite-nitrogen (NO_2^- -N), nitrate-nitrogen (NO_3^- -N), and dissolved oxygen (DO) in the overlying water before and after stabilization, whereas the ammonia-nitrogen (NH_4^+ -N) increased from 0.71 mg L⁻¹ to 1.14 mg L⁻¹. This result suggests that the release of nitrogen from the sediment is limited. After stabilization, NaNO_3 was added to each cylinder to obtain a high nitrate concentration for further investigating the removal capacity. As the amount of external NO_3^- -N was much higher than the NH_4^+ -N released from sediment, we suggested that coupled denitrification was not the main nitrate loss path during our experiments.

The batch tests were designed to illustrate the nitrate removal under different sediment-water interface conditions and its potential. The tests were divided into two phases. In phase I, the experiment had 5 treatments, and each treatment had two duplicates. For treatment 1[#] and treatment 2[#], the sediment surface was covered with gravel and zeolite (particle size of 4-8 mm, approximately 1-2 cm deep), respectively. For treatment 3[#], methanol was added as an additional carbon source (methanol/NO₃⁻-N mass ratio of 4:1). For treatment 4[#], the overlying water was mixed with a peristaltic pump at a flow rate of 25 mL min⁻¹. Treatment 5[#] was the control. In phase II, the overlying water was adjusted with methanol and NaNO₃ to maintain the methanol/NO₃⁻-N mass ratio at 4:1. During the experiment, when the TN and nitrate concentration decreased to a relatively low level, lake water with same methanol/NO₃⁻-N mass ratio was again added to each cylinder. A total of four rounds of continuous removal experiments were performed. Sampling was performed every 1-2 d. Before sampling, the overlying water was mixed for approximately 5 s with a peristaltic pump. Then, 15 mL water was sampled from the upper, middle and lower layer of the overlying water. The sample was mixed for further analysis. To maintain a constant water volume, deionized water was added after each sampling. During the entire experimental process, the pH value was maintained between 7.0 and 7.5.

2.2 Column experiment

The experimental reactor was a Y-shape structure, comprising two polymethyl methacrylate columns (He et al., 2013). The main column had a 20-cm-inner diameter and was 120 cm in height, and the side cylinder portion had a 20 cm-inner diameter and was 20 cm in height. Both ends of the columns were sealed with a plastic stopper. Two mechanical agitators, fixed on the stoppers, were used to stir the water. Prior to the experiment, sediment that was corrected and treated as described above was filled to the main column, maintaining a layer of 10 cm. Then, lake water was injected into the experimental apparatus for

a 100 cm deep, and stabilized for 24 h. The equivalent height of overlying water was approximately 1.2 m when taking the side cylinder portion into account. During the experiments, NaNO_3 was added to obtain a high nitrate concentration for evaluation. Ethanol, methanol, and *Hydrilla verticillata* and *Eichhornia crassipes* debris were added as carbon sources. To introduce the proper mixing conditions, the frequencies of the vertical and side agitator were maintained at 80 and 36 r min^{-1} , respectively. These conditions permitted mixing of the overlying water without disturbing the sediment surface because the suspended matter rarely increased when the agitators operated. The pH value of the overlying water was maintained at 7.0-8.0. The water temperature was approximately 30 ± 1 °C. Each day, approximately 20 mL water was sampled from the upper, middle and lower layers of the overlying water and was mixed for further analysis. For the methanol addition treatment, when the nitrate concentration of the overlying water decreased to a low level, nitrate and organic carbon sources were added again.

2.3 Wetland microcosm

The wetland microcosm was comprised of polymethyl methacrylate and consisted of two main units and three water distribution cells (Fig. 1). The first unit was a floating plants pond, primarily designed for denitrification. The pond was 45 cm \times 60 cm \times 70 cm (inner width, length, and height) in size, with a water depth maintained at 60 cm. The surface of the pond was covered by duckweed, and the bottom was filled with 10 centimeters of sediment. During the experiment, the overlying water was mixed with a pump with a flow rate of 120 mL min^{-1} . The second unit was a gravel bed filter, measuring 15 cm \times 60 cm \times 50 cm (inner width, length, and height). It was filled with 1- to 2-cm-diameter gravel, and an aeration device was installed at the bottom to provide aerobic conditions and allow for chemical oxygen demand (COD) removal and nitrification through contact with the surface of the gravel. To optimize the water flow, three water distribution cells with perforated baffles were located between the treatment units.

The source water was tap water augmented with NaNO₃ to a concentration of 20-25 mg NO₃⁻-N L⁻¹. The water was pumped into the wetland and then flowed through the floating plant pond and gravel bed filter. The water temperature during the experiment was approximately 26±1 °C. The main hydraulic loading rate of the wetland was maintained at 0.5 m d⁻¹ with a hydraulic retention time of approximately 32 hours.

