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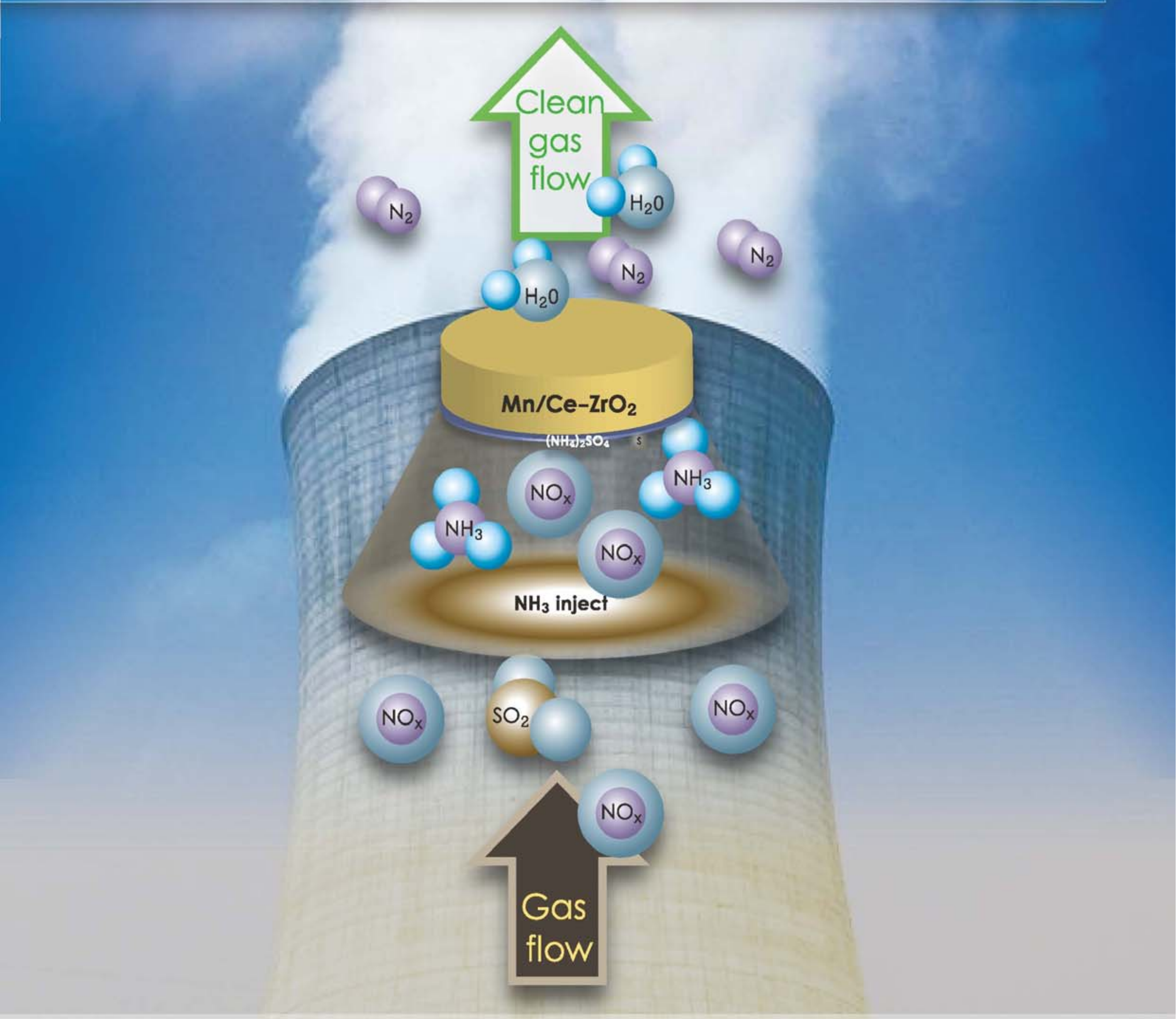
JOURNAL OF ENVIRONMENTAL SCIENCES

