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Nitrogen removal by bioaugmentation in constructed wetlands for rural domestic wastewater in autumn

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ABSTRACT

Two subsurface flow constructed wetlands planted with *Typha orientalis* (CCW) and *Phragmites* (RCW) were constructed to study the effect of the addition of *Paenibacillus* sp. XP1 on nitrogen removal from rural domestic wastewater in autumn (15–21℃). CCW inoculated by *Paenibacillus* sp. XP1 (CCW-XP1) had obvious improvement on ammonia (NH_3-N) and total nitrogen (TN) removal efficiency than RCW-XP1. The removal efficiency of TN in the CCW is similar to that of NH_3-N, and the maximal removal efficiency of 78% was achieved, doubled with the control group. The final removal efficiencies of the CCW-XP1 were found to be 73% for chemical oxygen demand, 94% for NH_3-N, and 78% for TN. The effect of hydraulic retention time (HRT) variation on treatment efficiency of CCW was also discussed. Statistical analyses indicated that the optimal HRT for NH_3-N concentrations achieving the GB18918-2002 standard (China) for Class I-B guideline of 8.0 mg/L was 4 days, while the NH_3-N removal of the control group had not meet the criteria until 18 days. In comparison with the control group, HRT of CCW-XP1 was shortened for more than 15 days. The CCW would be a cost-effective measure for N removal from rural domestic wastewater by bioaugmentation.

Keywords: Constructed wetlands; Domestic wastewater; *Paenibacillus* sp. XP1; Nitrogen removal

1. Introduction

Rural pollution has attracted increasing attention over the past decade for its important consequences on surface and groundwater quality [1]. The environmental pollution and hazards were caused by the direct discharge of rural domestic wastewater, since the main pollutants were nitrogen (N) and phosphors (P), which led to the eutrophication. Owing to the dispersed rural population in China and construction costs of sewage collectors, centralized wastewater treatment plants based on activated sludge or bacterial bed processes that are utilized in large and small cities are not suitable in rural areas [2].

Several proposed solutions for the treatment of diffuse sources of domestic wastewater have been applied to on-site treatment in spacious rural areas,

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including vermibiofilter, soil infiltration trenches, vegetation-based wastewater treatment, and constructed wetlands (CWs) [3–6]. Among these technologies, CWs are the most promising economical method for treating point and diffused sources of domestic wastewater in small rural communities employed in China. They require lower investment and operation costs while providing higher treatment efficiency and more ecosystem services than conventional wastewater treatment methods [7,8]. Subsurface flow constructed wetlands (SSFCWs), the most widely used alternative wastewater treatment facilities, are applied typically in small villages and farms for specific wastewater treatment [9], though with the obvious disadvantage of large area and the long hydraulic residence time (HRT). Kjellin et al. concluded that the flow pattern and the HRT in wetlands strongly influenced the treatment efficiency [10]. Therefore, the normal HRT or the mean HRT could be used to indicate the nutrient removal efficiency of CWs. Seeking a way to shorten the HRT of SSFCW systems is essential for domestic wastewater treatment in developing countries.

Microbial activity crucially contributes to N removal in wetlands prior to sedimentation, filtration, precipitation, volatilization, adsorption, and plant uptake [11]. Some have studied the relationship between microbial activity and wastewater treatment efficiency in CWs. The studies of Calheiros et al. on the microbial dynamics on two-stage series of SSFCWs treating tannery wastewater have indicated that a diverse and distinct bacterial community inhabited each CW and the different hydraulic loadings did not result in evident changes in the microbial communities [12]. Llorens et al. have assessed the different microbial reactions to organic removal in a SSFCW treating urban wastewater, and have suggested that the anaerobic bacteria contribution was higher than the anoxic and aerobic bacteria contribution [13]. In addition, microbes may play an important part in phosphorous (P) removal [14]. Meanwhile, nitrification of nitrobacteria coupled with denitrification of denitrifying bacteria is usually the most significant nitrogen (N) removal mechanism in the CW [15]. N removal is typically associated with specific microbial functional groups, thus denitrification can be enhanced by optimizing the activity of those groups [16,17].

