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Improvement in denitrification efficiency at low temperature with addition of redox mediators

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ABSTRACT

The efficiency of wastewater denitrification was determined in batch cultures at low temperatures (10°C and 15°C) by adding different redox mediators (RMs; anthraquinone-2,6-disulphonate [AQDS], 2-hydroxy-1,4-naphthoquinone [LAW] and 1,2-naphthoquinone-4-sulphonate [NQS]). The denitrification rate and nitrogen removal efficiency were found to increase with the addition of RMs at low temperatures compared with the control. At 10°C, the highest nitrogen removal efficiency was 11% (control: 5%) and maximum denitrification rate of 2.06 mgNO_x^-–N/g volatile suspended solid [VSS]·h was achieved with addition of NQS (control: 1.15 mgNO_x^-–N/g VSS·h). At 15°C, the maximum denitrification efficiency increased to 18.7% with addition of LAW (control: 10.7%) and the denitrification rate improved to 5.63 mgNO_x^-–N/g VSS·h with addition of NQS (control: 3.53 mgNO_x^-–N/g VSS·h). Investigation of the underlying mechanism revealed a typical redox reaction. Addition of different RMs improved the oxidation–reduction potential, sludge dehydrogenase activity, and electron transport system to some extent compared with the control. Furthermore, the high-throughput sequencing revealed the presence of denitrifying bacteria such as *Thauera* spp., *Pseudomonas* spp., and *Dechloromonas* spp. in the acclimated sludge.

Keywords: Low temperature; Redox mediator; Denitrification rate; Efficiency

1. Introduction

According to the "2015 Report on the State of China's Environment" [1], aquatic environment pollution in China is still severe, and eutrophication of slow-flow water such as lakes and reservoirs is not an optimistic situation. Urban sewage treatment plant is one of the major facilities to reduce the pollution of nitrogen and phosphorus. Activated sludge biological nitrogen removal is a cost-effective method that is commonly used in the actual sewage treatment process. However, temperature is one of the important environmental conditions affecting the effect of nitrogen removal. Furthermore, the sewage nitrogen removal efficiency tends to decrease particularly at low temperature in winter. An evaluation of the impact of different quinoid redox mediators (RMs) on the removal of nitrate in a denitrifying culture could provide a new research approach to solve the problem of low biological denitrification efficiency at low temperature in winter.

With the development of modern industry and agriculture, a large amount of nitrogenous wastewaters are discharged into the water bodies, thus deteriorating the water resources [2–4]. The biological nitrogen removal process from wastewater mainly comprises nitrification and denitrification, with nitrifying and denitrifying bacteria being the main participants. It has been shown that the microbial activity decreases at temperatures less than 15°C, and significantly decreases at temperatures less than 10°C, while the physiological activity becomes extremely weak below 4°C. Therefore, low temperature has a significant influence on the

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proliferation of microorganisms in activated sludge, especially those participating in denitrification [5]. In Northern China, where the temperature is for most of the year, with a frost period of as long as 3–6 months, the wastewater temperature is generally at 10°C. The optimum temperature for activated sludge wastewater treatment process is 20°C–35°C to ensure normal growth of microorganisms, and 15°C is considered as low temperature. A previous study found that the denitrifying bacteria were more sensitive than nitrifying bacteria when the temperature was decreased [6,7], indicating the necessity to find appropriate methods to improve denitrification process.

RM, also known as an electron shuttle, can be oxidized and reduced reversibly and can accelerate the reaction rate to several orders of magnitude [8]. In some cases, the presence of RM is a necessary condition for the reaction. Previous studies have reported that RMs mostly contain a quinone structure, which is considered to be a key group for electron transfer [9–11]. Quinones and humic substances are the major RMs discussed in the literature. It has been reported that quinone compounds and humic substances can serve as energy sources to promote the growth of denitrifying bacteria [12]. In addition, it has been demonstrated that quinone mediators can be used as electron acceptors in electron transport chain by denitrifying bacteria for respiration and can increase the nitrate and nitrite degradation rate by 1.14- to 1.63-fold [13]. Furthermore, Guo et al. [14] embedded anthraquinone in calcium alginate and obtained a new type of insoluble mediator for catalyzing biological denitrification process. This mediator increased the denitrification rate by nearly twofolds and reduced the redox potential by 20 mV compared with the control.

