Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.26627

211 (2021) 117–122
January

High-efficiency denitrification for steel wastewater treatment by immobilized bacteria

Lian-gang Hou[a], Jun Li[a,*], Fu-yin Sun[b], Xin-yu Zhang[b], Yang Liu[a]

[a]*College of Architecture and Civil Engineering, Beijing University of Technology, 100 Pingleyuan, Chaoyang District, Beijing 100124, China, emails: bjutlijun@sina.com (J. Li), houliangang@163.com (L.-g. Hou), liuyang@emails.bjut.edu.cn (Y. Liu)*
[b]*Engineering and Technology Co., Ltd., of Ansteel Group Cor., Ltd., Anshan, Liaoning 114021, China, emails: 429212205@qq.com (F.-y. Sun), 2482574183@qq.com (X.-y. Zhang)*

Received 31 May 2020; Accepted 18 September 2020

ABSTRACT

The performance of immobilized bacteria by waterborne polyurethane in treating steel wastewater with nitrate nitrogen was investigated. It found that the optimal C/N ratio was 5.0 using glucose as an electron donor and the hydraulic retention time was set to 6 h could meet the needs of denitrification in this study. The removal efficiency of immobilized denitrifying bacteria on nitrate–nitrogen in steel wastewater reached 80%, and the nitrate concentration in the effluent was less than 5 mg/L, indicating that microbial immobilization technology has a better treatment effect on steel wastewater. High-throughput sequencing shows that steel wastewater has a domesticated selection effect on the evolution of the immobilized bacteria, making the bacteria which adapt to the quality of the steel wastewater to be dominant bacteria. Through this study, we believe that steel wastewater can be treated in a targeted manner to achieve accurate decontamination by microbial immobilization technology.

Keywords: Immobilized bacteria; Denitrification; Steel wastewater; Nitrate; Wastewater treatment

1. Introduction

The steel industry is an important source of industrial wastewater discharge [1], and the steel wastewater contains a variety of pollutants which is highly biotoxic [2] such as phenol, ammonia, nitrogen compounds, etc [3]. Nitrogen compounds in steel wastewater can cause environmental problems such as eutrophication [4–6], and nitrate has been known to be hazardous to human health [7]. While conventional methods are inadequate for complete treatment, the advanced treatments, like membrane separation, remains relatively untapped in steel wastewater treatment [3]. At present, the steel wastewater is often treated biologically by microbial life metabolic processes to degrade toxic substances [8,9], commonly used microorganism carriers including activated sludge and biofilm [10–12]. Nevertheless, microorganism in these treatment methods is easy to lose and affect wastewater treatment efficiency [13,14]. Therefore, an alternative treatment technology for efficient steel wastewater treatment is urgently needed.

Microbial immobilization technology uses physical or chemical methods to limit free cells to a certain space area [15–18], which is a nutrient removal technology developed in recent years [19–23]. Microbial immobilization technologies have been proven to be used in biological wastewater processing to remove nitrate [24–26]. However, there are few reports [3] on using microbial immobilization technology for steel wastewater treatment.

The present study uses immobilized bacteria by waterborne polyurethane for treating steel wastewater, investigated the performance of denitrification bioreactor with immobilized bacteria. It investigated the feasibility of immobilized bacteria in the steel wastewater treatment, and the

* Corresponding author.

optimal bioreactor operation parameters were obtained from denitrification batch experiments. This research would provide a new method for steel wastewater treatment and promote the application progress of microbial immobilization technology in wastewater treatment.

2. Materials and methods

2.1. Immobilization procedure

According to our previous reports [27], we use cultured sludge [28] as microbial strains and waterborne polyurethane as immobilized material. Added 1% (w/V) potassium persulfate solution and 0.5% (w/V) N,N,N′,N′-tetramethylethylenediamine solution to induced polymerization. The mixture solidified for 5~10 min at 27°C ± 2°C and formed a solid jelly-bean-shaped block of hydrosol, then cut it into 3 mm × 3 mm × 3 mm cubes and obtain the immobilized bacterial particles.