Bioaugmentation is achieved by inoculating microbial strains or mixes of strains that are isolated from the same polluted site and grown in the selective media containing the pollutant to enhance microbial activities in removing undesired pollutants. Application of bioaugmentation by way of improving HRT in CW is a new technology. Some studies have primarily focused on the bacterial communities and their dynamic in the CW [18]. However, research on applied bioaugmentation in CW systems is scarce. Lin et al. introduced microbial compound to a reed wetland system and the ammonia (NH_3-N) removal was significantly enhanced [19]. Pei et al. used *Bacillus subtilis* FY99-01 strains to efficiently enhance microbial nitrate removal in the artificial riparian wetland of a river bend [20]. Their results suggested that nitrate (NO_3^--N) was removed more efficiently in summer (>35°C) than in winter (3.8–8.6°C) and the maximal removal efficiency (100%) of NO_3^--N was 36.1%.

A denitrifying bacterium isolated from the rhizosphere soil of cattail, with a high removal efficiency of NO_3^--N, was obtained and named *Paenibacillus* sp. XP1 in the laboratory test [21]. The goal of the present paper was to determine if bioaugmentation by *Paenibacillus* sp. XP1 can enhance the nitrogen removal in CWs in autumn. The main purpose of this study is as follows: (1) to determine whether microbial inoculums can act as an efficient way to enhance N removal at the mean temperature of 15–21°C in autumn; (2) to assess the COD and N removal for rural domestic wastewater by bioaugmentation in CWs planted with *Typha orientalis* and *Phragmites*, if the microbial inoculums has an active effect; and (3) to identify HRT by microbial inoculation of meeting the Class I-B criteria specified in the *Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants* (GB18918-2002) of China.

2. Materials and methods

2.1. System description

The experiments were carried out in Shandong Architectural University, a section surrounding villages, in the suburb of Jinan in Shandong Province, China (36.67′N, 117.0′E). The total system consisted of a wastewater containment tank and four subsurface flow constructed wetland mesocosms (Fig. 1). The tank and polyvinyl chloride (PVC) pipes were installed to collect and transfer the influent, the secondary effluent from a sewage disposal system, which is used to purify domestic wastewater from Shandong Architectural University. Each CW was 400 cm in length and 1 m width, with a depth of 130 cm, and packed padding of 100 cm. Their filter beds both consisted of four layers from top to bottom to a height of 10 cm with soil, 10 cm with silver sand, 10 cm with ceramic particles, 55 cm with fine detritus and 15 cm with cobblestones, which were used as the supporting layer. The soil came from wetlands of Nansi Lake in

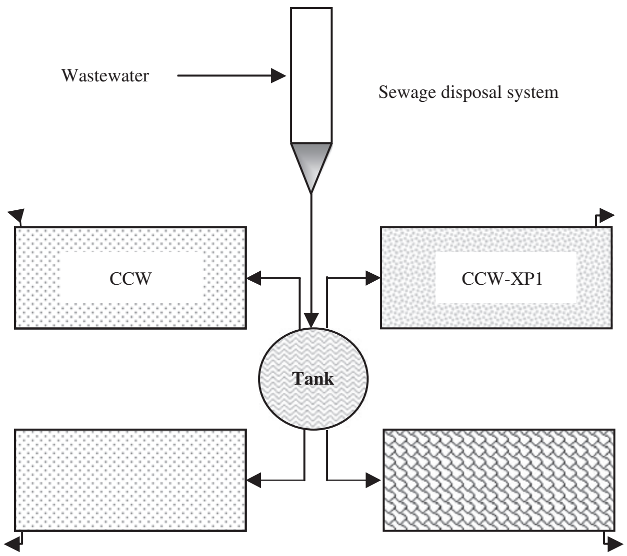

Fig. 1. Scheme of the constructed wetland mesocosms.

Shandong Province. The characteristics of the ceramic particles used in this study can be found in Jiang et al. [22]. Two CWs were planted with cattail (*Typha orientalis*) (CCW) in March 2010, while the other two were planted with reed (*Phragmites*) (RCW). After planting, the microcosms were kept flooded for two months until the macrophyte was well established. The system was started to dynamically operate from May 2010. The harvest period of plants was at the end of October, and the average air temperature was 18℃.