Although addition of RM could increase the rate of denitrification, does it produce the same effect at low temperatures? What types of RMs are more effective at low temperature? What is the degree of change in the denitrification rate and growth of denitrifying bacteria? These questions need further investigation. It has been reported that addition of denitrification consortium strengthened denitrification under low temperature in China [15]. The present study aimed to evaluate the impact of different quinoid compounds on the conversion of nitrate in a denitrifying culture. The study investigated the effects of different types of RMs on the biological nitrogen removal at low temperatures, and the underlying mechanism of using RMs was also examined.

2. Materials and methods

2.1. Wastewater and sludge

Activated sludge was collected from a sewage treatment plant in Tianjin, China. The sludge was acclimated in a lab-scale sequencing batch reactor (SBR) under denitrifying conditions. The initial characteristics of the sludge are shown in Table 1.

The composition of the basal medium used during biomass activation in the lab-scale reactor is shown in Table 2.

2.2. Experimental setup

Fig. 1 illustrates the denitrification reactor, which was a cylindrical vessel made of organic glass with a diameter of 170 mm, length of 360 mm, and effective volume of 7.6 L. Four reactors were constructed and numbered as 1#, 2#, 3#, and 4#, and sequence batch activated sludge process was employed to acclimate the sludge to low temperature. The reactor was operated for 12 h, including the stages of drainage (15 min), idle (60 min), water (15 min), reaction (420 min), and precipitation (270 min).

Table 1
Initial characteristics of the sludge

Parameter	Value
Temperature, °C	20
pH	7.6
VSS/SS	0.48
MLSS, mg/L	3512
SVI, mL/g	82

Note: VSS, volatile suspended solids; SS, suspended solids; MLSS, mixed liquid suspended solids; SVI, sludge volume index.

Table 2
Composition of the basal medium used in lab-scale reactor

Name	Percentage, %
$C_3H_5O_2Na$	16.2303
KNO_3	44.7859
KH_2PO_4	16.2303
$MgSO_4$	12.1727
$CaCl_2{\cdot}5H_2O$	10.1439
$FeSO_4$	0.3043
$CoCl_2{\cdot}6H_2O$	0.0088
EDTA	0.0548
$ZnSO_4$	0.0157
$MnSO_4$	0.0362
$Na_2MoO_4{\cdot}2H_2O$	0.0080
$CuSO_4{\cdot}5H_2O$	0.0091

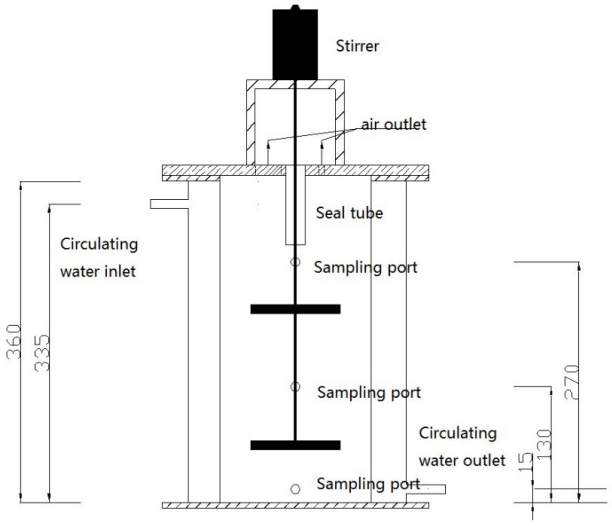

Fig. 1. Diagram of the denitrification reactor.