ISSN 1001-0742
CN 11-2629/X

April 1, 2013 Volume 25 Number 4

www.jesc.ac.cn



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Chinese Academy of Sciences

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 25 No. 4 2013

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Environmental health and toxicology

Environmental catalysis and materials

Serial parameter: CN 11-2629/X*1989*m*211*en*P*25*2013-4

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ScienceDirect

Journal of Environmental Sciences 2013, 25(4) 670–676

JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Removal of nitrogen from wastewater with perennial ryegrass/artificial aquatic mats biofilm combined system

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Received 07 June 2012; revised 04 November 2012; accepted 06 November 2012

Abstract

To develop a cost-effective combined phytoremediation and biological process, a combined perennial ryegrass/artificial aquatic mat biofilm reactor was used to treat synthetic wastewater. Influent ammonium loading, reflux ratio, hydraulic retention time (HRT) and temperature all had significant effects on the treatment efficiency. The results indicated that the effluent concentration of ammonium increased with increasing influent ammonium loading. The reactor temperature played an important role in the nitrification process. The ammonium removal efficiency significantly decreased from 80% to 30%–50% when the reactor temperature dropped to below 10°C. In addition, the optimal nitrogen removal condition was a reflux ratio of 2. The nitrate and ammonium concentration of the effluent were consistent with the HRT of the combined system. The chemical oxygen demand (COD) removal efficiency was at a high level during the whole experiment, being almost 80% after the start-up, and then mostly above 90%. The direct uptake of N by the perennial ryegrass accounted for 18.17% of the total N removal by the whole system. The perennial ryegrass absorption was a significant contributor to nitrogen removal in the combined system. The result illustrated that the combined perennial ryegrass/artificial aquatic mat biofilm reactor demonstrated good performance in ammonium, total N and COD removal.

Key words: nitrogen; perennial ryegrass; biofilm; wastewater treatment

DOI: 10.1016/S1001-0742(12)60099-0

Introduction

The high content of nitrogen in industrial and agricultural effluent discharged into the environment has caused several problems, such as eutrophication, the greenhouse effect, environmental toxicity and ecological damage (Toet et al., 2005; Hwang et al., 2009). Therefore, nitrogen removal has been one of the most important themes in wastewater treatment processes. Nitrogen removal is mainly achieved through nitrification/denitrification processes, such as the sequencing batch reactor activated sludge, anaerobic/anoxic/oxic, and biofilm processes (Ilies and Mavinic, 2001; Zhang et al., 2007). In aerobic conditions, nitrifying bacteria use O_2 to oxidize ammonia ion (ammonium) to nitrite, and then to nitrate, while the denitrifying bacteria convert nitrate into nitrite, and eventually to gaseous nitrogen using carbon sources (Zhang et al., 2007). Some other novel methods of nitrogen removal have been reported recently, such as simultaneous nitrification/denitrification, short-cut nitrification/denitrification, sharon/anammox and completely autotrophic nitrogen removal over nitrite. In recent years, there has been an increasing interest in biofilm processes for wastewater treatment. The use of biofilm-based reactors offers numerous advantages compared to conventional systems, such as lower space requirement and easy biosolid-liquid separation (Bassin et al., 2012). Biofilm reactors have been proven to be very reliable for nitrogen removal because of their high volumetric loading rates, low solids build-up and efficient oxygen transfer (Semmens et al., 2003; Terada et al., 2003).

Phytoremediation is considered as a simple but effective method for wastewater treatment which requires lower energy consumption. Phytoremediation plays an important role in the wastewater treatment system, with strategies such as constructed wetlands (Lee et al., 2009), oxidation ponds, and floating plant beds (Li et al., 2010). Recent studies showed that perennial ryegrass has a strong ability to absorb and fix nitrogen (Goh and Bruce, 2005). Perennial ryegrass can be the best adapted to moist, cold environments where temperatures are not extreme in

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the winter or summer (Hoffman et al., 2010). Common perennial ryegrass germinates quickly and could be used as a valuable forage and soil stabilization plant. The perennial ryegrass can regrow quickly after cutting. However, it is difficult to control the hydraulic retention time (HRT) and the pollution loading rate when the treatment system is applied at real field sites (Li et al., 2010).