2.2. Inoculation preparation

Paenibacillus sp. XP1 (Chinese patent number ZL 201110121394.2) was isolated in the School of Environmental Science and Engineering in Shandong University and preserved in the China Center for Type Culture Collection with the number of M 2011120 [20]. The LB medium (pH 7.2–7.4) for enrichment of *Paenibacillus* sp. XP1 was prepared by dissolving 10 g Tryptone, 5 g Yeast extract, and 10 g NaCl in 1 L of distilled water. LB medium was autoclaved for 30 min at 121℃. Sterile medium (100 mL) in 250 mL conical flasks was inoculated with 2 mL of freshly activated sludge and incubated stably at 35℃ for 24 h. The optical density of *Paenibacillus* sp. XP1 was measured at 600 nm (OD600) using a spectrophotometer (UV-2450, Shimadzu, Japan) at the end of incubation.

2.3. Inoculation procedure

Two CWs (a CCW and a RCW) inoculated with *Paenibacillus* sp. XP1 to treat the secondary effluent from sewage disposal system were conducted to determine the efficiencies of N removal from 29 October to 16 November in 2010 after harvesting. The other two (a CCW and a RCW) were as the control. About 9 L of the culture suspension ($OD_{600} = 1.5$) in a 10 L plastic drum was released into the CCW and the RCW, regarded as CCW-XP1 and RCW-XP1, respectively. The special injector (Chinese patent number ZL 20112485865.3) was used to add an equivalent volume of inoculums into the CWs in three different depths (0.35, 0.45, and 0.55 m).

2.4. Water sampling and analysis

The influent was sampled before the wastewater flowed into the CWs. A certain amount of outlets were fixed at the bottom on the other side of the CWs. All the outlets were closed when the CWs were filled with wastewater. After bioaugmentation, the effluent samples were taken from the lowest outlet on the first day, and then sampling interval was 3 days in an 18-day period. In the laboratory, the wastewater samples in triplicate were analyzed for chemical oxygen demand (COD), total nitrogen (TN), NH_3-N, and NO_3^--N using the standard methods [23]. The data were all averaged. COD was measured using the potassium dichromate method. TN, NH_3^+–N, and NO_3^--N were determined by a UV–vis spectrophotometer (Shimadzu Instrument Co. Ltd., UV-2450, Japan) using the Nessler's reagent spectrophotometric method, potassium persulphate oxidation-ultraviolet spectrophotometry, and ultraviolet spectrophotometry screening method, respectively. Dissolved Oxygen (DO) was monitored using the portable dissolved oxygen determining meter (DO-200, Lovibond, Germany). The pH of the effluent of each CCW was measured a pH meter (Shanghai Leici Instrument Co. Ltd., PHS-3C, China). Treatment efficiency was calculated as the percentage of removal as follows:

$$\text{Removal efficiency } (\%) = (C_i - C_e)/C_i \times 100$$

where C_i and C_e are the influent and effluent concentration in mg/L, respectively.

3. Results and discussion

3.1. Wastewater chemical properties

The quality of influent wastewater chemical properties was measured for four times a month. The influent concentrations were relatively stable. COD, NH_3-N, and TN were measured in this study ranging

40–60 mg/L, 124–146 mg/L, and 130–150 mg/L, respectively. NO_3^--N and NO_2^--N were kept at low concentrations of 0.1–1.5 mg/L and 0–0.2 mg/L, respectively. Similarly, for many CWs, NH_3-N is the dominant type of N with the percent of 88–95% in the influent. Wastewater pH was maintained at the range of 7.5–8.0 and did not change greatly. It is generally accepted that pH in the range 7.5–8.0 is more conducive to the nitrification process. Suitable DO concentration, to continue the nitrification process, was needed to keep in 1.0–1.5 mg/L [24]. Nitrification is not expected at DO below 0.3 mg/L [25]. DO of 0.5 mg/L produced no effect on ammonia oxidation (NH_3-N of 80 mg/L), while nitrite oxidation was strongly inhibited [26]. So, it is indicated that nitrification would be more active at the DO concentrations of 1.03 ± 0.11 mg/L in this study.

