Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2021.27837

242 (2021) 100–105
December

Shortcut nitrification–denitrification achieved by hydroxylamine addition in constructed rapid infiltration system

Xiuqin Kong*, Yiming Lian, Nini Zhang, Qianjun Tang, Yajing Zhao

School of Petrochemical Engineering, Lanzhou University of Technology, Lanzhou 730050, Gansu, China, Tel. +13893349788; email: xqkong2@126.com (X.Q. Kong), 2506208735@qq.com (Y.M. Lian), 914627791@qq.com (N.N. Zhang), 705197567@qq.com (Q.J. Tang), 1046569769@qq.com (Y.J. Zhao)

Received 13 April 2021; Accepted 18 September 2021

ABSTRACT

Shortcut nitrification–denitrification process is a promising method for nitrogen removal from domestic wastewater. In this study, hydroxylamine (NH_2OH) was adopted for the promotion of shortcut nitrification–denitrification in constructed rapid infiltration (CRI) system. Two CRI systems (C1, C2) were operated, C1 was the contrast no adding $NH_2OH·HCl$, and C2 was added $NH_2OH·HCl$ at gradient-increased concentration (3, 5, 10, 15, 20, 25, 30, 35 mg/L). Finally, shortcut nitrification–denitrification was successful achieved when the added concentration of NH_2OH increased to 20 mg/L. Under this condition, the chemical oxygen demand and ammonia nitrogen (NH_4^+–N) removal efficiencies reached 85.9% and 84.37%, respectively. The total nitrogen removal reached 75.4% and the nitrite accumulation rate increased to 87.2%. The main genus of nitrite-oxidizing bacteria (NOB), Nitrospira, was eliminated from the system, while the main genura of ammonium-oxidizing bacteria (AOB), Nitrosomonas, norank_f_Nitrosomonadaceae and Phycisphaera, and the main genura of denitrifying bacteria (DNB), Thauera, Ottowia, Pseudomonas and Paracoccus, was enhanced. Overall, NH_2OH addition is an excellent strategy to achieve Shortcut nitrification–denitrification in CRI system for domestic wastewater.

Keywords: Hydroxylamine; Shortcut nitrification–denitrification; Constructed rapid infiltration (CRI) system; Domestic wastewater

---

1. Introduction

Constructed rapid infiltration (CRI) system is a novel wastewater biofilm treatment technology, developed from traditional rapid infiltration system and constructed wetland system [1]. CRI system adopts the mixture of natural river sand, zeolite sand and marble sand that replaces conventional soil layers as the main packing media, which can improve hydraulic load [2]. In addition, a unique operation mode of wet-dry cycling is applied, which alternates the running environment between an aerobic and anaerobic environment, so cultivating a more diverse mixture of microorganisms [3]. Contaminants can be efficiently trapped and adsorbed by the packing media during the wetting period and then degraded by microorganisms in the biofilm during the drying period [4]. CRI system attracted more attention in recent years, owing to its advantages of good effluent quality, low construction cost, easy management, low energy consumption and no surplus sludge [4–6]. During the stable operation of CRI system on treating domestic sewage, the removal efficiencies of chemical oxygen demand (COD) and ammonia nitrogen (NH_4^+–N) can be maintained at approximately 85% and 90%, respectively [7]. However, the removal efficiency of total nitrogen (TN) is relatively poor and cannot meet the standard of relevant emission, due to the limitation of organic carbon for denitrification in the anaerobic environment of the CRI system [8–9].

---

* Corresponding author.

1944-3994/1944-3986

Therefore, various methods have been researched to improve the nitrogen removal efficiency of the CRI system, which include changing the water feeding patterns, adding extra carbon sources, changing the ratio of C/N in the influent, and optimizing the packing composition [10–11]. But those methods are all depend on complete nitrification–denitrification process which required the enough organic carbon for denitrification [12]. Thus, they would consume many carbon sources in the actual engineering.