Plant-biofilm systems have been used to treat wastewater locally, which could dramatically reduce the costs of sewage collection and the treatment system construction costs compared with conventional sewage treatment. A plant–biofilm oxidation ditch system was found to be quite satisfactory and stable for the treatment of municipal sewage and polluted lake water (Wu et al., 2006). In addition, a plant floating-bed employing plants, freshwater clams and biofilm carrier effectively removed total N (TN), total organic carbon, and chlorophyll-*a* (Li et al., 2010). Immobilized denitrifying bacteria and aeration were added into the Canna floating bed, which showed that these enhancements substantially improved the nitrogen removal efficiency of the floating beds (Sun et al., 2009). However, plant-biofilm combination systems have not been used to treat other sewage. In addition, there has been no major optimization of operational parameters incorporated in this treatment system. Therefore, process optimization is an important objective of this study. Improving removal efficiency with minimum cost is an environmental objective that must be carried out.

In present study, perennial ryegrass and biofilm were used together to treat synthetic wastewater. The objective of the present research was to explore the removal potential and character of nitrogen by a perennial ryegrass/artificial aquatic mat biofilm combined system and to investigate the contributions of artificial aquatic mat biofilm and perennial ryegrass to contaminant removal.

1 Materials and methods

1.1 Experimental design

One anaerobic/aerobic baffled biofilm cuboids reactor made of polyvinylchloride was operated (**Fig. 1**). The reactors had five closets of which the left three were anaerobic and the right two were oxygenated by aeration. Each closet had an inner length of 200 mm, width of 300 mm, height of 500 mm and the liquid volume of 150 L. Artificial aquatic mats (Yisheng Co., Ltd., Hebei, China) (**Table 1**) were used as support materials for biofilm. Each closet had 30 strips which had a length of 450 mm. The artificial aquatic mats accounted for 23% of the total reactor volume. Influent was controlled by peristaltic pump. The surface of the reactor was planted with perennial ryegrass (*Lolium perenne L.* Daytona). The reactor discharged tail water by a serrated overflow weir and then collected the effluent in a barrel after sedimentation.

The seed sludge for the reactor was taken from an aeration tank of the Sibao Municipal Wastewater Treatment Plant (Hangzhou City, Zhejiang, China). The MLSS of the sludge medium were 338.7 mg/L. The dissolved oxygen in the right two zones was controlled to 0.5–1.0 mg/L with the rotameter.

Artificial aquatic mat media was hung on the frame of the perennial ryegrass floating bed before start-up of the reactor. The seed sludge slurry was added into the