3.2. *COD_{Cr} removal in the CCW*

Organic compound is obviously removed in CWs. An important factor for organic matter mineralization is the supply of oxygen and alternative electron acceptors in the wastewater [27,28]. Insoluble organic matter as sediment storage can be used by tiny creatures. Soluble organic matter can be adsorbed by the biofilm and microbial metabolic processes. The higher efficiency of organic pollutant removal by several bacterial groups at favorable oxygen conditions was observed in a microcosm study [29]. In this study, COD_{Cr} in the influent fluctuated with the average value of 46 mg/L and decreased gradually in the initial 4 days after bioaugmentation in CCW-XP1 and RCW-XP1 (Fig. 2). In CCW-XP1, COD_{Cr} increased significantly to 71 mg/L on the first day because of the bacteria added, which was cultured in LB medium of beef extra and peptone. The maximal removal efficiency in the initial four days reached 65% in CCW-XP1 and 52% in RCW-XP1. Denitrification was found to be limited by the organic carbon supply, especially by the easily degradable fraction of this component of the wastewater in CWs [30] while autotrophic nitrifiers depend on the inorganic carbon pool present in the system [27]. So, the variance of COD was negative correlated to the microbial metabolism as well as N removal. As shown in Fig. 2, organic matter measured as COD_{Cr} has shown removal of 73% in the CCW-XP1 and 55% in RCW-XP1 in the 18 day test, while 64% (CCW) and 54% (RCW) of the control group. COD_{Cr} removal did not change significantly during the operation period. The organic matter content of influent wastewater had already met the standards of national and local governments (China), the Class I-B criteria of GB18918-2002 ($COD_{Cr} < 60$ mg/L). COD_{cr} removal

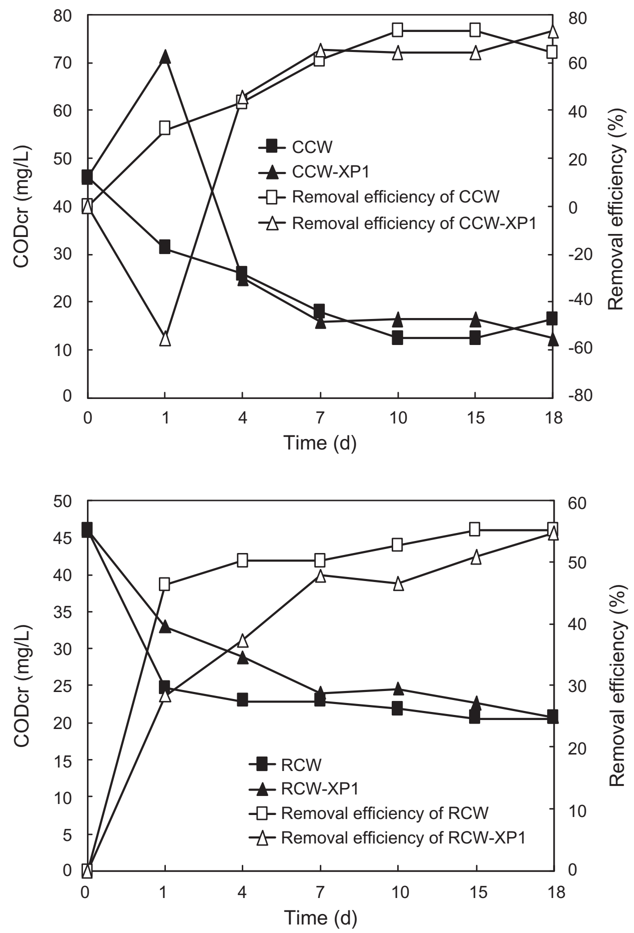

Fig. 2. COD_{cr} removal during an 18-day test in the autumn of 2010.

efficiency was not as high as expected in the CWs inoculated. Additionally, insignificant change in COD_{Cr} was observed between the microbial inoculated group and the control group, indicating that bioaugmentations by *Paenibacillus* sp. XP1 had less obvious impact on COD removal, when COD_{Cr} range was less than 46 mg/L in this study. Denitrification in wetlands was increased by sufficient organic carbon increase, since denitrifiers basically consist of heterogeneous bacteria [31], and the COD_{Cr}/NO_3^--N ratio strongly influenced NO_3^--N reduction [32]. It is likely that carbon source was additionally needed in order to ensure the microbial activity, if the COD_{Cr} ranged in a lower concentration in practice.