2.4 Analysis methods

The samples were analyzed for TN and COD_{cr}, and the filtered samples were analyzed for NH₄⁺-N, NO₃⁻-N, NO₂⁻-N. All analytical measurements were recorded according to Standard Methods (APHA, 1995). According to results of the batch tests (Fig. 2), the nitrate removal was estimated by the following formula: $C=C_0e^{-kT}$, where C is the TN or NO₃⁻-N concentration in the control and treatments, k is the specific removal rate, and T is the incubation time. The error bars was analyzed by using SPSS13.0 statistical soft.

3. Results and discussion

3.1 Nitrate removal under different sediment-water interface conditions and its potential

Fig. 2 shows nitrate removal under different sediment-water interface conditions. The reduction of TN and NO₃⁻-N was well fitted by an exponential curve. The coefficients of determination (R²) ranged from 0.87 to 0.99, suggesting the reaction followed the first-order reaction model. The amount of NO₃⁻-N removal accounted for the majority amount of TN removal (always above 80%), suggesting that denitrification was the primary pathway of nitrogen loss during the experiment. The incubations with methanol as the carbon source showed the highest specific NO₃⁻-N removal rate of 1.27d⁻¹, and within 3 days, the NO₃⁻-N concentration decreased from 9.8 mg L⁻¹ to almost 0 mg L⁻¹. Of the other four treatments without additional carbon, the treatment with mixing had the highest TN and NO₃⁻-N removal

rate (0.15d^{-1} and 0.17d^{-1} respectively), indicating that mixing promotes nitrate removal. To test our presumption that mass diffusion across sediment-water interface influences nitrate removal, the sediment surface of two incubations was covered with bed media, gravel and zeolite. As anticipated, the covering inhibited the nitrate removal as the specific removal rates of these two treatments were much lower than the incubations with the natural water-sediment interface.

Because denitrification is a microbial-mediated process, NO_3^- -N removal at the sediment-water interface may increase after acclimation of denitrifying bacteria. Therefore, we conducted four rounds of continuous removal experiments with a methanol/ NO_3^- -N mass ratio of 4:1 to investigate the nitrate removal potential (Fig.3). An increase in NO_3^- -N removal was maintained during the first three rounds of the experiments (0-4 d, 5-7 d and 8-10 d), and for the 4th round, the average NO_3^- -N removal rate increased to $2.31\text{g m}^{-2}\text{d}^{-1}$. Comparatively, the incubation without additional carbon showed a low efficiency of nitrate removal, with an average removal rate of $0.21\text{g m}^{-2}\text{d}^{-1}$. After 13 days of incubation, we assume that the denitrifying bacteria were acclimated, and the nitrate removal potential in water-sediment interface was at its limit. Thereafter, the overlying water was adjusted to a higher NO_3^- -N concentration of 20mg L^{-1} , with a methanol/ NO_3^- -N mass ratio of 4:1. The frequency of sampling was shortened to hours to reflect the dynamics of the nitrate removal. As shown in Fig. 4, the result confirmed that the removal of TN and NO_3^- -N was well fitted to a first-order reaction model.

3.2 The combined effect of mixing and carbon addition on nitrate removal

The combined effect of mixing and carbon addition on nitrate removal was investigated through column experiments. During phase I of the experiments, methanol was added as a carbon source (methanol / NO_3^- -N mass ratio of 4:1). One treatment was mixed, and the other treatment was not. As shown in Fig.5, the results verified that mixing accelerated nitrate removal. After 9 days of acclimation,

the NO_3^- -N removal increased to a relatively high level. During the next three rounds of the experiment, the NO_3^- -N and TN in carbon addition plus mixing column decreased rapidly, with an average removal rate of $5.68 \text{ g m}^{-2} \text{ d}^{-1}$ and $5.17 \text{ g m}^{-2} \text{ d}^{-1}$, respectively. Comparatively, in the carbon addition without mixing column, a much lower rate of removal of NO_3^- -N and TN was maintained, with an average removal rate of $2.91 \text{ g m}^{-2} \text{ d}^{-1}$ and $2.72 \text{ g m}^{-2} \text{ d}^{-1}$. The positive effect of mixing on nitrate removal can be explained by the increase of the diffusive NO_3^- and carbon supply across the water-sediment interface as mentioned above.