2.2. Reactor setup

The schematic of the denitrification bioreactor is shown in Fig. 1, the bioreactor was made of a Plexiglas cylinder with an operating volume of 85.0 L. Added 17.0 L (20%, v/V) immobilized bacterial particles into the reactor. In this study, a peristaltic pump was used to control the hydraulic retention time (HRT), and the bioreactor was operated by continuous water inflow and outflow. Glucose was used as an external carbon source for the denitrification process. In batch experiments, set the HRT to 8 h, and set the C/N ratio to 2.0, 3.0, 3.5, 4.0, 5.0, and 6.0, respectively. During the long-term experiment, set the C/N ratio to 5.0 and the HRT was set to 8h for the first 15 d and 6 h after 15 d. The operation temperature was controlled 25°C ± 2°C.

Steel wastewater used in this work was obtained from a wastewater treatment plant that treats steel wastewater of Anshan Iron and Steel Group (Liaoning Province, China). The results of wastewater quality analysis are shown in Table 1. The nitrate concentration in steel wastewater was about 25 mg/L, and chemical oxygen demand (COD) was about 50 mg/L.

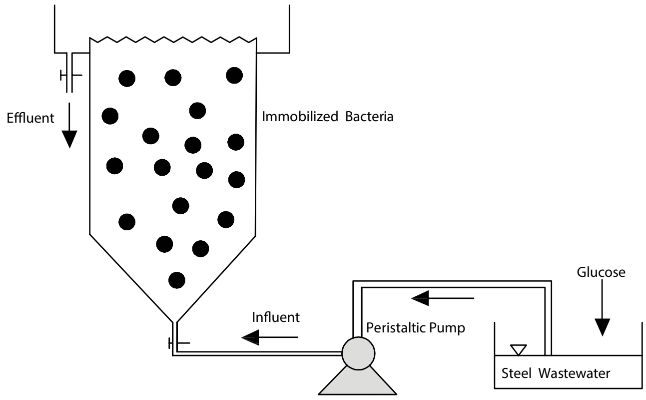

Fig. 1. Schematic diagram of the denitrification bioreactor with immobilized bacteria.

Table 1
Water quality analysis of the steel wastewater

Test index	Contents
Phenol	60 μg mL^{-1}
Nitrate	25 ± 2 mg L^{-1}
Nitrite	0.02 mg L^{-1}
Ammonia	0.1 mg L^{-1}
Total nitrogen	25 mg L^{-1}
pH	7.2
Total phosphorus	0.2 mg L^{-1}
Iron	1.65 mg L^{-1}
Aluminum	0.52 mg L^{-1}
Manganese	0.028 mg L^{-1}
Calcium	53.91 mg L^{-1}
Cyanide	0.1 mg L^{-1}
COD	50 ± 5 mg L^{-1}
Chroma	10
Turbidity	10
Alkalinity	120 mg L^{-1}
Hardness	450 mg L^{-1}
Dissolved oxygen	1.35 mg L^{-1}

2.3. Analytical methods

Samples for water quality measurements were collected and analyzed every day. The concentrations of NO_3^-–N, NO_2^-–N and COD in influent and effluent were analyzed according to standard methods for the Examination of Water and Wastewater [29]. All tests were repeated more than twice and the temperature and pH were measured near the midway of the bioreactor by using a WTW analyzer (Multi 3620IDS, Germany). The scanning electron microscope procedure of the sample was carried out according to the description in Dong et al. [17].

2.4. Microbiological analysis

High-throughput sequencing of 16S rRNA gene fragments was used to analyze the microbial communities in immobilized particle samples. Bacterial genomic DNA was extracted using an E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Inc., United States) after the samples pretreated following the manufacturer's instructions. The PCR amplification used the universal bacteria primers which incorporated the Miseq platform the V3-V4 hypervariable regions. The PCR products were sequenced on the Miseq 2 × 300bp platform by Sangon Biotech, Shanghai, China.

3. Results and discussion

3.1. Effects of C/N ratios and HRT on the denitrification efficiency in batch experiments

Fig. 2 shows the changes in nitrate and COD concentrations at different C/N ratios. As shown in Fig. 2, the nitrate removal rate was 32.4% when no carbon source was added (C/N = 2.0). In this research, the nitrate concentration in steel

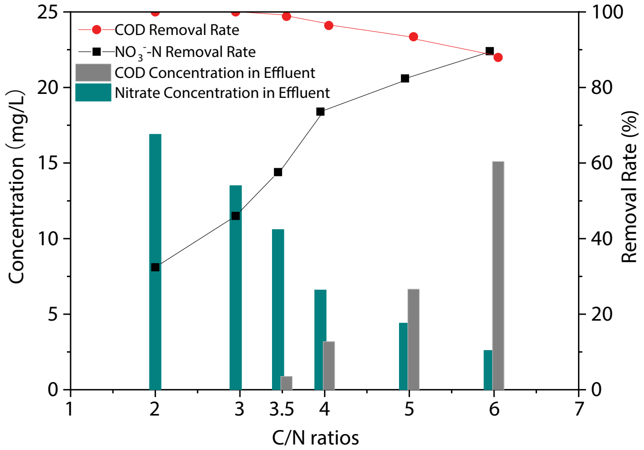

Fig. 2. Changes of nitrate and COD under different carbon–nitrogen ratios.