The temperature of the reactors was controlled using the coolant circuit (Model IL-008-02, Shanghai STIK All Instrument Equipment Co., Shanghai, China). The chemical oxygen demand (COD) was about 260 mg/L and the concentration of nitrate was 85–130 mg/L. From their respective anaerobic stock solutions, 100 μM anthraquinone-2,6-disulfonate (AQDS), 1,2-naphthoquinone-4-sulfonate (NQS), and 2-hydroxy-1,4-naphthoquinone (LAW) were added to reactor 2#, 3#, and 4#, respectively, to study the influence of different RMs on sewage biological denitrification at low temperatures of 10°C and 15°C. Reactor 1# was used as the control.

2.3. Analytical procedures

The analyses of nitrate, nitrite, mixed liquor suspended solids, mixed liquor volatile suspended solids, and total nitrogen were conducted in accordance with the standard methods [16]. The oxidation–reduction potential (ORP) was measured using platinum electrode [17], and environmental microbiological assay was conducted using high-throughput sequencing [18–20].

2.4. Calculations

The ratios, rates, and specific rates of total nitrogen, nitrate, nitrite, and enzyme activity were calculated as follows:

$$\text{Denitrification rate} = \frac{\Delta(NO_x^-) - N \times 60}{\Delta t \times VSS} \left(\text{mg } NO_x^- - N/\text{g VSS} \cdot \text{h}\right) \quad (1)$$

$$NO_x^-\text{–N} = NO_3^-\text{–N} + 0.6 \times NO_2^-\text{–N} \quad (2)$$

where $\Delta(NO_x^-\text{–N})$ is the difference in nitrogen concentration between the two samples (mg/L), Δt is the difference in time between two sampling time points (min), and VSS is the concentration of volatile suspended solids (g/L).

$$\text{Dehydrogenase activity} = ABC \quad (3)$$

where A represents the triphenyltetrazolium chloride concentration (g/mL), B denotes the training time correction (h, i.e., reaction time divided by 60 min), and C indicates color dilution ratio. When $A > 0.8$, through appropriate dilution, its value was reduced to <0.8.

The activity of iodonitrotetrazolium–electron transport system (INT–ETS) was determined as follows:

$$U^T = \frac{A_{485}V}{K^T W t} \quad (4)$$

where U^T is the activity of INT–ETS (TF/g h), A_{485} denotes the absorbance of the supernatant fluid at the wavelength of 485 nm, V is the extractant volume (mL), K^T denotes the slope, w represents sludge dry weight (g), and t indicates training time (h).

3. Results and discussion

3.1. Variations in nitrogen

Fig. 2 illustrates the variations in nitrate, nitrite, and nitrogen removal efficiency of the reactor at different temperatures in the presence of different RMs. The degrees of improvement in nitrogen removal efficiency varied with different RMs, and addition of RMs significantly improved the nitrogen removal efficiency compared with the control. At 660 min of operation and 15°C, the nitrogen removal efficiency reached 18.7% with the addition of LAW, which was 1.7-fold higher than that in the control (10.7%). The highest nitrogen removal efficiency was achieved with the addition of NQS at 10°C. At 10°C, the nitrogen removal efficiency reached 11% with the addition of NQS, which was 1.9-fold higher than that in the control (5.8%).

The concentration of nitrate in the reactor decreased gradually with the reaction duration. The initial nitrate concentration was about 130 mg/L, and at the end of a reaction cycle (660 min), the lowest nitrate concentration in reactor 4# (with added LAW) at 10°C was 90.9 mg/L, while that in the control was 105.6 mg/L. At 15°C, the lowest nitrate concentration in reactor 3# (with added NQS) was 56.44 mg/L, while that in the control was 92 mg/L. Thus, compared with the control, the concentration of nitrate declined by 13.9% and 38.7% in reactors 4# and 3#, respectively.