The main objective of phase II was to determine the influence of the different carbon sources combined with mixing. Different carbon sources, including the debris of the floating plant *Eichhornia crassipes* and the submerged plant *Hydrilla verticillata* and ethanol were added to the mixing columns. As shown in fig.6, *Hydrilla verticillata* promotes NO_3^- -N removal compared to control column. However, in the column with *Eichhornia crassipes*, the lowest NO_3^- -N removal was observed. Additional studies suggested that a large amount of NH_4^+ was released with the decomposition of *Eichhornia crassipes*. This may be the reason for the low removal of NO_3^- -N. For the ethanol addition (ethanol / NO_3^- -N mass ratio of 4:1) plus mixing column, the NO_3^- -N removal was the highest. The NO_3^- -N decreased from 7.4 mg L^{-1} to 0.6 mg L^{-1} , with a removal rate of $1.6 \text{ g m}^{-2} \text{ d}^{-1}$ during the first 5 days. On the 10th day, ethanol and NO_3^- -N was added again, and a much higher removal rate was obtained. This increase in the removal rate was similar to the batch test, indicating that the denitrifying bacteria were gradually acclimated. Thereafter, another three rounds of removal experiments were conducted with the same ethanol / NO_3^- -N mass ratio. During these treatments, the removal rate of NO_3^- -N and TN was as high as $15.0\text{-}16.5 \text{ g m}^{-2} \text{ d}^{-1}$.

The column experiments show that although the decomposable plant material had a positive effect on

denitrification, the increase was very limited. Comparatively, molecular organic compounds have high bioavailability and are easy to metabolize. Studies had shown that adding molecular organic compounds can significantly increase the nitrate removal in wetlands. For example, Kozub and Liehr (1999) performed experiments on wetland microcosms, and they observed that adding acetate as an external carbon source led to significantly increased denitrification rates. However, the reported nitrate removal capacity was always below $5 \text{ g m}^{-2} \text{ d}^{-1}$. As suggested in our column experiment, ethanol and methanol addition plus mixing significantly improved the nitrate removal. After 14 days of acclimation, the nitrate removal rate increased approximately 10 fold, from $1.6 \text{ g m}^{-2} \text{ d}^{-1}$ to $16.5 \text{ g m}^{-2} \text{ d}^{-1}$. Comparatively, the ethanol addition treatment showed a higher nitrate removal capacity than the methanol treatment.

3.3 Wetland microcosm

To verify the feasibility of enhancing nitrate removal by carbon addition plus mixing, a small-scale experiment was conducted with a wetland microcosm. A steady state was achieved after 10 days of operation, and the removal efficiency in the two units remained almost constant for the following 25 days. The concentrations of pollutants in the influent and effluents were averaged, and the values for the pollutant load, pollutant removal, removal efficiency, and removal of pollutant load were calculated to confirm the removal performance of the different units. The results are summarized in Table 1. In general, the system performed well for TN and NO_3^- -N removal, and the removal efficiencies of TN and NO_3^- -N all exceeded 90% at a hydraulic loading rate of 0.5 m/d. The floating plant pond also exhibited significant nitrogen removal capacity. Approximately 89.0% of the TN load and 91.1% of the NO_3^- load were eliminated in the floating plant pond. The removal rate of NO_3^- -N and TN was as high as 10.0 - $12.0 \text{ g m}^{-2} \text{ d}^{-1}$, suggesting that denitrification was successfully achieved. By comparison, the highest reduction rate of our wetland microcosm was approximately $12 \text{ g m}^{-2} \text{ d}^{-1}$, much higher than the majority of values

achieved in wetlands and ponds. Therefore, as shown with the successful operation of this small-scale wetland, simple engineering measures can efficiently remove nitrate.

3.4 Implications for practice

In wetlands and ponds, the overlying water is usually aerobic, and the bottom water DO declines steeply when it approaches to sediment. As a result, an oxic-anoxic layer forms at the sediment-water interface. Such oxic-anoxic layer, although as shallow as 10 mm, has a remarkable biological activity, and can favor both coupled and decoupled denitrification (Sweerts et al., 1991; Jensen, et al., 1993; Wilson, et al., 2013). According to recent studies, steep vertical profiles of nitrate and carbon are likely to occur at sediment-water interface, and further limit the benthic denitrification (Reinhardt, et al., 2006; Wilson, et al., 2013). Our investigation provides evidence that such vertical profiles can be artificially controlled by carbon addition plus vertical mixing, and thus benefits the nitrate removal. However, the vertical profiles of parameters across sediment-water interface in response to the manipulation should be further investigated.

On the other side, sediment-water interface can be considered as a kind of natural substrate that denitrifying bacteria has already attached on it. Therefore, when additional carbon is supplied, it takes a short time to acclimate and to achieve a high nitrate removal. Such rapid response favors engineering practice. Moreover, for practical application, the proper way to mix water should be fixed out. There are two essential issues for the engineering design including: the mixing device should be able to mix a large amount of overlying water at low cost; and meanwhile it should not significantly increase the DO in bottom water. Accordingly, air lifting devices which previously used for artificial destratification in deep reservoirs may be modified to meet the requirement (Heo and Kim, 2004).