Shortcut nitrification–denitrification is a novel type of nitrogen removal process, which has distinctive advantages compared to full nitrification–denitrification, such as saving dissolved oxygen (DO) and requiring less organic carbon source [12]. Shortcut nitrification–denitrification can convert NH_4^+–N into nitrite nitrogen (NO_2^-–N) and then directly into nitrogen by denitrifying bacteria (DNB), such as, Thauera, Bacillus, Ottowia, Spirillum, Pseudomonas Micrococcus and Paracoccus [13]. Whereas, the key to achieving shortcut nitrification–denitrification is to achieve shortcut nitrification, which demands to inhibit or wash out nitrite-oxidizing bacteria (NOB), such as, Nitrococcus, Nitrospina, and at the same time without effects on ammonium-oxidizing bacteria (AOB), such as Nitrosomonas, Nitrosospira, Nitrosococcus, and Nitrosovibrio [14]. To inhibit nitrite oxidization, the methods of increasing concentrations of free ammonium (FA) and free nitrous acid (FNA), decreasing concentration of DO, adding suitable inhibitor have been successfully applied [15–17]. Fang et al. [12] studied the addition of 5 mM potassium chlorate ($KClO_3$) in influent could support efficient shortcut nitrification in CRI. Sukru et al. [18] found that nitrite accumulation rate (NAR) above 76% was achieved at the nitrogen loading rate (NLR) of 830 g NH_4^+–N/m^{3}d with salt free wastewater. Sun et al [19] studied the shortcut nitrification process could be achieved in SBR through the synergetic effects of FA inhibition, the TN removal efficiency can reach 99.2%. However, these methods will introduce other substances or consume lots of energy in practical applications.

Hydroxylamine (NH_2OH) is an intermediate of nitration, the related chemical reactions are shown as follows in Eqs. (1) and (2) [20]:

$$NH_3 + 2H^+ + 2e^- + O_2 \rightarrow NH_2OH + H_2O \quad \Delta G = -127.6 \text{ kJ/mol} \tag{1}$$

$$NH_2OH + H_2O \rightarrow NO_2^- + 2H^+ + 2e^- \quad \Delta G = -198.5 \text{ kJ/mol} \tag{2}$$

During nitritation process NH_4^+–N is initially oxidized to NH_2OH by ammonia monooxygenase (AMO), and then the NH_2OH is further converted to nitrite by hydroxylamine oxidoreductase (HAO) [21]. Although NH_2OH is an intermediate of nitritation, it has been found that NH_2OH can enhance shortcut nitrification owing to selective inhibition of AOB and NOB by hydroxylamine [22]. For example, Okabe et al. [23] found NH_2OH can effectively promote the initiation of nitritation in SBR system. Kuai and Verstraete [24] found that the NH_2OH-amended sludge removal efficiency of NH_4^+–N and NO_2^-–N that was six times greater than that of the control (no NH_2OH). Xu et al. [25] found that 10 mg/L NH_2OH dose in an aerobic granule reactor could induce a stable shortcut nitrification. Li et al. [26] studied NAR above 95% could be achieved in 5 d with limited addition of NH_2OH. These results suggest that NH_2OH can enhance nitration and nitrite accumulation, but the NH_2OH concentration and dose strategy are different, which should be optimized based on effectiveness for real application.

In view of the lack of carbon source for denitrification, and low TN removal efficiency, the principal goal of this study was to explore the operational feasibility of achieving shortcut nitrification–denitrification through the NH_2OH addition. The objectives of this study were to select the optimum NH_2OH concentration through batch tests, investigate the nitrogen removal rate with and without NH_2OH addition. The results of the current study would be helpful to the engineering application of the nitrogen removal.

2. Materials and methods

2.1. Experimental wastewater

The influent wastewater used in this study was synthetic wastewater, with water quality parameters as shown in Table 1.

2.2. Experimental equipment

Two separate CRI columns with a total height of 120 cm and a diameter of 14 cm were used in this experiment. Both of the columns had a support layer with 5.0 cm at the bottom followed by the packing layer of 100 cm and then a 5.0 cm protective layer. The packing layer of experimental column was filled with 90% river sand (diameter 0.5–1.0 mm), 5% marble sand (diameter 1.0–2.0 mm) and 5% zeolite sand (diameter 1.5–2.0 mm), the support layer and protective layer filled with pebbles. For preparation, the natural river sand was washed and dried. The influent wastewater was pumped into columns through distributing pipes at the top, the effluent was collected through the outlet at the bottom. The structure of column is shown in Fig. 1.