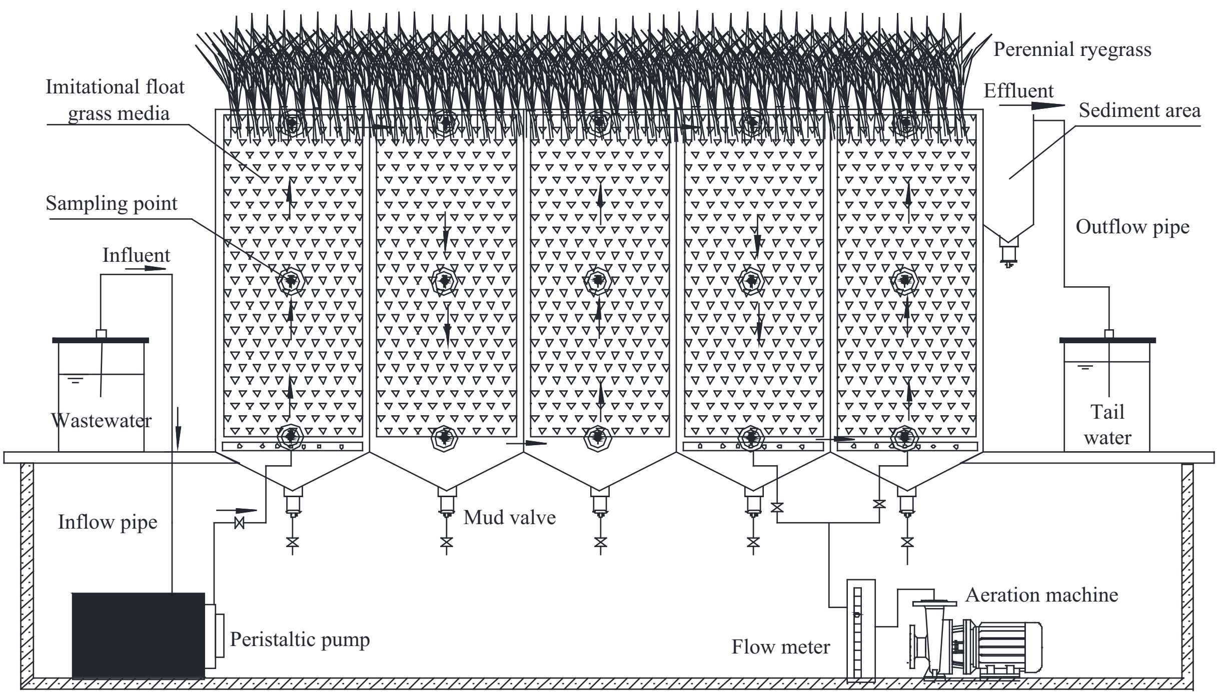

Fig. 1 Perennial ryegrass/artificial aquatic mats biofilm combined system design.

Table 1 Technical parameters of artificial aquatic mats

Carrier	Specification			Terry height	Finished weight (kg/m^3)	Surface area (m^2/m^3)	Void (%)	Film weight (kg/m^3)
	Diameter	Spacing	Row spacing					
Artificial aquatic mats	20–40	100	100	8–12	1–2	3200–6500	99	100–300

reactor. In the initial 60 days, system was set under ambient temperature. From the day 61 to the end of the experiment, heating rods was used to keep a suitable temperature in the cold winter, in order to keep the temperature at 23 ± 2°C.

A synthetic wastewater consisting of NH_4HCO_3 as ammonium and glucose ($C_6H_{12}O_6$) as chemical oxygen demand (COD) was used as the carbon and nitrogen sources, respectively for the reactor. The C/N ratio was maintained at five. The experiments were conducted in three phases. In Phase I, the plant floating bed-biofilm reactor was started by stepwise increasing the feed of influent ammonium and COD. In Phase II, the effect of the reflux ratio in the system was investigated. In Phase III, the effect of HRT was investigated (**Table 2**). At the end of the experiment the fresh mass of perennial ryegrass and the concentration of nitrogen in the dry perennial ryegrass were measured to ascertain the contribution of perennial ryegrass to nitrogen removal.

Table 2 Operating conditions and synthetic wastewater composition during three experimental phases

	Phase I	Phase II	Phase III
Influent ammonium (mg/L)	5–120	81–92	83–90
Influent COD (mg/L)	25–700	430–525	400–470
Reflux ratio	1	2–4	2
HRT (day)	10	10	5–12
Start day	1	140	206
End day	140	206	263

COD: chemical oxygen demand; HRT: hydraulic retention time.

1.2 Sample analysis

To monitor the nitrogen removal performance in the reactor, concentrations of ammonium, nitrite, nitrate, COD and TN were routinely measured according to Standard Methods (APHA, 1998). The value of pH was detected with a pH analyzer (Mettler Toledo, SG2, Switzerland).

The perennial ryegrass was cut from the initial (22 September, 2009) to the middle operation period (4 January, 2010) when its height was around 25–30 cm, and subsistence caudexes was about 5 cm. The biomass and nitrogen concentration of the harvested perennial ryegrass were analyzed by a high precision balance (Mettler Toledo, PB303-N, Switzerland). TN concentration of perennial ryegrass was determined by the Kjieldhal method after digestion with H_2SO_4-H_2O_2 (Thao et al., 2008). The direct uptake rates of N by the perennial ryegrass were the ratio of TN contained in the perennial ryegrass to TN removed by the combined system.