The nitrite concentration gradually increased with the reaction duration, and reached the maximum value at the end of the reaction cycle (660 min). At 15°C, the maximum concentration of nitrite in reactor 3# (with added NQS) was 66.2 mg/L, which was 1.9-fold higher than that in the control (35.4 mg/L). At 10°C, the maximum concentration of nitrite in reactor 4# (with added LAW) was 36.8 mg/L, which was 1.5-fold higher than that noted in the control (24.3 mg/L). Thus, it can be concluded that the RMs could improve denitrification under the conditions employed in this study by transforming nitrate to nitrite, and then nitrite to nitrogen. As shown in Figs. 2(b) and (e) compared with the control, addition of RMs increased nitrite generation and accelerated the conversion of nitrate to nitrite in the denitrification process, thus increasing the likelihood of conversion of nitrite to nitrogen. Similar results have also been reported by Guo et al. by using immobilized 1,5-dichloroanthraquinone [22].

The results obtained in the present study revealed that the consumption of nitrate could be significantly improved at low temperature by adding RMs. It must be noted that although residual nitrate existed after a specific reaction period even at high nitrate consumption rate, it required much longer time to achieve the same result in the control. Thus, it can be concluded that the effect of RMs varied at different temperatures, and the reason for higher nitrate consumption at higher temperature could be attributed to the changes in microbial activity resulting from a decrease in temperature.

3.2. Changes in the biological denitrification rate

As shown in Fig. 3, the RMs could increase the denitrification rate. At 10°C (Fig. 3(a)), in reactors 3# and 4#