2.3. Experimental conditions

The experiment was performed under 25.3°C ± 1.8°C, the pH value was kept constant at 7.5 ± 0.3. The CRI columns were periodically fed with experimental wastewater using a hydraulic load of 1.0m/d and wet/dry (W/D) ratio

Table 1
Water quality parameters of the influent

Water quality parameters	Concentration (mg/L)
Chemical oxygen demand	252.36 ± 25.54
NH_4^+–N	48.37 ± 5.84
NO_2^-–N	0.87 ± 0.44
NO_3^-–N	0.37 ± 0.14
Total nitrogen	50.21 ± 5.92
pH	7.5 ± 0.3
Temperature (°C)	25.3 ± 1.8

of 1:3 (3 h of dosing and 9 h of resting) at 12 h per cycle. The selection of operating parameters was based on previous laboratory research and practical engineering [27–30]. The scanning electron microscopy (SEM) images of the packing material face were shown in Fig. 2. The blank packing material face was smooth (Fig. 2a), while the packing material face formed with biofilm had a lamellar film (Fig. 2b). The column C1 was the contrast without adding $NH_2OH \cdot HCl$, which was operated for 48 d. The column C2 was added $NH_2OH \cdot HCl$ at gradient increased concentration (3, 5, 10, 15, 20, 25, 30, 35 mg/L) and each concentration gradient was run for 6 d. COD, NH_4^+–N, NO_2^-–N, NO_3^-–N and TN concentration of influent and effluent were measured every day to evaluate the efficiencies of NH_2OH addition.

2.4. Analytical methods

Every day, triplicate samples were collected and subsequently mixed for further analysis. The concentrations of COD, NH_4^+–N, NO_2^-–N, NO_3^-–N, and TN in the water samples were determined according to the Chinese National Standard Methods (SEPA of China, 2002). Above, concentration of COD in the water was determined by the potassium dichromate method; concentrations of NH_4^+–N, NO_2^-–N, NO_3^-–N and TN were determined by the UV spectrophotometer (UV-5200, China), and the pH value was determined by the pH analyzer (PHS-3C, China). The biofilm of the filling medium was prepared by the glutaraldehyde fixation method and observed by using SEM (JSM-6701F, Japan). Illumina Miseq sequencing was used to analyze the microbial community in the CRI system when the dosage of NH_2OH is optimal. The NAR was calculated as eq. (3).

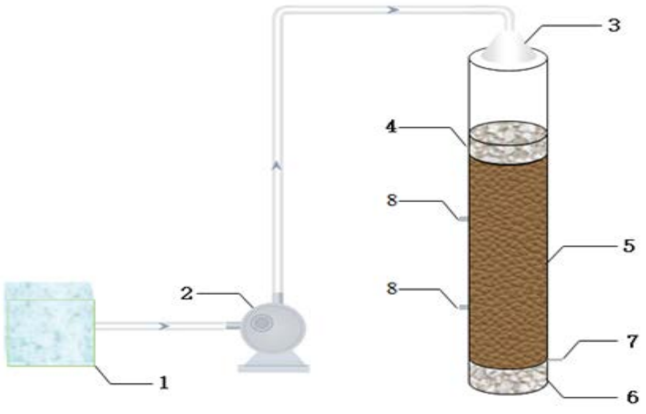

Fig. 1. The structure of column (1. Feeding tank, 2. Feeding pump, 3. Distributing pipes, 4. Protective layer, 5. Packing layer, 6. Support layer, 7. Effluent outlet, 8. Sampling port).

$$\text{NAR} = \frac{\left[NO_2^- - N\right]_{\text{eff}}}{\left[NO_2^- - N\right]_{\text{eff}} + \left[NO_3^- - N\right]_{\text{eff}}} \times 100\% \tag{3}$$

where $[NO_2^-–N]_{eff}$ and $[NO_3^-–N]_{eff}$ were the concentrations of NO_2^-–N and NO_3^-–N in effluent, respectively, mg/L.