2 Results

2.1 Effect of influent concentration

During the first 19 days of the start-up period, the ammonium removal efficiency was around 52.6%–78.1%, meanwhile, the oxidation of ammonium to nitrate were observed but without nitrite accumulation (**Fig. 2**). From the 20th day, the ammonium removal efficiency rose to 84.5%–94.8% (Stage One). The ammonium removal

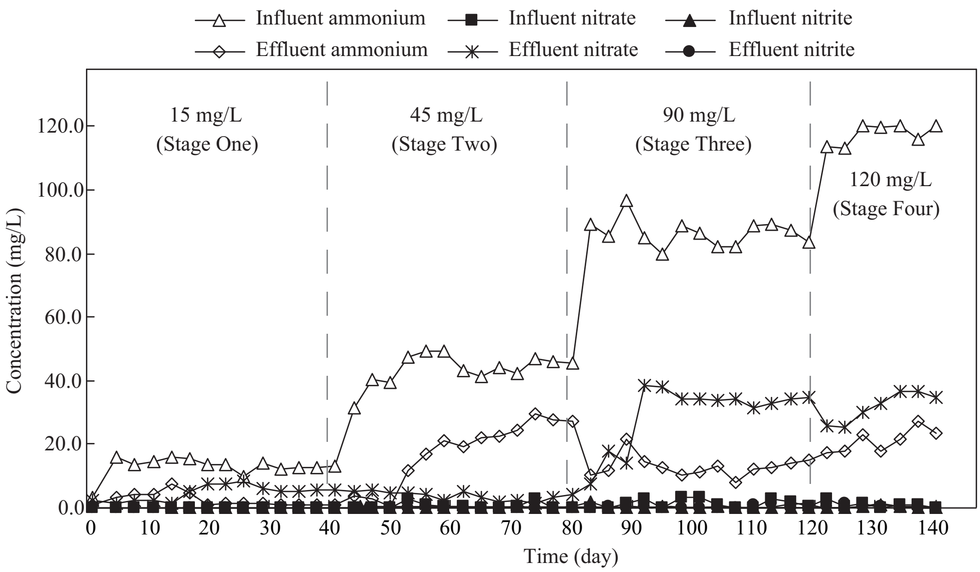

Fig. 2 Variation of ammonium, nitrate and nitrite during the phase I of perennial ryegrass/artificial aquatic mats biofilm reactor. Influent ammonium: 15, 45, 90, and 120 mg/L at four stages.

efficiency was dramatically reduced to 37.1% when the influent ammonium increased to 45 mg/L (Stage Two). Then it returned to 77.7%–90.4% when the influent ammonium increased to 90 mg/L (Stage Three). However, when the influent ammonium concentration increased to 120 mg/L, the average ammonium removal efficiency decreased to 82.2% (Stage Four).

2.2 Effect of reflux ratio and hydraulic retention time

The effect of the reflux ratio and HRT for nitrogen removal was observed by studying the behavior of ammonium, nitrite, nitrate and TN concentration in the influent and effluent (**Fig. 3**). For the different reflux ratios, the ammonium removal efficiency was similar and over 90%. In addition, the TN removal efficiency decreased from 60.2% ± 4.0% with reflux ratio of 2 to 47.9% ± 8.0% with reflux ratio of 3, and then increased to 51.6% ± 8.4% with reflux ratio of 4. The HRT did not observably influence the ammonium removal efficiency, which was always around 86.5%–92.7%. When the HRT increased from 5 to 8 days, the TN removal efficiency remained at 45.3% ± 7.8%. However, the TN removal efficiency increased to 57.9% ± 4.2 % for HRT of 12 days.