4 Conclusions

On the basis of the results we conclude that:

1) The sediment-water interface has a great potential for nitrate removal, and adding additional carbon plus mixing significantly increases the nitrate removal capacity. A first-order reaction model can be used to fit the removal of TN and NO_3^- -N at water-sediment interface.

2) Adding *Hydrilla verticillata* debris as a carbon source increased nitrate removal, whereas adding *Eichhornia crassipe* decreased it. Adding ethanol plus mixing greatly improved the removal performance, with the removal rate of NO_3^- -N and TN reaching 15.0-16.5 $\text{g m}^{-2} \text{d}^{-1}$.

3) The small-scale wetland experiment showed that the NO_3^- -N removal rate of the wetland maintained 10.0-12.0 $\text{g m}^{-2} \text{d}^{-1}$ with a hydraulic loading rate of 0.5 m/d. This is almost the highest removal rate ever reported for free-surface wetland or ponds, suggesting carbon addition plus mixing is a simple but effective measure to improve nitrate removal in wetlands or ponds that receive effluents from sewage plants or agricultural non-point sources.

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Figures and Table

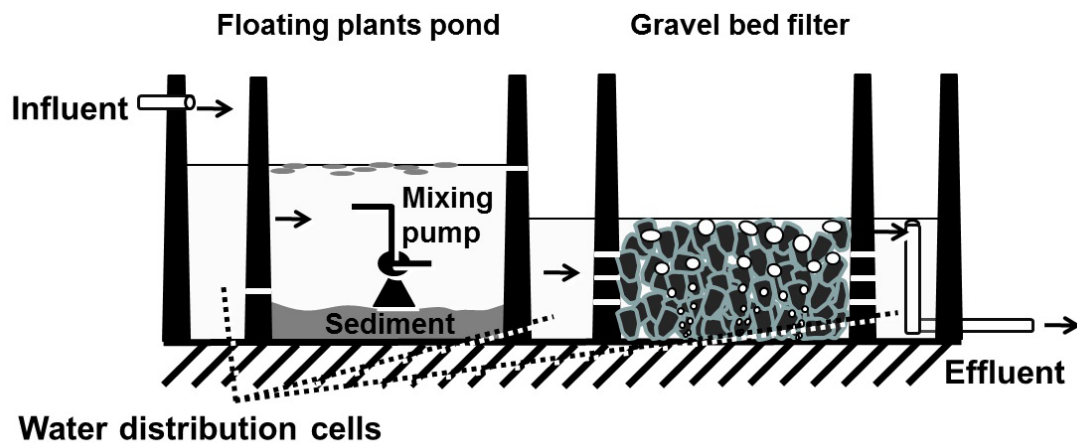


Fig. 1. Schematic diagram of the wetland microcosm experimental set-up.