3. Results and discussion

3.1. Effect of NH_2OH on the removal efficiency of COD

The effect of NH_2OH on removal efficiency of COD in the CRI system was investigated. The results are shown in Fig. 3. There was no significant effect in the removal efficiency of COD when the added concentration of NH_2OH was less than 30 mg/L, the average COD removal efficiency maintained 86.3% ± 2.6%. When the added concentration of NH_2OH was 35 mg/L, the COD removal efficiency

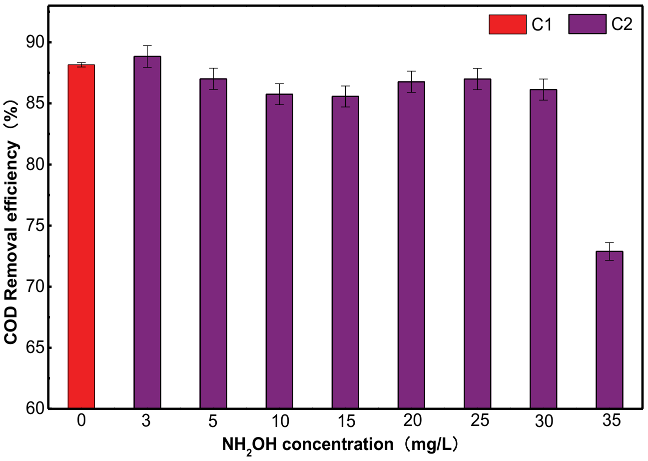

Fig. 3. Chemical oxygen demand removal efficiency of CRI system.

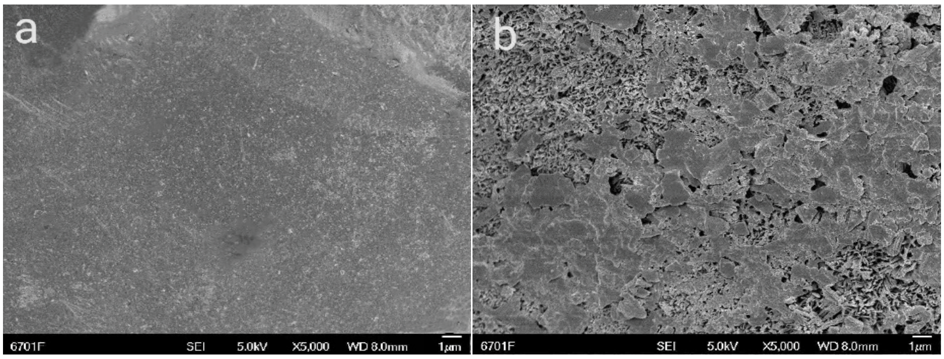

Fig. 2. SEM images of packing material face. (a) Blank packing material face and (b) packing material face formed with biofilm.

reduced to 72.4%. The possible reason was the concentration of NH_2OH added greater than 30 mg/L will inhibit the activity of catalase [31]. Therefore, NH_2OH concentration should be controlled below 30 mg/L.

3.2. Effect of NH_2OH on the removal efficiency of NH_4^+–N

The NH_4^+–N removal efficiency at different NH_2OH concentration was measured, as shown in Fig. 4. It was characterized by the following three stages. When the added NH_2OH concentrations were 3 mg/L and 5 mg/L, the removal efficiency of NH_4^+–N was slightly higher than the control column C1, the possible reason was that NH_2OH may cause floc disaggregation, enhancing ammonia oxidation because of mass transfer limitations reduced [32]. There was no significant difference on the NH_4^+–N removal efficiency when the NH_2OH concentration increased from 10 to 20 mg/L. The removal efficiency of NH_4^+–N from 83.37% decreased to 58.82%, when the NH_2OH concentration increased from 20 to 35 mg/L. It is stated that the less NH_2OH concentration can promote the removal of NH_4^+–N, but more than 20 mg/L, which has a significant inhibition on the removal of NH_4^+–N.

3.3. Effect of NH_2OH on NAR and TN removal efficiency in CRI system

In order to evaluate the occurring of shortcut nitrification by NH_2OH addition in the CRI system, the nitrite accumulation was assessed. The results can be seen in Fig. 5. When NH_2OH concentration was less than 20 mg/L, with the concentration increase, NAR increased from initial 1.3% to 87.2%. NH_2OH induced selective inhibition on NOB, hindered the further oxidation of nitrite, which resulted in an increase of NAR. When NH_2OH concentration was more than 20 mg/L, with the concentration increase, NAR decreased gradually, which was consistent with the Chen's conclusion [31]. These results indicated that the optimal added concentration of NH_2OH was 20 mg/L in CRI system to achieve the shortcut nitrification.