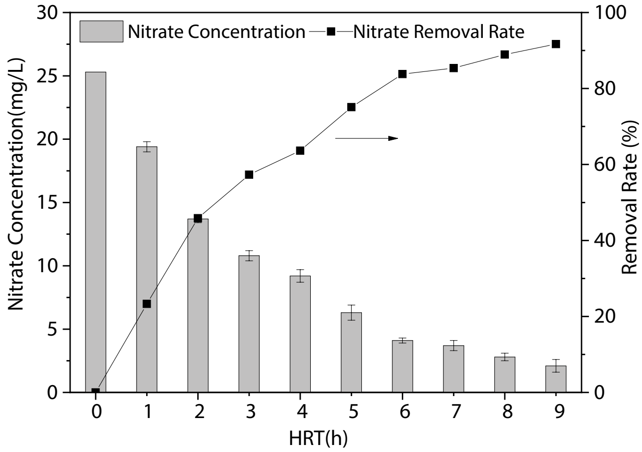

Fig. 3. Changes of nitrate under different HRT.

wastewater was about 25 mg/L, and the COD was about 50 mg/L according to Table 1. Denitrification requires a C/N ratio of more than 2.86 in theory [30], and the COD in the steel wastewater cannot meet the requirements of denitrification in this study. When the C/N ratios were 3.0, 3.5, 4.0, 5.0, and 6.0, the nitrate removal rates were 46.0%, 57.6%, 73.6%, 82.4% and 89.6%, respectively. When C/N ratio was 5.0, the nitrate concentration in the effluent was 4.4 mg/L and the COD concentration was 6.65 mg/L, 82.4% nitrate was removed and most of the glucose was utilized by denitrification in the steel wastewater. Therefore, adding glucose to maintain the C/N ratio to 5.0 could meet the demand of denitrification and without causing a waste of carbon sources in this study, and this is consistent with previous reports [31–33].

HRT affects the reaction time of nitrate and denitrifying bacteria and affects the removal efficiency of pollutants [34]. Fig. 3 shows the changes in nitrate removal rate with HRT. According to Fig. 3, it can be seen that the nitrate removal rate reached 63.63% after 4 h, 83.79% after 6 h, and about 88.93% after 8 h. When HRT was too short, the reaction was incomplete, while it will waste time when HRT was too long. Extending HRT blindly was meaningless and an appropriate HRT for denitrification was required, this is consistent with other people's reports [35]. We believe that when the immobilized denitrifying bacterial was used for steel wastewater treatment, the HRT was set to 6 h could meet the needs of denitrification according to this study.

3.2. Performance of denitrification bioreactor with immobilized bacterial

Fig. 4 shows the changes of NO_3^-–N concentration from steel wastewater with the immobilized denitrifying bacteria treatment. As shown in Fig. 4, the nitrate removal rate in the bioreactor gradually increased in the first 15 d. On the 15th day, the removal rate reached 77.87%, it shows that the immobilized denitrifying bacteria gradually adapt to the steel wastewater quality and become the dominant bacterial species [9,36]. On the 16th day, the HRT was shortened from 8 to 6 h, and the nitrate removal rate decreases temporarily. Nitrate was removed stably from the 19th day, the removal rate reached 80% and the nitrate concentration in the effluent was less than 5 mg/L. When the HRT was shortened, the rapid recovery of the nitrate removal rate indicates that the immobilized bacteria in this study have a strong ability to resist the impact of water quality load. The results of nitrate removal indicated that the immobilized denitrifying bacteria used in this study has a good effect of denitrification on steel wastewater, and bacteria immobilized technology can be used for steel wastewater treatment. Other reports have also proved that the bacterial immobilization technology has better effects in wastewater treatment, such as Dong et al. [17] reported that waterborne polyurethane immobilized activated sludge has a high-efficiency denitrification effect for the treatment of acrylonitrile wastewater. We believe that different immobilized bacteria can be used to treat different pollutants in steel wastewater, and steel wastewater can be treated in a targeted manner to achieve accurate decontamination. It is hoped that this research will lay the foundation for the application of immobilized bacteria technology in steel wastewater.