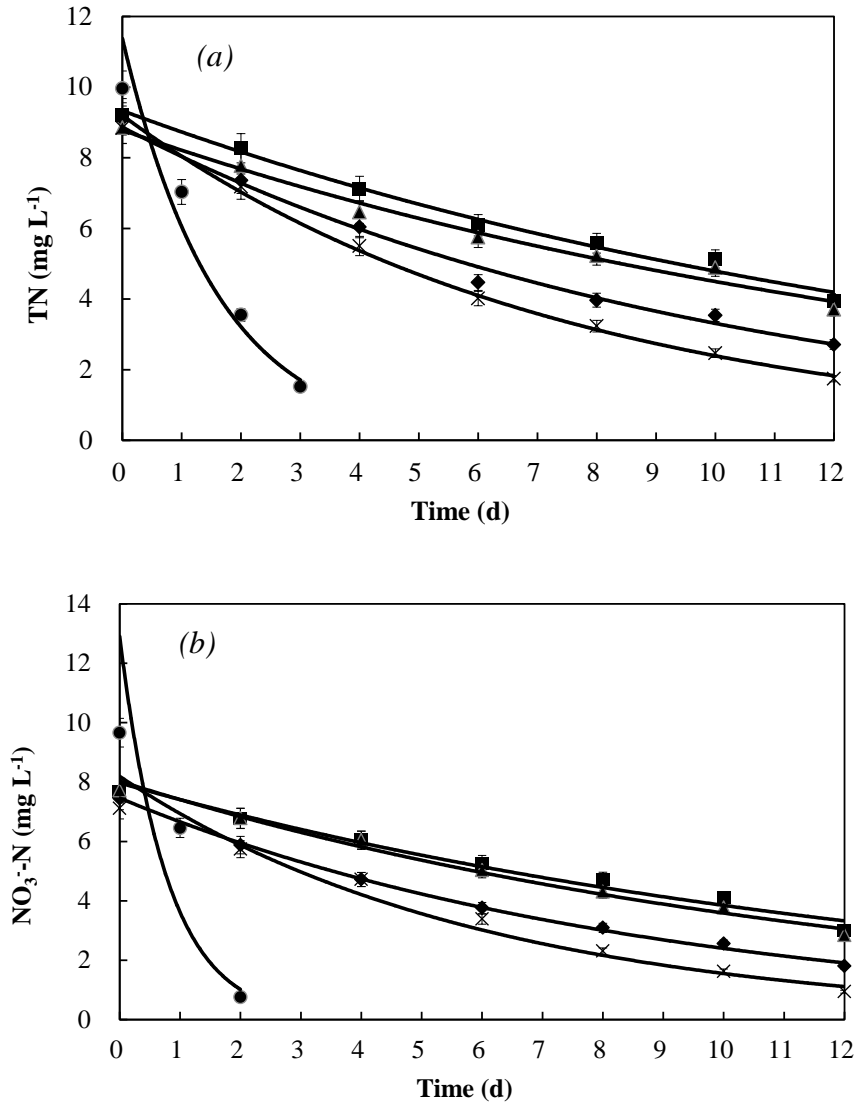


Fig. 2. The nitrate removal under different sediment-water interface conditions and exponential curve

fitting (a) Exponential curve fit for TN concentration as a function of time, -●- for carbon addition,

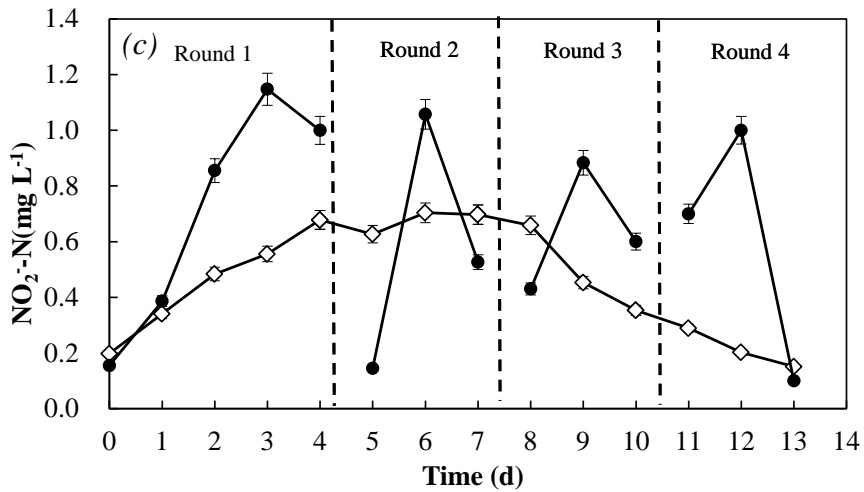
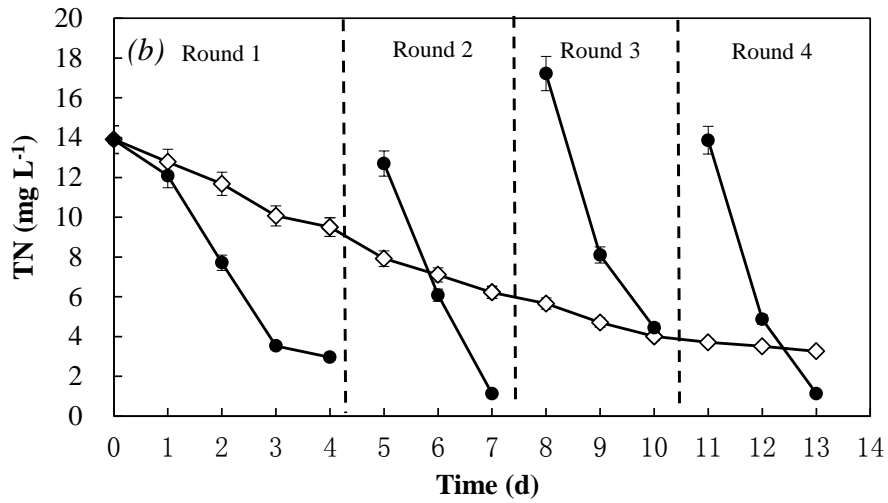
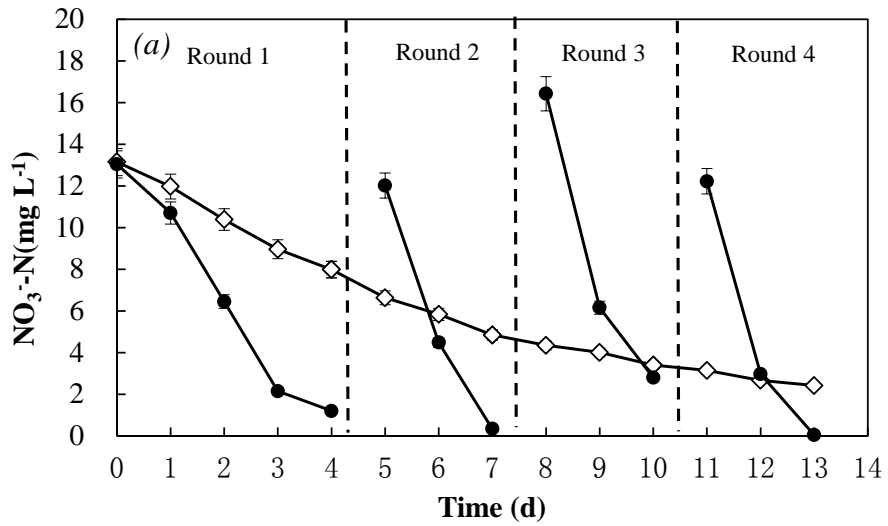
$C=11.38e^{-0.63T}$, $R^2=0.97$; -x- for mixing, $C=9.43e^{-0.15T}$, $R^2=0.99$; -▲- for zeolite covering, $C=9.47e^{-0.071T}$,