For insight into the effect of NH_2OH addition to shortcut nitrification–denitrification for domestic wastewater treatment, the TN removal efficiency was analyzed. The effect of NH_2OH on TN removal efficiency are shown in Fig. 5. In the meanwhile. The trend of TN removal efficiency with NH_2OH concentration was consistent with the trend of NAR. As NH_2OH concentration was 20 mg/L, TN removal efficiency was 75.4%, which was the best. Then, TN removal efficiency declined to 58.27% when NH_2OH concentration was 35 mg/L. Suggesting that TN removal efficiency and NAR were highly correlated. In C2, when NH_2OH concentration was 20 mg/L, NAR increased to the maximum, meanwhile, the activity of DNB was enhanced, preferentially used the limited carbon source in wastewater as hydrogen donor and directly converted nitrite into nitrogen, which maximized TN removal efficiency and achieved shortcut nitrification–denitrification. Compared with C1 reactor without NH_2OH, the TN removal efficiency of C2 was improved 41.72%.

Combined with the data presented in Fig. 3 COD removal efficiency and Fig. 4 the NH_4^+–N removal efficiency, it could concluded that the optimal added concentration of NH_2OH was 20 mg/L to achieve the shortcut nitrification–denitrification in CRI system for domestic wastewater treatment.

3.4. Bacterial community analysis

To further investigate the mechanisms of the CRI system for nitrogen removal, the bacterial community was measured and analyzed. Two samples were collected when the concentration of NH_2OH were 0 mg/L (C1) and 20 mg/L (C2), respectively, and analyzed by Illumina MiSeq sequencing to illustrate the reason of achieving shortcut nitrification–denitrification on microbiology. Results can be seen in Fig. 6. On the genus level, AOB consisted of Nitrosomonas, norank_f_Nitrosomonadaceae and Phycisphaera Nitrosomonas, norank_f_Nitrosomonadaceae were common AOB, and Phycisphaera also had the function of oxidizing ammonia [33], while NOB only consisted of Nitrospira. The DNB consist of Thauera, Ottowia, Pseudomonas and Paracoccus. Thauera was a typical DNB [34]. The aggregate results showed that the relative abundance of AOB in the total bacterial community when the added concentration of NH_2OH

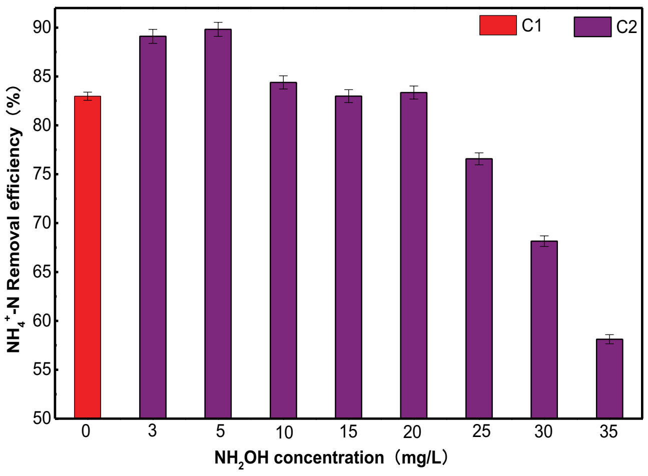

Fig. 4. The NH_4^+–N removal efficiency of CRI system.

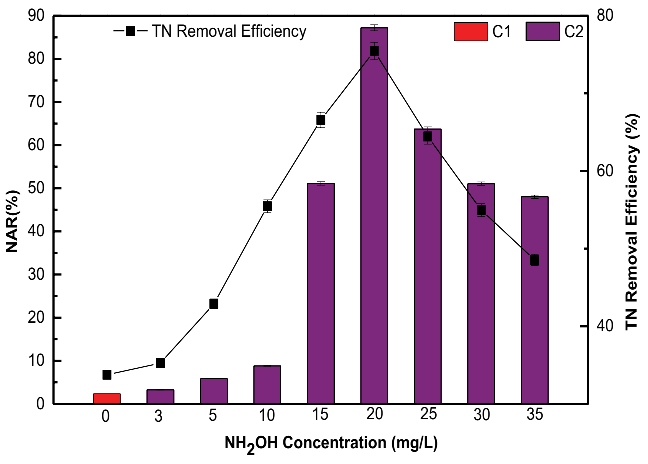

Fig. 5. NAR and TN removal efficiency of CRI system.