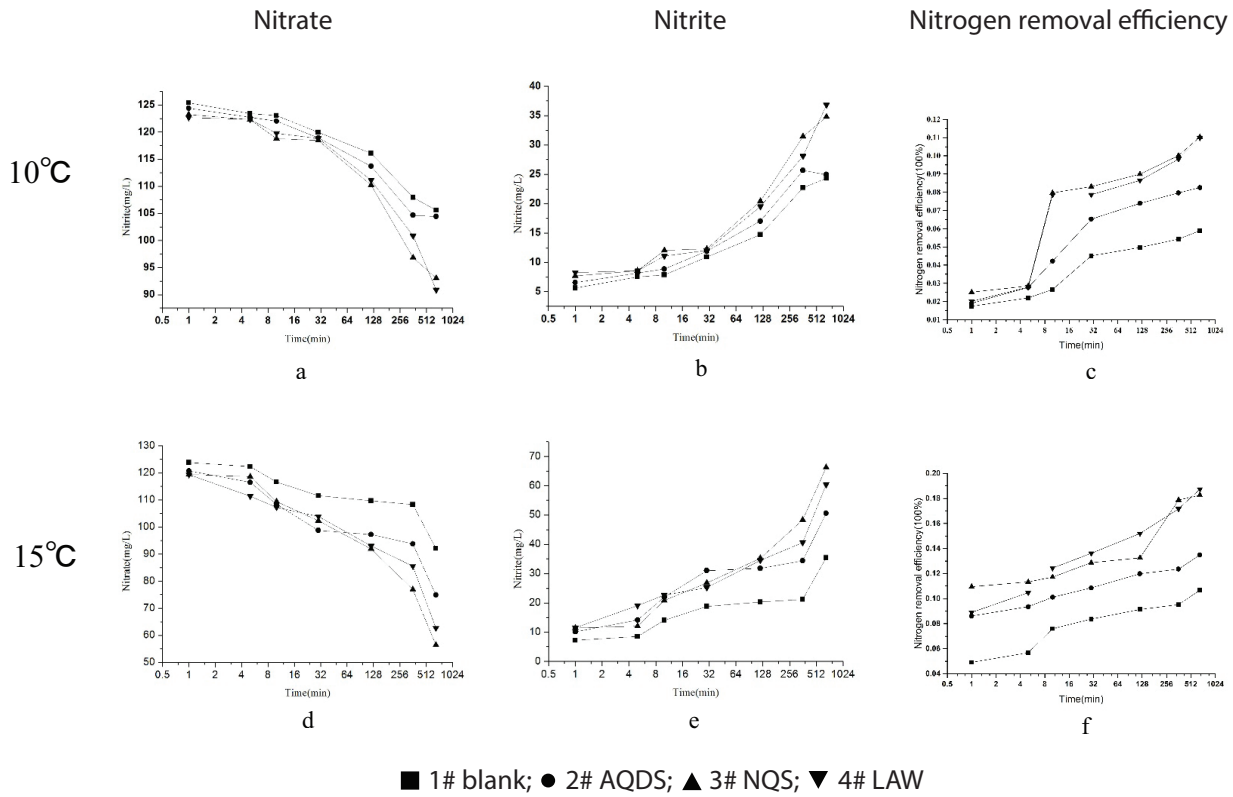

Fig. 2. Variations in nitrogen. (a) The change of nitrate with time at 10°C ; (b) the change of nitrite with time at 10°C ; (c) the change of nitrogen removal efficiency with time at 10°C; (d) the change of nitrate with time at 15°C; (e) the change of nitrite with time at 15°C; and (f) the change of nitrogen removal efficiency with time at 15°C.

(with added NQS and LAW, respectively), maximum denitrification rate of 2.06 and 1.52 mgNO_x^-–N/g VSS·h was reached at 10 min, respectively, whereas the control and reactor 2# (with added AQDS) presented a maximum denitrification rate of 1.15 and 1.01 mgNO_x^-–N/g VSS·h, respectively, at 5 min. Thus, addition of NQS increased the denitrification rate by 1.79-fold compared with that in the control. Furthermore, at 15°C, a denitrification rate of 5.63 and 3.53 mgNO_x^-–N/g VSS·h was achieved at 10 min in reactor 3# (with added NQS) and control, respectively, revealing that NQS addition increased the denitrification rate by 1.59-fold (Fig. 3(b)).

These findings showed that RMs accelerated the denitrification process. Besides increasing the rate of reduction of nitrate to nitrite, addition of RM also improved the reduction of nitrite. Nevertheless, the effects of different quinoid compounds on the removal of nitrate under denitrifying conditions varied. LAW increased the nitrogen removal efficiency by 1.8-fold at 15°C. In contrast, NQS, which is reduced by nitrate at the highest rate at 10°C, increased the nitrogen removal efficiency by twofolds under denitrifying conditions. However, AQDS, which is reduced by nitrate at a modest rate, did not significantly affect the nitrogen removal efficiency. Therefore, it is reasonable to assume that the rate of reduction of quinones by nitrate is high under denitrifying conditions.

3.3. Reaction mechanism analysis

Based on the above-mentioned results, it can be concluded that RMs can improve the removal of nitrate and generation of nitrite at low temperatures. However, the underlying mechanism involved in the improvement of denitrification efficiency with the addition of RMs, including the chemical and biological aspects must be investigated. Fig. 5 illustrates the potential role of quinones during the conversion of nitrate under denitrifying conditions [23]. The data obtained indicated that the quinoid RMs evaluated in this study could be reduced by nitrate at low temperatures, and that the rate of reduction varied among the RMs. The corresponding hydroquinones of the quinoid RMs are considered as model quinoid compounds [24]. In the first step of denitrification, the non-specific enzyme reduces the RM_{ox} (the oxidized RM) to the reduced intermediate that has high activity and certain degree of stability. In the second step, the reduced intermediate is reoxidized by NO_x^-, and then returned to its original state [25]. Thus, RMs play a mediated role in denitrification.

3.3.1. Changes in the ORP

ORP is one of the necessary parameters for effective denitrification, during which electrons are transferred from

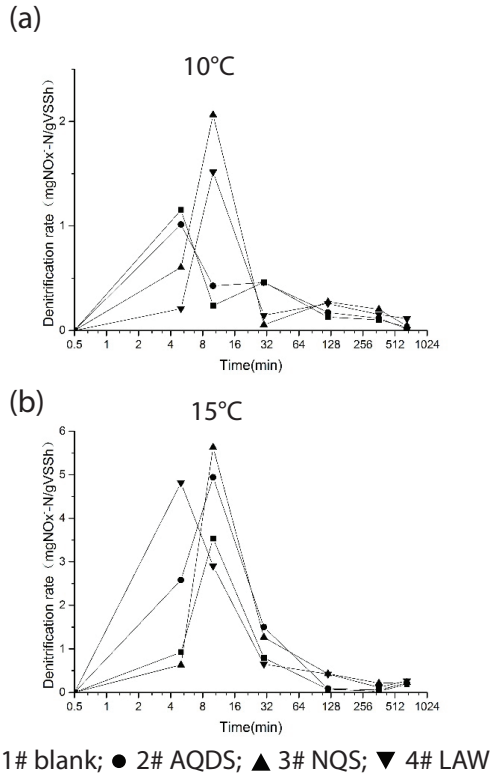

Fig. 3. Changes in the denitrification rate.