$R^2=0.99$; -■- for gravel covering, $C=8.72e^{-0.066T}$, $R^2=0.99$; -◆- for control, $C=8.97e^{-0.10T}$, $R^2=0.99$.

(b) Exponential curve fit for NO_3^- -N concentration as a function of time, -●- for carbon addition,

$C=12.91e^{-1.27T}$, $R^2=0.87$; -x- for mixing, $C=8.19e^{-0.17T}$, $R^2=0.97$; -▲- for zeolite covering, $C=8.03e^{-0.081T}$,

$R^2=0.98$; -■- for gravel covering, $C=7.97e^{-0.073T}$, $R^2=0.96$; -◆- for control, $C=7.46e^{-0.11T}$, $R^2=0.99$.



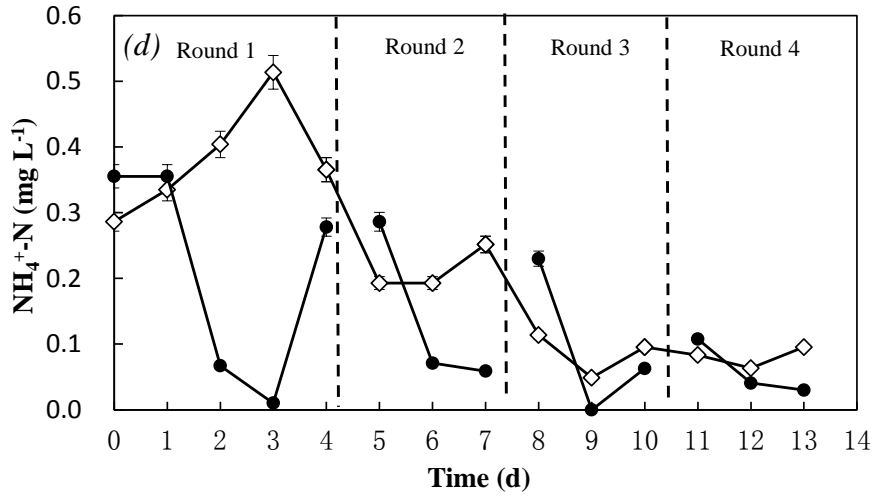


Fig. 3. Enhancement of nitrate removal by carbon addition. (a) $\text{NO}_3^- \text{-N}$; (b) TN; (c) $\text{NO}_2^- \text{-N}$; (d) $\text{NH}_4^+ \text{-N}$,