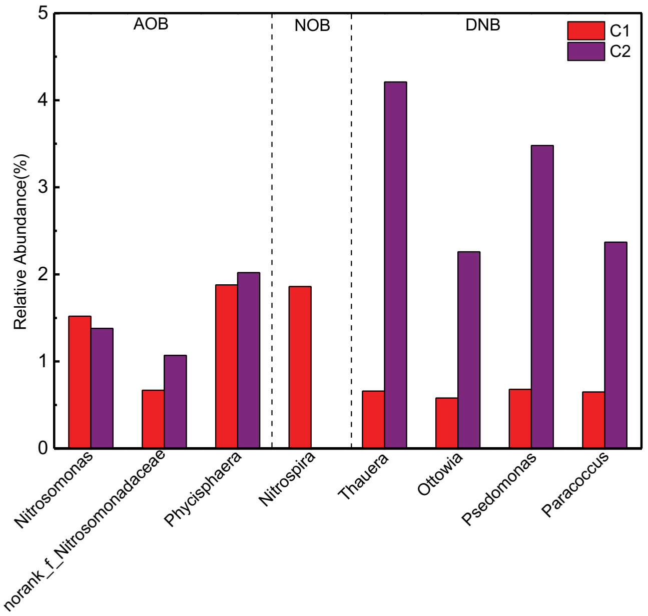

Fig. 6. Relative abundance of functional microorganisms on the genus level.

were 0 mg/L (C1) and 20 mg/L (C2) retained 4.07%–4.47%, respectively, while the relative abundance of NOB declined from 1.86% (C1) to be ignore as 0(C2), suggested that NOB was eliminated in C2 when the added concentration of NH_2OH was 20 mg/L. The DNB relative abundance of Thauera, Ottowia, Pseudomonas and Paracoccus increased from 0.66%, 0.58%, 0.68%, 0.65% in C1 to 4.21%, 2.26%, 3.48%, 2.37% in C2, respectively. The obviously increase due to the fact that the increase of substrate concentration in denitrification reaction. Suggesting that the denitrification reaction in C2 was enhanced when the added concentration of NH_2OH was 20 mg/L. Based on the results of high-throughput sequencing, when the added concentration of NH_2OH was 20 mg/L, NOB was seriously inhibited which caused NAR to increase to 87.2%, then the denitrifying bacteria was enhanced, the nitrite was converted directly to nitrogen and TN removal efficiency increased to 75.4%, which indicated that the shortcut nitrification–denitrification was achieved.

4. Conclusions

This study investigated the operational feasibility of achieving shortcut nitrification–denitrification in CRI system through the NH_2OH addition. The results indicated that the optimal added concentration of NH_2OH was 20 mg/L to achieve shortcut nitrification–denitrification on treating the domestic wastewater in CRI system. As the added concentration of NH_2OH was 20 mg/L, NAR and the removal efficiency of TN could reach 87.2% and 75.4%, respectively. NOB (Nitrospira) was seriously inhibited and DNB (Thauera, Ottowia, Pseudomonas and Paracoccus) was enhanced, meanwhile, COD and NH_4^+–N removal efficiencies reached 85.9% and 84.37%, respectively. Therefore, the low TN removal efficiency of CRI system was improved, which could be applied to wastewater treatment with denitrification rquirements.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (grant number 51268034). The authors would like to thank the assistant for sampling collection and analysis and for making useful suggestions. The authors would also like to thank the anonymous reviewers for making useful suggestions.

Author contributions

Xiuqin Kong conceived and designed the experiments; Yiming Lian performed the experiments; Nini Zhang analyzed the data; Qianjun Tang and Yajing Zhao contributed reagents and analysis tools; Yiming Lian wrote the paper.