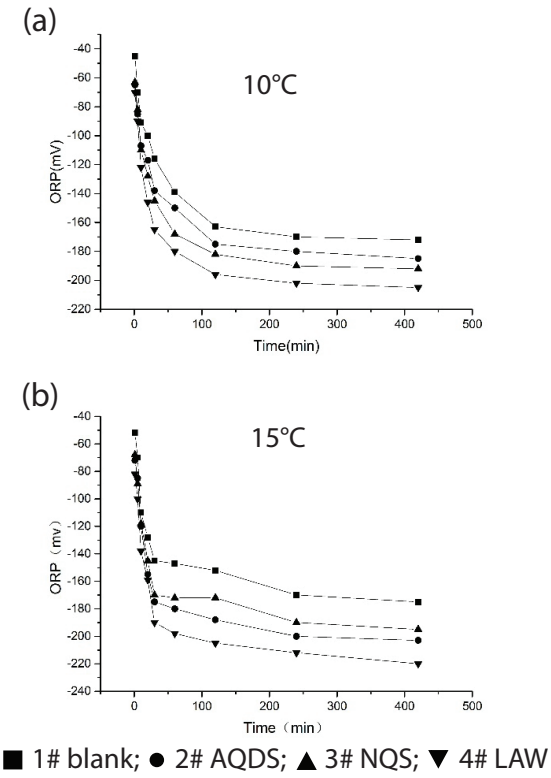

Fig. 4. Changes in the oxidation–reduction potential (ORP).

a reducing agent to an oxidizing agent until the reaction is in balance. The ORP changes are shown in Fig. 4. In the present study, the ORP value in the reactors was negative. The ORP value significantly declined in reactor 4# (with added LAW) at 10°C and 15°C. At 10°C and 420 min, the ORP values in reactor 4# (with added LAW) varied from −70 to −205 mV (which decreased to −135 mV), which was lower by −33 mV compared with that in the control (−45 to −172 mV, which decreased to −127 mV). At 15°C and 420 min, the ORP values in reactor 4# (with added LAW) varied from −82 to −220 mV (which decreased to −138 mV), which was lower by −45 mV compared with the control (−52 to −175 mV, which decreased to –123 mV). The reason for the decrease in the ORP values could be the reduction of the oxide in the solution, including NO_x^-, as well as the negligible amount of oxygen during the denitrification reaction [26]. Thus, addition of quinone RMs rapidly changed the denitrification system into a lower reducing environment, which was more conducive to NO_x^- reduction [27]. The results of ORP analysis proved that the catalytic reaction of RMs is a redox reaction, suggesting the process of receiving and losing electrons or the skew of shared electron pair. Nevertheless, the question of whether addition of RMs could accelerate the transfer of electrons needs to be investigated.

3.3.2. Analysis of sludge dehydrogenase and ETS activities

In the biological wastewater treatment process, the biological oxidation of organic substrates is completed by a series of biochemical reactions of microorganisms. In biological cells, dehydrogenase catalyzes the oxydehydrogenation

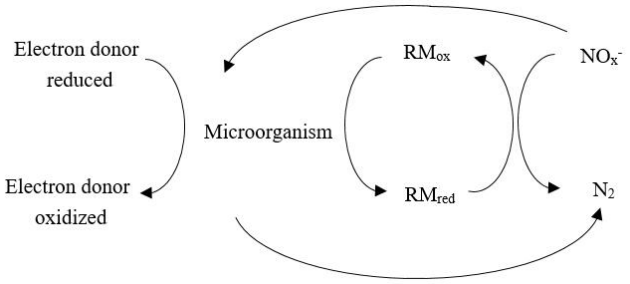

Fig. 5. Mechanism underlying reactions of nitrogenous compounds mediated by quinones under denitrifying conditions.