2.3 COD and temperature

During the entire experiment, the effluent COD averaged 37.8 mg/L and removal efficiency averaged 85.0% (**Fig. 4**). The COD removal efficiency was around 60.5% when the influent COD concentration was under 100 mg/L. However, it increased to 70% and remained above 90% when the influent COD concentration increased from 100 to 700 mg/L. The ammonium removal efficiency was significantly decreased by a sudden temperature drop on 53rd day, and it was only about 30%–50% between the 59th and 80th day (**Fig. 5**). In addition, the ammonium removal efficiency rebounded to a normal level (80%) after the temperature was raised by a heating rod.

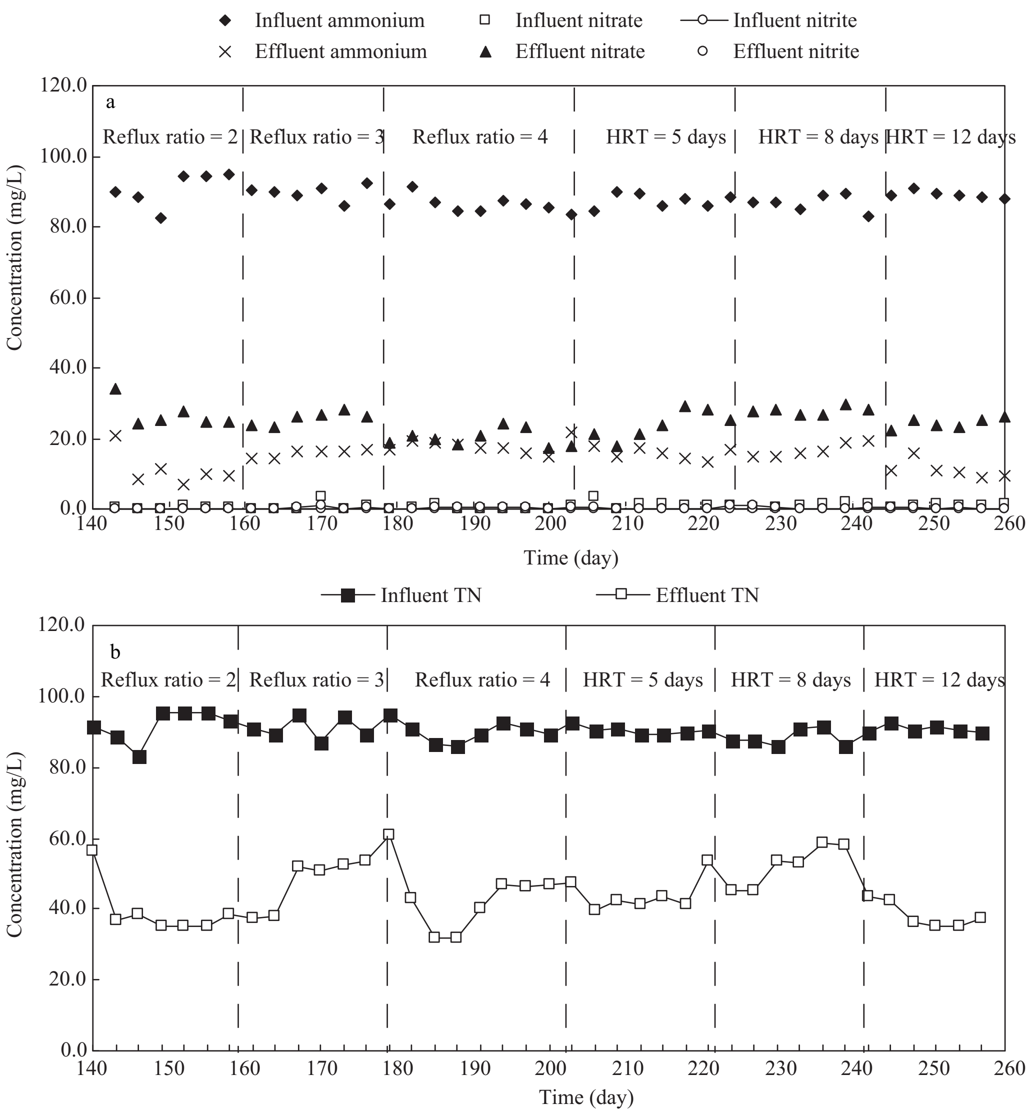

Fig. 3 Effect of reflux ratio and HRT on nitrogen removal in perennial ryegrass/artificial aquatic mats biofilm reactor. TN: total N.