-●- Carbon addition, -◇-Control

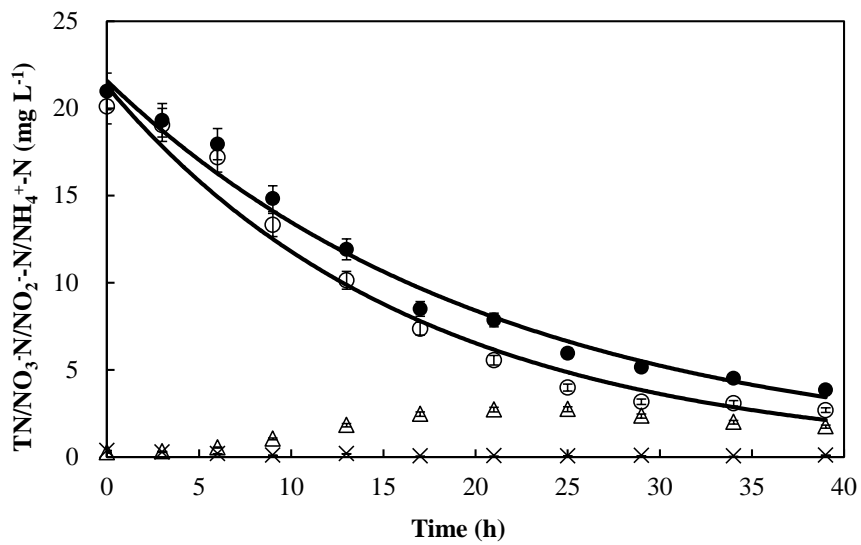


Fig. 4. Dynamic of N removal after acclimation -●- Exponential curve fit for TN as a function of time,

$y=21.57e^{-1.13x}$, $R^2=0.98$; -○- Exponential curve fit for $\text{NO}_3^- \text{-N}$ as a function of time, $y=21.27e^{-1.42x}$,

$R^2=0.97$; -△- $\text{NO}_2^- \text{-N}$; -X- $\text{NH}_4^+ \text{-N}$.

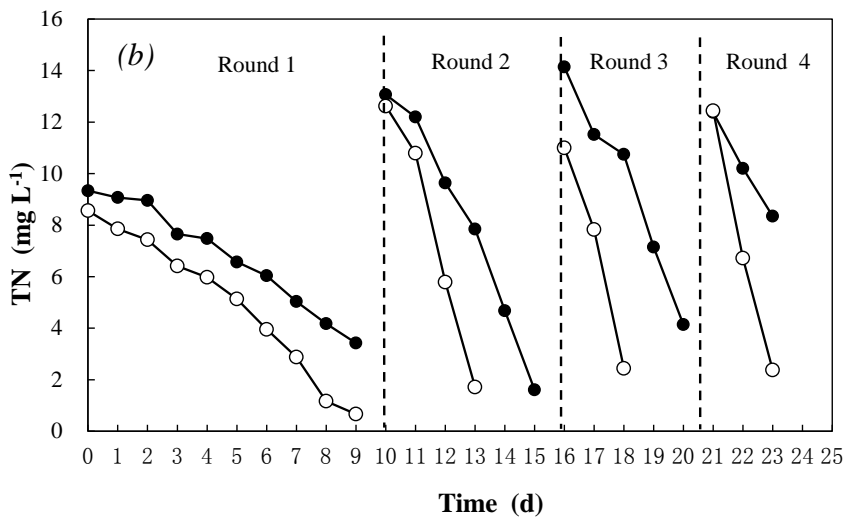
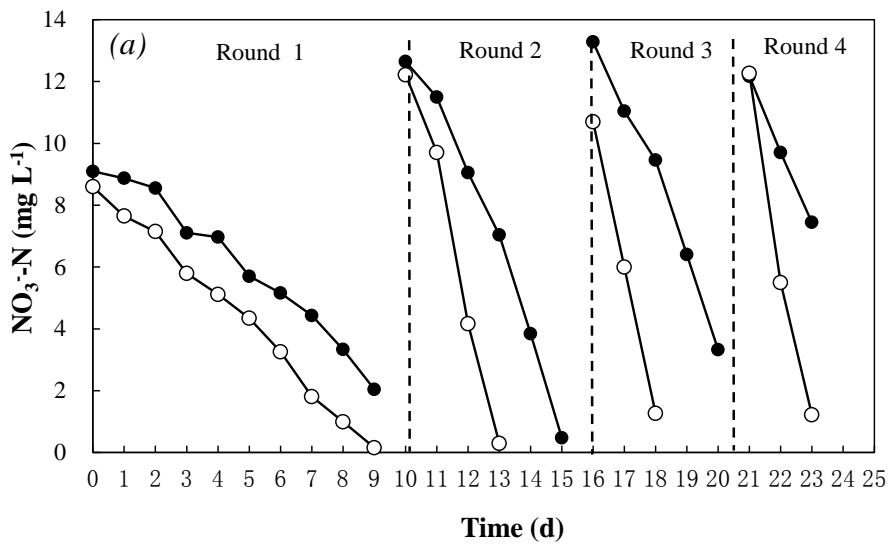


Fig. 5. Influence of mixing on NO₃⁻-N (a) and TN (b) removal. -●- Carbon addition; -○- Carbon addition plus mixing.