of organic matter and transfers electron to the terminal electron acceptor (O_2, NO_3^-, SO_4^{2-}, etc.) to achieve mineralization of organic pollutants. Addition of mediators to activated sludge can boost the metabolic rate of microorganisms by improving the enzymatic activity. Although the dehydrogenation rate of the matrix increases with higher dehydrogenase activity, the hydrogen that slips off will get transferred along the electron transport chain and release energy, thus increasing the ETS activity. Therefore, to a large extent, the activities of dehydrogenase and ETS in an organism can be used to reflect the state of its activity.

The activities of sludge dehydrogenase and ETS at 10°C were evaluated. As shown in Figs. 6 and 7, addition of RMs not only improved the activities of ETS and dehydrogenase but also shifted the peak time forward. In reactor 4# (with added LAW), the maximum ETS activity

(625.2 μgINFF/mg VSS·h) appeared at 5 min, which was 2.3-fold higher than that noted in the control (277.8 μgINFF/mg VSS·h, at 10 min). The dehydrogenase activity in the four reactors was as follows: reactor 4# > reactor 3# > reactor 2# > reactor 1#. The maximum dehydrogenase activity in reactor 4# (with added LAW) was 42.0 μgTF/mg VSS·h, at 5 min, which was 1.8-fold higher than that noted in the control (24.0 μgTF/mg VSS·h, at 10 min).

3.3.3. Microbiological analysis

Fig. 8 shows the microbial community structure of the sludge samples (1# to 9#). Sample 1# represents the initial sludge sample taken from the unacclimated sludge, samples 2# to 5# indicate the activated sludge through a period of acclimation at 20°C, and samples 6# to 9# denote acclimated sludge at 10°C. It must be noted that no RMs were added to samples 2# and 6#, AQDS was added to samples 3# and 7#, NQS was added to samples 4# and 8#, and LAW was added to samples 5# and 9#. As can be seen from Fig. 8, the microbial community composition of the inoculated sludge was different from that of the cultured sludge, both at low and room temperatures.

The activated sludge had a good effect on denitrification after cultivation and acclimation. The common dominant species in the nine samples were *Proteobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Planctomycetes* and *Acidobacteria*. In addition, *Thauera*, *Pseudomonas*, and *Dechloromonas*, which possess denitrification function, were also found [28–30]. It must be noted that *Dechloromonas* was not detected in samples 2# and 6# (without added RM) but was found in the rest of the samples. Furthermore, the growth of *Thauera* spp. was limited in the cultured sludge at low temperature compared with that in sample 6#, and increased significantly at 20°C with the addition of quinones. *Pseudomonas* spp. were found in activated sludge cultured with RMs and not in sample 1# (Table 3).

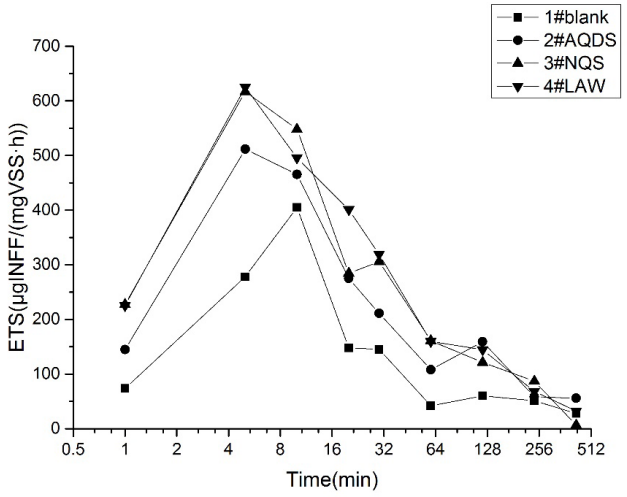

Fig. 6. Change in the electron transport system (ETS) activity.