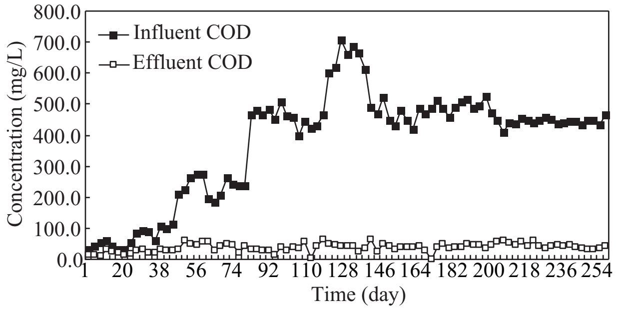

Fig. 4 Variation of COD concentration in the influent and effluent of the reactor.

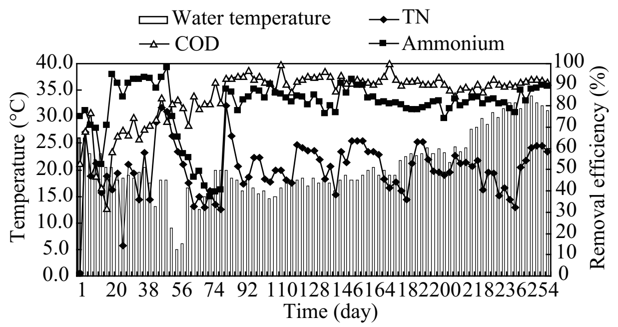

Fig. 5 Variation of TN, COD and ammonium and removal rate with the water temperature in the reactor.

Table 3 Proportions of total nitrogen uptake by perennial ryegrass in different zones and total nitrogen removal in the system

Zone	Harvested fresh biomass (g)	Harvested biomass dry (g)	Plant uptake TN (g)	TN removal (g)	Plant uptake (%)
First anaerobic	89.41	16.33	0.60		
Second anaerobic	95.36	16.89	0.71		
Anoxic	84.04	14.62	0.59		
First aerobic	171.94	50.23	2.28		
Second aerobic	130.22	29.89	1.38		
Total	570.96	127.96	5.55	30.54	18.17

2.4 Ryegrass biomass and nutrients concentration

The first aerobic grid had the largest ryegrass biomass, and its biomass was 171.94 g during the whole process. In order of quantity, the next largest ryegrass biomass was the second aerobic grid, second anaerobic grid, first anaerobic grid and anoxic grid (**Table 3**). The moisture content of the ryegrass was 78.9% ± 5.1%. The quantity of TN absorbed by the perennial ryegrass was 5.55 g during the whole process. The TN was 2.28 g for the first aerobic grid, which was about 1.65 times that of the second aerobic grid. The direct uptake rates of N by the perennial ryegrass accounted for 18.17% of the TN removal by the whole system.

3 Discussion

3.1 Influence factors of water quality

In this study, the perennial ryegrass and artificial aquatic mat biofilm combined system was operated under different influent ammonium loadings, reflux ratios, HRT and temperatures. The ammonium concentration of the effluent increased with the ammonium loading of the influent. During the 40 days of Stage One (Phase I), the system showed excellent removal of ammonium. During the initial 10 days of Stage Two, it was found that the ammonium load had no negative influence on the ammonium nitrogen removal of the system. However, the ammonium removal efficiency decreased notably after a few days of temperature drop below 10°C and it rebounded to a normal level after the temperature was raised by a heating rod. The decreasing trend in nitrification rate was similar to the decreases previously found by Head and Oleszkiewicz (2004). This would result from the activity inhibition of nitrogen removal microbes at low temperature. The suitable temperature range for nitrifiers to live is 20 to 35°C. The activity increases with reaction temperature, and nitrification is very limited when the wastewater temperature is below 10°C (Kim et al., 2006). The temperature played an important role in the nitrification process. During the 60 days of Stages Three and Four, the effluent ammonium concentration was maintained at 10.4–27.3 mg/L. The system performed with excellent nitrification ability, and the nitrification rate was in the range of 84.0% ± 3.5%.