Conflicts of interest

The authors declare no conflict of interest.

References

[1] X.J. Fan, Y.S. Fu, F. Liu, D. Xue, W.L. Xu, Total nitrogen removal efficiency of improved constructed rapid infiltration system, Technol. Water Treat., 10 (2009) 70–72, 85.

[2] J.T. He, Z.S. Zhong, M.G. Tang, New method of solving contradiction of rapid infiltration system land using, Geoscience, 15 (2001) 339–345.

[3] W.L. Xu, Y.N. Yang, C. Cheng, Treat Phoenix River water by constructed rapid infiltration system, J. Coastal Res., 73 (2015) 386–390.

[4] W.L. Xu, J.Q. Zhang, Y. Liu, Degradation of organic matter and ammonia nitrogen in constructed rapid infiltration system, Fresenius Environ. Bull., 20 (2011) 1685–1690.

[5] W.C. Ronald, C.R. Sherwood, K.B. Robert, Applying treatment wastewater to land, Bio. Cycle, 4 (2001) 32–35.

[6] J.T. He, Z.S. Zhong, M.G. Tang, H.H. Chen, Experimental research of constructed rapid infiltration wastewater treating system, China Environ. Sci., 22 (2002) 239–243.

[7] L. Yang, F.L. Kong, M. Xi, Y. Li, S. Wang, Environmental economic value calculation and sustainability assessment for constructed rapid infiltration system based on energy analysis, J. Cleaner Prod., 167 (2017) 582–588.

[8] L. Wang, Z.P. Yu, Z.J. Zhao, The removal mechanism of ammoniac nitrogen in constructed rapid infiltration system, China Environ. Sci., 26 (2006) 500 –504.

[9] J.B. Zhang, Study on Constructed Rapid Infiltration for Wastewater Treatment, Doctoral Thesis, China University of Geosciences, Beijing, China, 2002.

[10] Z.X. Song, H.Z. Zhang, Z.L. Wang, Y.H. Ping, G.Y. Liu, Q. Zhao, Treating sewage by strengthened constructed rapid infiltration system, Chin. J. Environ. Eng., 10 (2016) 3491–3495.

[11] Q.L. Fang, W.L. Xu, G.H. Xia, Z.C. Pan, Effect of C/N ratio on the removal of nitrogen and microbial characteristics in the water saturated denitrifying section of a two-stage constructed rapid infiltration system, Int. J. Environ. Res. Public Health, 15 (2018) doi: 10.3390/ijerph15071469.

[12] Q.L. Fang, W.L. Xu, Z.J. Yan, L. Qian, Effect of potassium chlorate on the treatment of domestic sewage by achieving shortcut nitrification in a constructed rapid infiltration system, Int. J. Environ. Res. Public Health, 15 (2018) 670, doi: 10.3390/ijerph15040670.

[13] Z. Wang, L. Zhang, F. Z. Zhang, H. Jiang, S. Ren, W. Wang, Nitrite accumulation in comammox-dominated nitrification–denitrification reactors: effects of DO concentration and hydroxylamine addition, J. Hazard. Mater., 384 (2020) 121375, doi: 10.1016/j.jhazmat.2019.121375.

[14] M. Arnaldos, Y. Amerlinck, U. Rehman, T. Maere, S. V. Hoey, W. Naessens, From the affinity constant to the half-saturation

index: understanding conventional modeling concepts in novel wastewater treatment processes, Water Res., 70 (2015) 458–470.

[15] A.C. Anthonisen, R.C. Loehr, T.B.S. Prakasam, E.G. Srinath, Inhibition of nitrification by ammonia and nitrous-acid, J. Water Pollut. Control Fed., 48 (1976) 835–852.

[16] S.P.A. Villaverde, E García, F. Fdz-Polanco, Influence of pH over nitrifying biofilm activity in submerged biofilters, Water Res., 31 (1997) 1180–1186.

[17] T. Yamamoto, K. Takaki, T. Koyama, K. Furukawa, Novel partial nitritation treatment for anaerobic digestion liquor of swine wastewater using swim-bed technology, J. Biosci. Bioeng., 102 (2006) 497–503.