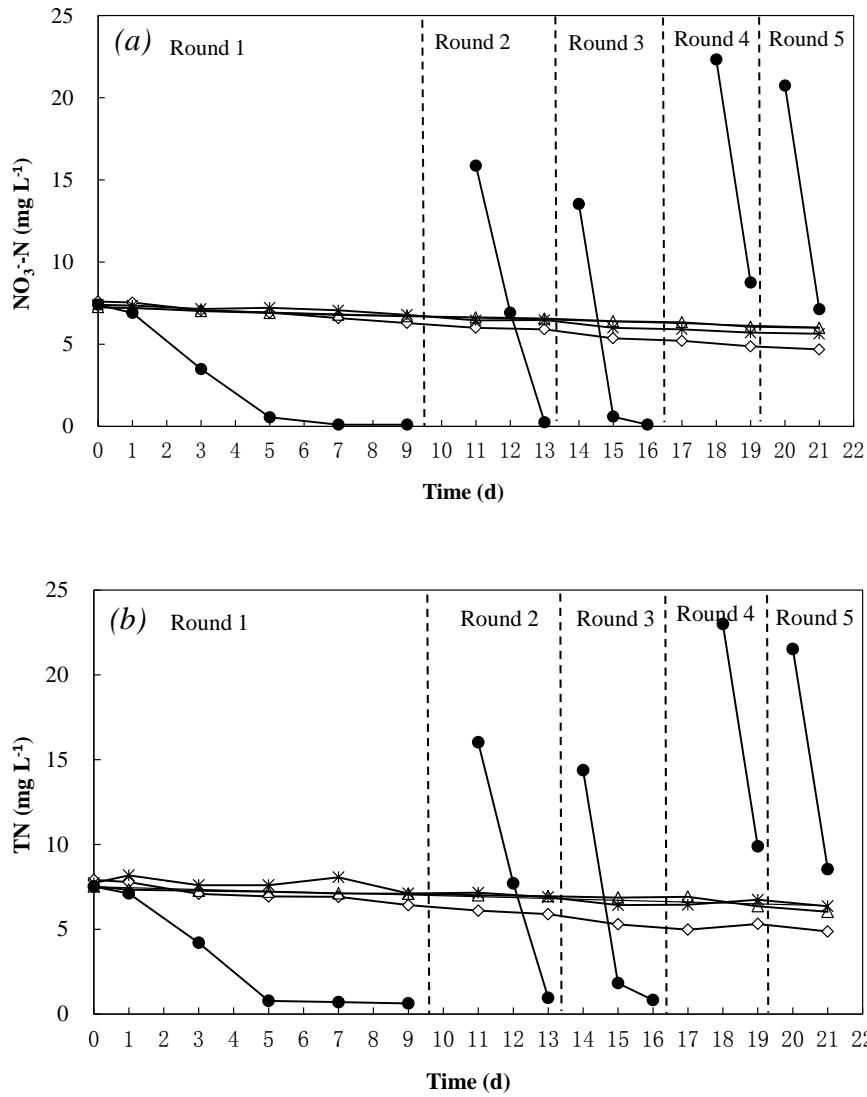


Fig. 6. Influence of carbon sources on NO_3^- -N (a) and TN (b) removal. -●-:Ethanol; -△-:*Eichhornia crassipes*; -◇-:*Hydrilla verticillata*; and -*:-:Control

Table1 Removal performance of the entire purification system and each treatment unit

Sampling locations (n=25)	TN	NO_3^- -N	NO_2^- -N	NH_4^+ -N
Entire treatment system				
Influent Concentration	25.5±1.3	23.6±1.1	0.2±0.1	0.1±0.1
Effluent concentration	2.2±0.5	1.3±0.7	0.6±0.3	0.1±0.2

Pollutant Load ($\text{g m}^{-2}\text{d}^{-1}$)	12.75	11.80	/	/
Pollutant removal ($\text{g m}^{-2}\text{d}^{-1}$)	11.65	11.15	/	/
Removal efficiency (%)	91.4	94.5	/	/
Floating plant pond				
Effluent concentration	2.8±0.2	2.1±0.1	0.5±0.1	0.1±0.1
Removal efficiency (%)	89.0	91.1	/	/
Pollutant removal ($\text{g m}^{-2}\text{d}^{-1}$)	11.35	10.75	/	/
Gravel bed filter				
Effluent concentration	2.2±0.5	1.3±0.7	0.6±0.3	0.1±0.2
Removal efficiency (%)	21.4	38.1	/	/
Pollutant removal ($\text{g m}^{-2}\text{d}^{-1}$)	0.90	1.20	/	/