3.3. Analysis of microbial community in immobilized particles

In the research process, the immobilized bacterial particles in the bioreactor were structurally stable in the steel wastewater by visually observing, without breaking or disintegration, and have good mechanical stability (Fig. 5). It can be seen from Fig. 5, the bacterial flora in the immobilized bacterial particles was densely clustered, and the number of the bacterial flora was large. The short rod-shaped [27] or spherical bacteria were tightly coupled with the immobilized material, so the bacterial cells were not easy to flow and the adhesion was good. Scanning electron microscopy pictures show good growth of bacteria in the immobilized bacteria particles, which ensures the efficiency of wastewater treatment. This shows that the immobilized bacterial particles prepared by waterborne polyurethane have good mechanical strength and suitable for steel wastewater treatment.

The types and relative abundance of microorganisms in the immobilized bacteria particles was analyzed at the genus level. After 40 d of steel wastewater treatment with

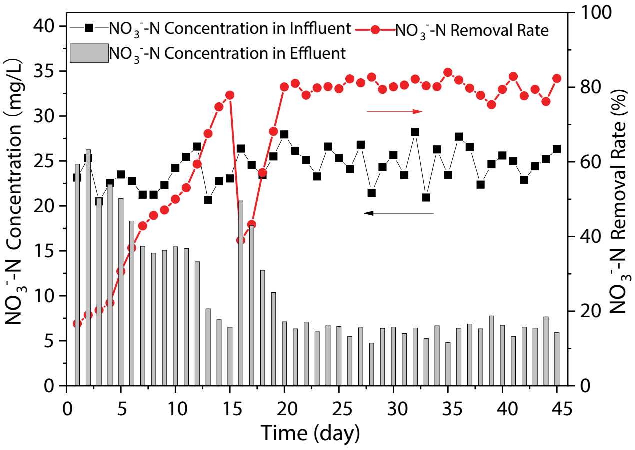

Fig. 4. The removal efficiency of nitrate in steel wastewater by immobilized denitrifying bacteria.

immobilized bacteria, 30–50 immobilized bacteria particles were randomly taken from the bioreactor, shredded, and 16S sequenced after pretreatment. According to our previous reports [28], the sludge used in the preparation of immobilized bacterial particles in this study mainly consists of *Diaphorobacter* (relative abundance was about 34%) and *Paracoccus* (relative abundance was about 25%). The preparation of immobilized bacterial particles was performed in a sterile environment, so it can be considered the bacteria in the immobilized bacterial particles when the reactor started mainly consist of these two bacteria in this study. It can be seen from Fig. 6 that the type and relative abundance of the predominant bacterial groups in the immobilized bacterial particles have changed significantly after 40 d in steel wastewater. At this time, the bacteria with a relative abundance of more than 10% in the immobilized bacterial particles were mainly *Comamonas* (42.99%), *Simplicispira* (13.23%), and *Paracoccus* (10.66%). Although the presence of *Diaphorobacter* and *Paracoccus* in the immobilized bacterial particles can still be detected, their relative abundances have decreased significantly. After 40 d operations, they have become a non-dominant bacterial group in the immobilized particles. We speculate that the activity of this microorganism was affected by the quality of steel wastewater, and its relative abundance has decreased.

The unique constitution of microbial communities may play a key role in immobilized particles enhancement of nitrate biodegradation in steel wastewater, such as the synergistic relationship of *Comamonas* and *Simplicispira* [37], *Comamonas* and *Paracoccus* [38], competition relationship of *Simplicispira* and *Paracoccus* [9,39]. *Comamonas* has a good degradation effect on a variety of pollutants. It is reported [40,41] that *Comamonas* degraded not only organic pollutants but also inorganic pollutants. In this study, the presence of organic substances such as phenol and inorganic substances such as nitrate in steel wastewater was beneficial to the growth of *Comamonas*. The special water quality environment of steel wastewater plays a domesticating role in the evolution of *Comamonas* and making it enriched and grown in the immobilized bacterial particles, which results in its high relative abundance and becomes