3.3. *N removal in the CCW*

Needless to say, biological nitrification-denitrification is the most studied process for N removal from wastewater [33]. N removal in CWs has mostly been assumed to be a result of the combination of nitrification-denitrification [34]. However, N removal efficiency reported for SSFCW is variable, ranging from high removals of over 90% [35] to removals as low as 11% [36]. Generally, denitrification is still believed to account for more than half of N removal in CWs [35]. The removal efficiencies of NH_3-N, NO_3^--N, and TN

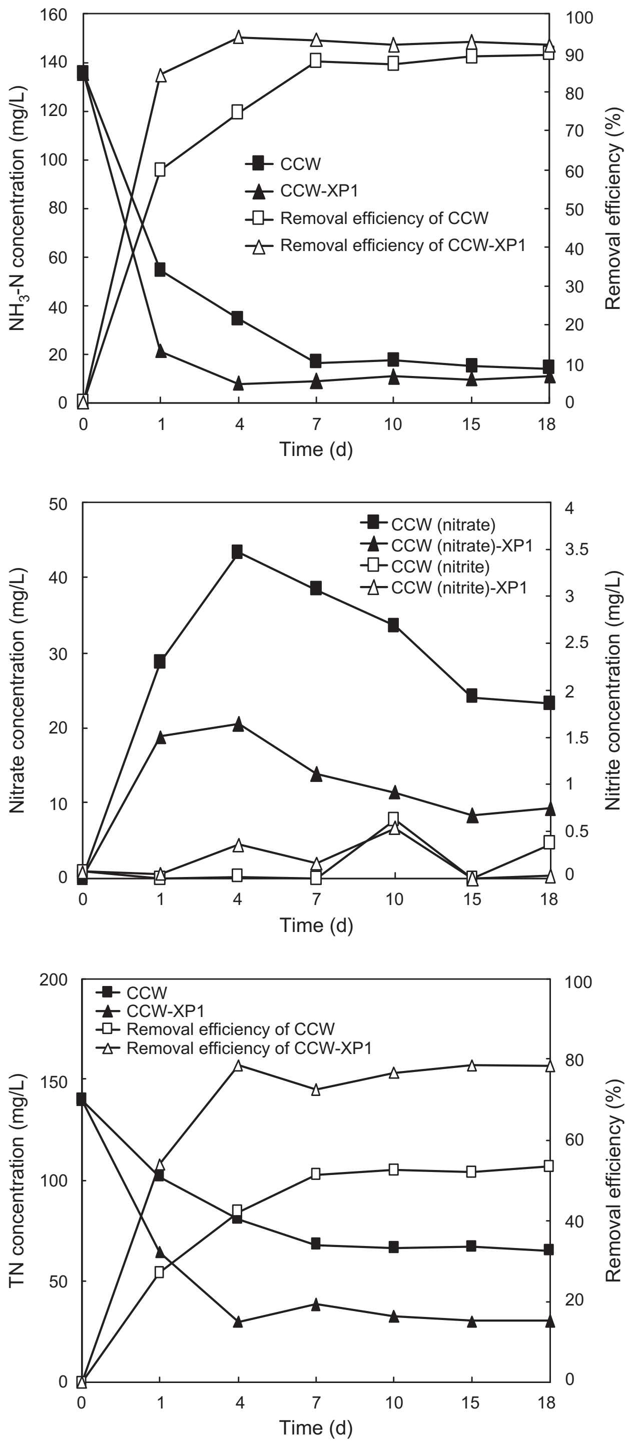

Fig. 3. Variations of nitrogen compounds in the CCW during an 18-day test in the autumn of 2010.