An increase of the reflux ratio from 2 to 3 had a greater effect of ammonium transformation than a reflux ratio increase from 3 to 4. The ammonium concentration of the effluent was very low possibly due to the low ammonium load, which was caused by a higher reflux ratio. The highest TN removal period was during the time when the reflux ratio was 2. The results obtained indicate that by using a reflux ratio of 2, good denitrification could be obtained (Baeza et al., 2004). However, a feasible reflux ratio (for example, 2) is necessary to obtain a very low nitrate concentration in the effluent, while the TN removal efficiency was lower for a reflux ratio of 4. Because an increase of reflux ratio implies more energy consumption, meanwhile nitrate could be used as electron acceptor instead of oxygen (1 mg of NO_3^--N is equivalent to 2.86 mg of oxygen) by denitrifying microorganisms in anoxic conditions (Baeza et al., 2004).

HRT had a pronounced effect on the rate of nitrification: the concentration of effluent ammonium decreased with longer HRT. At a longer HRT, ammonia could have a sufficient contact time to be converted to nitrite then to nitrate (Yan and Hu, 2009). It was a surprise that an increase of the HRT from 8 to 12 days had a greater effect on TN removal than an increase from 5 to 8 days. However, an increase in HRT decreases nitrate and ammonium concentrations in the effluent and hence improves the nitrogen removal efficiency, even though the economic cost increases simultaneously.

High COD removal efficiency in the entire experiment process showed good performance in organic carbon removal. These results indicated that changes in organic loading in the influent did not affect COD removal efficiency significantly.

3.2 Ryegrass biomass and nutrients concentration

Cutting the perennial ryegrass regularly could promote the biomass accumulation and photosynthetic capacity. About 2–5 cm of stubble was the best cutting height for perennial ryegrass, which could be a technical reference for ecological bed management. The TN content of the perennial ryegrass showed a regular variation. The TN of the grass in the anoxic and anoxic grid did not show significant differences, while it varied significantly for both of the aerobic grids. It is believed that the aerobic zone provided the critical conditions for larger biomass of perennial ryegrass. Some plants (eg. *B. monnieri* and *Azolla* spp.) prefer NO_3^- to NH_4^+ for uptake when both nitrogen forms are supplied (Fang et al., 2007). The nitrate produced from nitrification in the aerobic zone improved the absorption efficiency of nitrogen. In addition, aeration supplied oxygen for root respiration in the aerobic zone, which significantly promoted the growth of perennial ryegrass indirectly. The direct uptake rates of N by the perennial ryegrass accounted for 18.17% of the TN removal, while the contribution of the plant direct absorption was negligible (Wu et al., 2006). The average TN content in the perennial ryegrass growing in the combined system was 4.2%, which was higher than that reported by Li et al. (2011) in a perennial ryegrass-microorganism combined system.

4 Conclusions

The combined perennial ryegrass/artificial aquatic mat biofilm system was quite satisfactory and stable for the treatment of wastewater in removing COD and NH_4^+-N. Generally, the ammonium concentration of the effluent increased with the ammonium loading of the influent during the entire experiment. The temperature played an important role in the nitrification process. The highest TN removal was achieved when the reflux ratio was 2. The nitrate and ammonium concentration of the effluent were consistent with that expected from variations in HRT. The combined system had a good performance in organic carbon removal. It is worthwhile to mention that 18.17% of the TN was removed by the direct uptake rates of N by the perennial ryegrass.

Acknowledgments

This work was supported by the China National Critical Project for Science and Technology on Water Pollution Prevention and Control (No. 2008ZX07101-006). We thank Professor Jiayang Cheng from the North Carolina State University, USA, and the anonymous reviewers for their contributions to the manuscript.

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Journal of Environmental Sciences (Established in 1989)
Vol. 25 No. 4 2013

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
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Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742

9 771001 074130 04