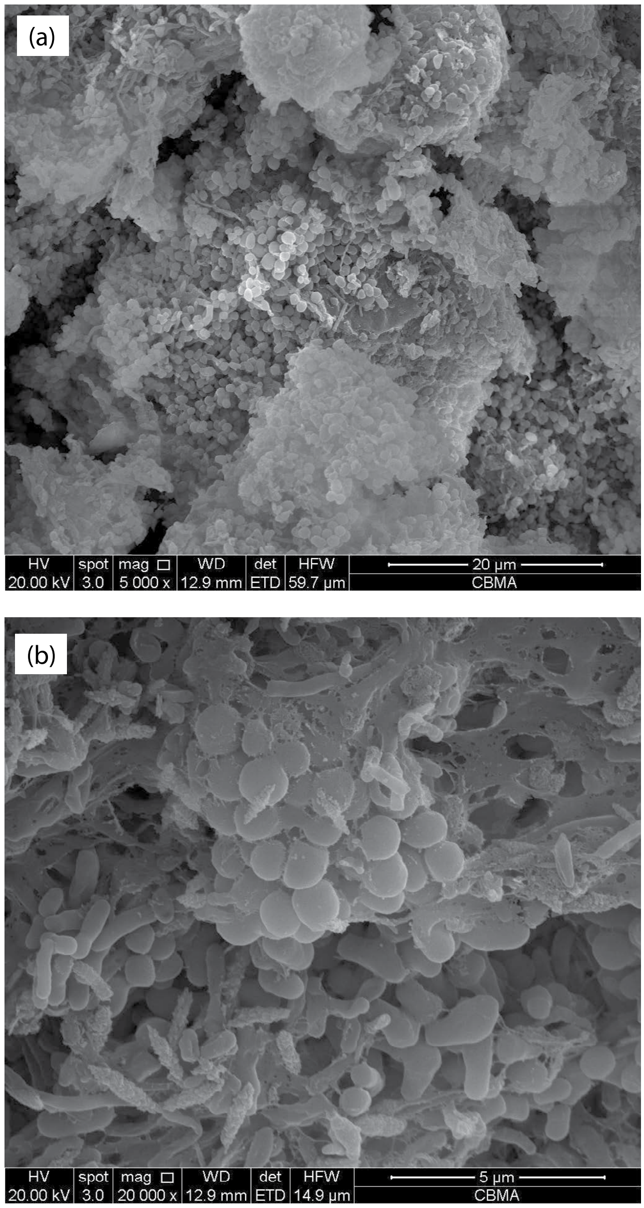

Fig. 5. Scanning electron microscopy of denitrifying immobilized particles.

one of the dominant bacterial groups. *Simplicispira* has a denitrifying effect and can convert NO_3^- to N_2 [42], it is reported that this type of bacteria was suitable for existence in an attached form. In this study, the special space environment within the immobilized bacterial particles makes the bacteria difficult to flow, which is more suitable for *Simplicispira* attachment, and it is conducive to their proliferation. Combining with the removal rate of nitrate in Fig. 3, it is known that *Simplicispira* plays an important role in the denitrification of steel wastewater treatment. *Paracoccus* is an important type of denitrifying bacteria, which can effectively transform different forms of inorganic nitrogen [43]. It could convert NO_2^-–N and NO_3^-–N by denitrification. The relative abundance of this bacterium was significantly reduced in the immobilized bacterial particles,

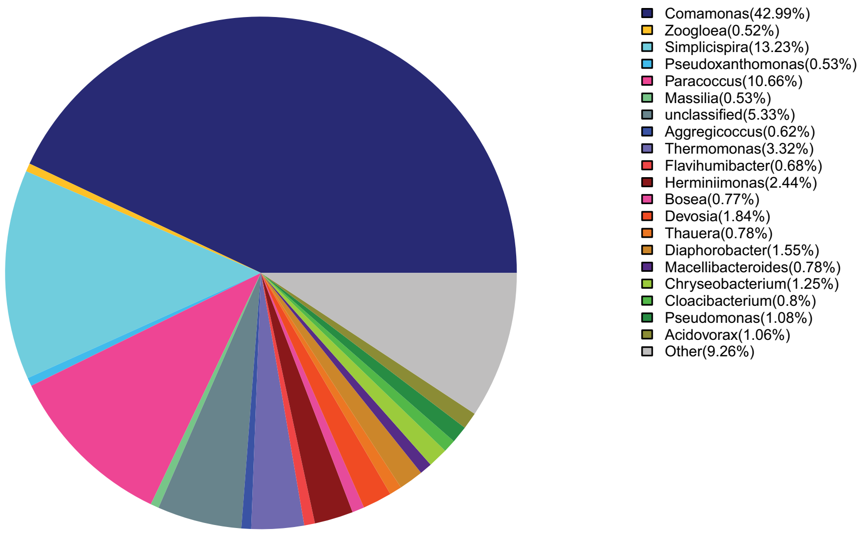

Fig. 6. 16sRNA analysis of the immobilized bacterial particles, genus identified based on sequencing are shown.