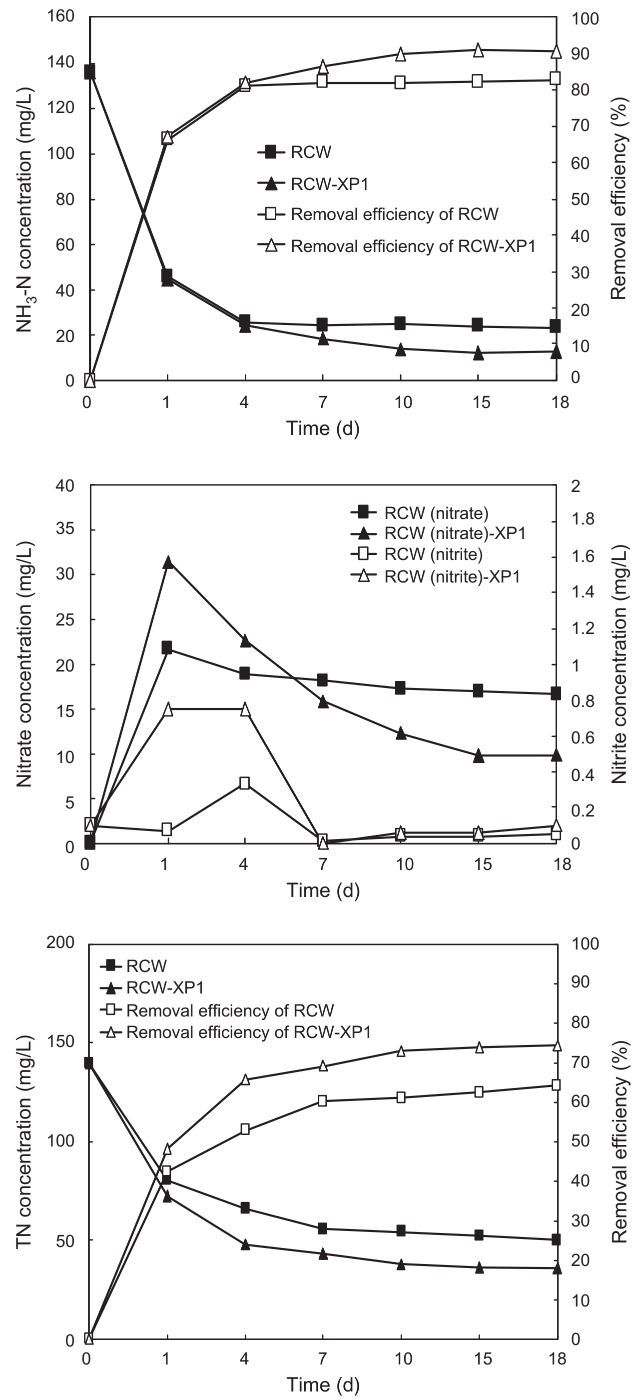

Fig. 4. Variations of nitrogen compounds in the RCW during an 18-day test in the autumn of 2010.

were described during an 18 day test. The pattern and efficiency of TN removal by bioaugmentation were partially reflected by different N species (Figs. 3 and 4).

Nitrification is the aerobic oxidation of NH_3-N to nitrite (NO_2^--N), and then to NO_3^--N. The process is performed by nitrifying bacteria [37]. Nitrification takes place in all types of CWs [2]. It is regulated by the temperature, since ammonium oxidizers grow faster than NO_2^--N oxidizers at temperatures above 15 and at 25℃ the NO_2^--N oxidizers can be dislodged by ammonium oxidizers [27]. Kern also reported the negative effect of low temperatures on the number of nitrifying bacteria and the nitrification process during the winter period [38]. Currently, the minimum temperature of nitrification was uniform. Oleszkiewica et al. analyzed the nutrient removal from wastewater at a cold temperature and found that nitrification was still occurred at the low temperature, but was severely reduced below 7℃ [39]. Besides, the optimal pH for nitrification is inconclusive. Antoniou et al. concluded that the optimum pH is in the range of 7.0–8.2 [40]. Kuschk et al. reported that the denitrification process was completed in summer and was significantly inhibited below 15℃ [36]. The average air temperature was 18℃ in our test period, which would benefit for nitrifying and denitrifying.

Denitrification is promoted by higher NO_3^--N concentrations [30], while nitrification is dependent on NH_3-N concentration in wastewater [27]. The influent NH_3-N concentrations fluctuated with the average value of 136 mg/L are approximated to the TN of 140 mg/L. Fig. 3 also shows that in the CCW-XP1, the concentration of NH_3-N decreased to 7.8 mg/L (NH_3-N <15 mg/L in the Class I-B criteria of GB18918-2002) during the fourth day. The efficiency of NH_3-N removal was about 94%. And, in Fig. 4, there is no significant change for NH_3-N removal between RCW inoculated with XP1 and the control. Furthermore, Vymazal indicated that the lack of oxygen available for nitrification limits the N removal process in the majority of CWs since NH_3-N is the dominant N species in sewage [41]. The NH_3-N removal in CCW was not achieved the criteria at the end of the whole period. In comparison with the control group, the HRT of NH_3-N achieved to the I-Class B criteria of GB18918-2002 (NH_3-N <15 mg/L), was greatly shorten. In comparison with the control group, HRT of CCW-XP1 was shortened for more than 15 days.