[18] A. Sukru, Erdal, Simsek, Influence of salinity on partial nitrification in a submerged biofilter, Bioresour. Technol., 118 (2012) 24–29.

[19] H.W. Sun, Q. Yang, G.R. Dong, H.X. He, S.J. Zhang, Y.Y. Yang, Achieving the nitrite pathway using FA inhibition and process control in UASB-SBR system removing nitrogen from landfill leachate, Sci. China Chem., 53 (2010) 1210–1216.

[20] Z.B. Yao, D.J. Zhang, P.Y. Xiao, S.C. Peng, P.L. Lu, Long-term addition of micro-amounts of hydrazine enhances nitrogen removal and reduces NO and NO_3^- production in a SBR performing anammox, J. Chem. Technol. Biotechnol., 91 (2016) 514–521.

[21] F.H.J. Willie, T. Akihiko, P. Franck, L.R. Xavier, K. Ken, M. Mustafa, The effect of hydroxylamine on the activity and aggregate structure of autotrophic nitrifying bioreactor cultures, Biotechnol. Bioeng., 102 (2010) 714–724.

[22] I. Schmidt, C. Look, E. Bock, Ammonium and hydroxylamine uptake and accumulation in Nitrosomonas, Microbiology, 150 (2004) 1405–1412.

[23] S. Okabe, M. Oshiki, Y. Takahashi, H. Satoh, Development of long-term stable partial nitrification and subsequent anammox process, Bioresour. Technol., 102 (2011) 6801–6807.

[24] L. P. Kuai, W. Verstraete, Ammonium removal by the oxygen-limited autotrophic nitrification–denitrification system, Appl. Environ. Microbiol., 64 (1998) 4500–4506.

[25] G. Xu, X. Xu, F. Yang, S. Liu, Y. Gao, Partial nitrification adjusted by hydroxylamine in aerobic granules under high DO and ambient temperature and subsequent Anammox for low C/N wastewater treatment, Chem. Eng. J., 213 (2012) 338–345.

[26] J. Li, Q. Zhang, X.Y. Li, Y.Z. Peng, Rapid start-up and stable maintenance of domestic wastewater nitritation through short-term hydroxylamine addition, Bioresour. Technol., 278 (2019) 468–472.

[27] D.S. Yu, Experimental Study on Artificial Rapid Infiltration System Treating Urban Initial Runoff Pollution, Master Thesis, Xi'an University of Architecture and Technology, Xi'an, China, 2017.

[28] L.Y. Fang, Research on Effect of Constructed Rapid Infiltration System for Distributed Rural Sewage Treatment, Master Thesis, Hebei Agricultural University, Hebei, China, 2014.

[29] Y.H. Ping, Y.H. Zhang, Z.L. Wang, Q.Z. Wang, Z.X. Song, Non-saturated layer of constructed rapid infiltration system about removing COD and ammonia nitrogen, Chin. J. Environ. Eng., 9 (2015) 5837–5842.

[30] W.J. Ma, Experimental study on treating rural sewage with artificial fast subsurface infiltration system, Technol. Water Treat., 40 (2014) 91–94.

[31] J. Chen, J.Q. Zhang, H.Y. Wen, Q. Zhang, X. Yang, J. Li, The effect of hydroxylamine inhibition and pH control on achieving shortcut nitrification in constructed rapid infiltration system, Acta Scientiae Circumstantiae, 36 (2016) 3728–3735.

[32] T. Kindaichi, S. Okabe, H. Satoh, Y. Watanabe, Effects of hydroxylamine on microbial community structure and function of autotrophic nitrifying biofilms determined by in situ hybridization and the use of microelectrodes, Water Sci. Technol.: J. Int. Assoc. Water Pollut. Res., 49 (2004) 61.

[33] J.B. Liu, Z.Y. Tian, P.Y. Zhang, G.L. Qiu, Y. Wu, Influence of reflux ratio on two-stage anoxic/oxic with MBR for leachate treatment: performance and microbial community structure, Bioresour. Technol., 256 (2018) 69–76.

[34] R. Du, Y. Peng, S. Cao, B. Li, S. Wang, and M. Niu, Mechanisms and microbial structure of partial denitrification with high nitrite accumulation, Appl. Microbiol. Biotechnol., 100 (2016) 2011–2021.