from 25% at the beginning to 11% after 40 d of operation. In summary, it can be seen that the flora in the immobilized bacterial particles has undergone significant changes under the action of the steel wastewater through high-throughput sequencing, and the bacteria that adapt to the quality of wastewater has become the dominant bacteria.

4. Conclusions

This study used immobilized bacteria to treat steel wastewater and found that the optimal C/N ratio was 5.0 using glucose as an electron donor and the HRT was set to 6 h could meet the needs of denitrification. The removal efficiency of immobilized denitrifying bacteria on nitrate in steel wastewater reached 80%, and the nitrate concentration in the effluent was less than 5 mg/L, indicating that microbial immobilization technology has a better treatment effect on steel wastewater for nitrate removal. The steel wastewater has a domesticating selection effect on the evolution of the immobilized bacteria, making the bacteria which adapt to the quality of wastewater to be dominant bacteria. This research would provide a new method for steel wastewater treatment and promote the application of microbial immobilization technology in wastewater treatment.

Acknowledgment

This work was supported by the Major Science and Technology Program for Water Pollution Control and Treatment of China (No. 2015ZX07202-013; No.2017ZX07103-001).

References

[1] Z.C. Guo, Z.X. Fu, Current situation of energy consumption and measures taken for energy saving in the iron and steel industry in China, Energy, 35 (2010) 4356–4360.
[2] J. Ernesto Ramírez, S. Esquivel-González, J. René Rangel-Mendez, S.L. Arriaga, M. Gallegos-García, G. Buitrón, F.J. Cervantes, Biorecovery of metals from a stainless steel industrial effluent through denitrification performed in a novel anaerobic swirling fluidized membrane bioreactor (ASFMBR), Ind. Eng. Chem. Res., 59 (2020) 2725–2735.
[3] P. Das, G.C. Mondal, S. Singh, A.K. Singh, B. Prasad, K.K. Singh, Effluent treatment technologies in the iron and steel industry - a state of the art review, Water Environ. Res., 90 (2018) 395–408.
[4] Y. Fernández-Nava, E. Marañón, J. Soons, L. Castrillón, Denitrification of wastewater containing high nitrate and calcium concentrations, Bioresour. Technol., 99 (2008) 7976–7981.
[5] U. Sandhya, D. Banerjee, I.J. Singh, P.K. Wattal, Denitrification of high sodium nitrate bearing effluents using flow-through bioreactor, Desal. Water Treat., 38 (2012) 52–58.
[6] W.L. Huang, B.G. Zhang, C.P. Feng, M. Li, J. Zhang, Research trends on nitrate removal: a bibliometric analysis, Desal. Water Treat., 50 (2012) 67–77.
[7] J.Y. Jung, S.J. Bae, W.J. Lee, Nitrate reduction by maghemite supported Cu-Pd bimetallic catalyst, Appl. Catal., B, 127 (2012) 148–158.
[8] G.P. Sun, J.F. Wan, Y.C. Sun, H.S. Li, C. Chang, Y. Wang, Enhanced removal of nitrate and refractory organic pollutants from bio-treated coking wastewater using corncobs as carbon sources and biofilm carriers, Chemosphere, 237 (2019)124520–124528.
[9] M.M.M. Kuypers, H.K. Marchant, B. Kartal, The microbial nitrogen-cycling network, Nat. Rev. Microbiol., 16 (2018) 263–276.
[10] E. Raper, R. Fisher, D.R. Anderson, T. Stephenson, A. Soares, Nitrogen removal from coke making wastewater through a pre-denitrification activated sludge process, Sci. Total Environ., 666 (2019) 31–38.
[11] E. Marañón, I. Vázquez, J. Rodríguez, L. Castrillón, Y. Fernández, Coke wastewater treatment by a three-step activated sludge system, Water Air Soil Pollut., 192 (2008) 155–164.
[12] E.C. Li, S.G. Lu, Denitrification processes and microbial communities in a sequencing batch reactor treating nanofiltration (NF) concentrate from coking wastewater, Water Sci. Technol., 76 (2017) 3289–3298.
[13] P.L. McCarty, What is the best biological process for nitrogen removal: when and why?, Environ. Sci. Technol., 52 (2018) 3835–3841.
[14] J.L. Tang, X.C. Wang, Y.S. Hu, Y.H. Pu, J. Huang, H.H. Ngo, Y.G. Zeng, Y.Y. Li, Nutrients removal performance and sludge properties using anaerobic fermentation slurry from food waste as an external carbon source for wastewater treatment, Bioresour. Technol., 271 (2019) 125–135.
[15] S. Naahidi, M. Jafari, M. Logan, Y. Wang, Y.F. Yuan, H.J. Bae, B. Dixon, P. Chen, Biocompatibility of hydrogel-based scaffolds for tissue engineering applications, Biotechnol. Adv., 35 (2017) 530–544.