As shown in Figs. 3 and 4, the initial concentrations of NO_3^--N and NO_2^--N were 0.2 and 0.08 mg/L, respectively. However, in the CCW and RCW without addition of the strain, the denitrification plays little effect. As Fig. 3 shows, the concentrations of NO_3^--N increased quickly to 43.6 mg/L in the first 4 days, and then gradually decreased to 25 mg/L much higher than the CCW-XP1 at the end. In the CCW-XP1, the amount of NO_3^--N is increased from 0.2 to 19.8 mg/L within 4 days, and then decreased up to 10.5 mg/L during the last 14 days. Prolonging HRT excessively would result in anoxic or low oxygen concentrations in the wetland unit [42]. So, 7 days later, as the time going, the nitrification was limited and the NO_3^--N concentration was maintained at a certain level. Presumably, part of NO_3^--N was transformed to NO_2^--N under anoxic condition and strains preferentially utilized NO_2^--N as an electron donor and finally N_2 to the atmosphere during denitrification. The removal efficiency of TN in the CCW-XP1 is similar to that of NH_3-N, and a maximum removal efficiency of 78% was achieved, doubled with the control group (Fig. 3), while the TN removal efficiency of 50% in the RCW-XP1 (Fig. 4).

Table 1 summarizes the comparison of the treatment effects on rural wastewater, among many SSFCWs in the similar size. The well-controlled pilot-scale CWs were more used to do research. The removal efficiency (100%) of TN in different CWs was influenced by influent wastewater quality and wastewater temperature. High temperature, at a range of 30–35℃ [43], was more efficient for TN removal than low temperature at 14℃ [44]. It is concluded that microbial growth and survival in the CWs may be significantly sensitive to wastewater temperature, so the TN removal efficiency was reduced following the decreasing temperature. In comparison with other

Table 1
TN removal efficiency (100%) comparation between our CWs and other SSFCWs

Type of CWs	Temperature (℃)	Size	Influent TN (mg/L)	HRT (d)	Effluent TN-removal (%)	References
Reed	15–30	2 × 0.5 × 0.6	20.88–51.33	4	60	Fu et al. [45]
Reed	30–35	3 × 1.5 × 1	3.15–7.23	4	72.2	Zhang et al. [43]
Cattail	15–30	2 × 0.5 × 0.6	20.88–51.33	4	61	Fu et al. [45]
D. Sanderiana	14	3 × 1 × 0.8	65	5	65	Luo et al. [44]
Cattail	15–21	4 × 1 × 1.3	140	4	56	This test
Cattail-XP1	15–21	4 × 1 × 1.3	140	4	78	This test

CWs, the TN removal efficiency of CCW-XP1 was the maximum, though the concentration of TN in the influent was the highest.

This data suggest that bioaugmentation in the CWs could be efficient for N removal and shorten the HRT for wastewater treatment. However, it could not be utilized as a dynamic treatment unit because the limited area needs long HRT to treat wastewater.

4. Conclusions

This study showed that CCW enhancement by *Paenibacillus* sp. XP1 had obvious improvement on NH_3-N and TN removal efficiency than RCW-XP1. The removal efficiency of TN in the CCW is similar to that of NH_3-N, and the maximum removal efficiency of 78% was achieved, doubled with the control group. The final removal efficiencies of the CCW-XP1 were found to be 73% for COD, 94% for NH_3-N, and 78% for TN. The optimal HRT for NH_3-N concentrations achieving the GB18918-2002 standard (China) for Class I-B guideline of 8.0 mg/L was 4 days, while the NH_3-N removal of the control group had not meet the criteria until 18 days. In comparison with the control group, HRT of CCW-XP1 was shortened for more than 15 days. The CCW would be a cost-effective measure for N removal of rural domestic wastewater by means of bioaugmentation. The growth rate of the inoculated organisms in the CCW was a critical factor for bioaugmentation. So, the further work is to prove whether the strain germinates well or not. And also the time of repeated inoculation is needed to define definitely.

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