[16] A. Freeman, M.D. Lilly, Effect of processing parameters on the feasibility and operational stability of immobilized viable microbial cells, Enzyme Microb. Technol., 23 (1998) 335–345.

[17] H.H. Dong, W. Wang, Z.Z. Song, H. Dong, J.F. Wang, S.S. Sun, Z.Z. Zhang, M. Ke, Z.J. Zhang, W.-M. Wu, G.Q. Zhang, J. Ma, A high-efficiency denitrification bioreactor for the treatment of acrylonitrile wastewater using waterborne polyurethane immobilized activated sludge, Bioresour. Technol., 239 (2017) 472–481.

[18] H.A. Ahmad, S.-Q. Ni, S. Ahmad, J. Zhang, M. Ali, H.H. Ngo, W.S. Guo, Z.W. Tan, Q. Wang, Gel immobilization: a strategy to improve the performance of anaerobic ammonium oxidation (anammox) bacteria for nitrogen-rich wastewater treatment, Bioresour. Technol., 313 (2020) 123642–123654.

[19] T.-H. Hsia, Y.-J. Feng, C.-M. Ho, W.-P. Chou, S.-K. Tseng, PVA-alginate immobilized cells for anaerobic ammonium oxidation (anammox) process, J. Ind. Microbiol. Biotechnol., 35 (2008) 721–727.

[20] R.R. Nair, P.B. Dhamole, S.S. Lele, S.F. D'Souza, Biotreatment of high strength nitrate waste using immobilized preadapted sludge, Appl. Biochem. Biotechnol., 151 (2008) 193–200.

[21] R. Aoyagi, R. Sato, A. Terada, H. Tokuyama, Novel composite gel beads for the immobilization of ammonia-oxidizing bacteria: fabrication, characterization, and biokinetic analysis, Chem. Eng. J., 342 (2018) 260–265.

[22] J.L. Xue, Y.N. Wu, K. Shi, X.F. Xiao, Y. Gao, L. Li, Y.L. Qiao, Study on the degradation performance and kinetics of immobilized cells in straw-alginate beads in marine environment, Bioresour. Technol., 280 (2019) 88–94.

[23] N. Li, H.C. Xu, Y.P. Yang, X.M. Xu, J.L. Xue, Preparation, optimization and reusability of immobilized petroleum-degrading bacteria, Environ. Technol., (2019) 1703826–1703826.

[24] Y.M. Dong, Z.J. Zhang, Y.W. Jin, J. Lu, X.H. Cheng, J. Li, Y.-Y. Deng, Y.-N. Feng, D.N. Chen, Nitrification characteristics of nitrobacteria immobilized in waterborne polyurethane in wastewater of corn-based ethanol fuel production, J. Environ. Sci., 24 (2012) 999–1005.

[25] F. Ma, Y.L. Sun, A. Li, X.N. Zhang, J.X. Yang, Activation of accumulated nitrite reduction by immobilized *Pseudomonas stutzeri* T13 during aerobic denitrification, Bioresour. Technol., 187 (2015) 30–36.

[26] X.Y. Xu, C.P. Lv, X.L. You, B. Wang, F.Y. Ji, B.B. Hu, Nitrogen removal and microbial diversity of activated sludge entrapped in modified poly(vinyl alcohol)–sodium alginate gel, Int. Biodeterior. Biodegrad., 125 (2017) 243–250.

[27] L.G. Hou, J. Li, Y. Liu, Microbial communities variation analysis of denitrifying bacteria immobilized particles, Process Biochem., 87 (2019) 151–156.

[28] L.G. Hou, J. Li, Z.M. Zheng, Q. Sun, Y.T. Liu, K. Zhang, Cultivating river sediments into efficient denitrifying sludge for treating municipal wastewater, R. Soc. Open Sci., 6 (2019) 190304–190304.

[29] E.W. Rice, R.B. Baird, A.D. Eaton, L.S. Clesceri, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C., 2012.

[30] K. Bernat, I. Wojnowska-Baryła, A. Dobrzyńska, Denitrification with endogenous carbon source at low C/N and its effect on P(3HB) accumulation, Bioresour. Technol., 99 (2008) 2410–2418.

[31] L. Pelaz, A. Gómez, A. Letona, G. Garralón, M. Fdz-Polanco, Nitrogen removal in domestic wastewater. Effect of nitrate recycling and COD/N ratio, Chemosphere, 212 (2018) 8–14.

[32] H.D. Hu, K.W. Liao, J.J. Geng, K. Xu, H. Huang, J.F. Wang, H.Q. Ren, Removal characteristics of dissolved organic nitrogen and its bioavailable portion in a postdenitrifying biofilter: effect of the C/N ratio, Environ. Sci. Technol., 52 (2018) 757–764.

[33] X.Y. Fan, H.-q. Li, P. Yang, B. Lai, Effect of C/N ratio and aeration rate on performance of internal cycle MBR with synthetic wastewater, Desal. Water Treat., 54 (2015) 573–580.

[34] J. Torno, C. Naas, J.P. Schroeder, C. Schulz, Impact of hydraulic retention time, backflushing intervals, and C/N ratio on the SID-reactor denitrification performance in marine RAS, Aquaculture, 496 (2018) 112–122.

[35] Y.D. Guo, L. Guo, M. Sun, Y.G. Zhao, M.C. Gao, Z.L. She, Effects of hydraulic retention time (HRT) on denitrification using waste activated sludge thermal hydrolysis liquid and acidogenic liquid as carbon sources, Bioresour. Technol., 224 (2017) 147–156.

[36] G. Guo, Y.Y. Wang, T.W. Hao, D. Wu, G.-H. Chen, Enzymatic nitrous oxide emissions from wastewater treatment, Front. Environ. Sci. Eng., 12 (2018) 10–22.

[37] O. Auguet, M. Pijuan, H. Guasch-Balcells, C.M. Borrego, O. Gutierrez, Implications of downstream nitrate dosage in anaerobic sewers to control sulfide and methane emissions, Water Res., 68 (2015) 522–532.

[38] T. Zhang, M.-F. Shao, L. Ye, 454 Pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants, ISME J., 6 (2012) 1137–1147.

[39] J.L. Wang, L.B. Chu, Biological nitrate removal from water and wastewater by solid-phase denitrification process, Biotechnol. Adv., 34 (2016) 1103–1112.

[40] Q. Ma, Y.Y. Qu, W.L. Shen, Z.J. Zhang, J.W. Wang, Z.Y. Liu, D.X. Li, H.J. Li, J.T. Zhou, Bacterial community compositions of coking wastewater treatment plants in steel industry revealed by Illumina high-throughput sequencing, Bioresour. Technol., 179 (2015) 436–443.

[41] D. Chen, K. Yang, H.Y. Wang, High nitrate removal by autohydrogenotrophic bacteria in a biofilm-electrode reactor, Desal. Water Treat., 55 (2015) 1316–1324.

[42] M.Z. Siddiqi, W. Sok, G.M. Choi, S.Y. Kim, J.-H. Wee, W.T. Im, *Simplicispira hankyongi* sp. nov., a novel denitrifying bacterium isolated from sludge, Antonie Van Leeuwenhoek, 113 (2020) 331–338.

[43] A. Olaya-Abril, J. Hidalgo-Carrillo, V.M. Luque-Almagro, C. Fuentes-Almagro, F.J. Urbano, C. Moreno-Vivián, D.J. Richardson, M.D. Roldán, Exploring the denitrification proteome of *Paracoccus denitrificans* PD1222, Front. Microbiol., 9 (2018) 1–12.