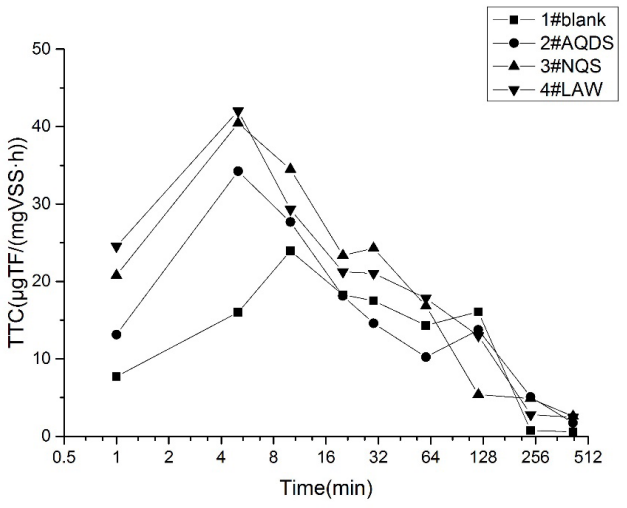

Fig. 7. Changes in the dehydrogenase activity.

4. Conclusions

- Quinoid RMs may significantly contribute to the removal of nitrogen under denitrifying conditions by increasing the rate and efficiency of removal of contaminants. It was observed that the RMs could increase the denitrification rate and nitrogen removal efficiency by 1.9- and 2-fold with the addition of NQS at 10°C, and 1.4- and 1.8-fold with the addition of LAW at 15°C.
- The ORP values decreased by around −33 mV with the addition of LAW at 10°C, and −45 mV with the addition of LAW at 15°C.
- Some bacteria with denitrifying function, such as *Thauera* spp., *Pseudomonas* spp., and *Dechloromonas* spp., were found.

Besides, the results obtained in the present study also revealed that the denitrification process including nitrate respiration and biochemical reduction reaction is accompanied by electron transfer.

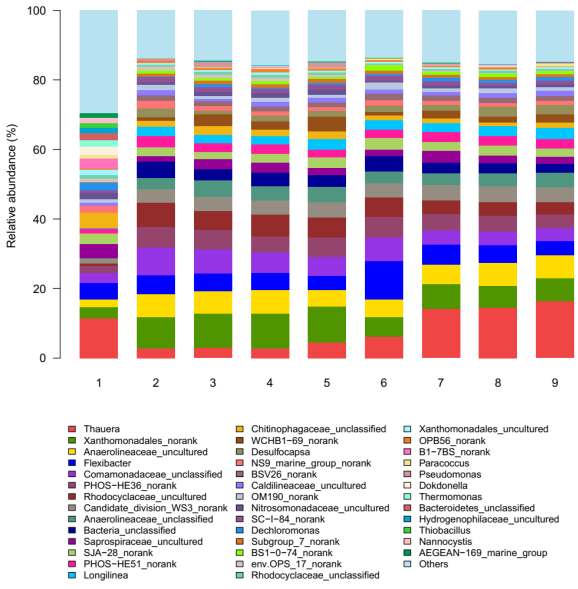

Fig. 8. Microbial community structure (genus).

Table 3
Composition of the microbial community in the samples

Genus	1	2	3	4	5	6	7	8	9
Dechloromonas	2%	0%	1%	1%	1%	0%	1%	0%	1%
Thauera	11%	6%	14%	15%	16%	3%	4%	4%	5%
Pseudomonas	0	0	0	0	0	1%	2%	1%	1%

Acknowledgments

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References

[1] 2015 Report on the State of China's Environment, Environmental Protection Department, China, 2016.
[2] J. Wang, H. Lu, G.H. Chen, G.N. Lau, W.L. Tsang, M.C. van Loosdrecht, A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment, Water Res., 43 (2009) 2363–2372.
[3] H. Chen, S. Liu, F. Yang, Y. Xue, T. Wang, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal, Bioresour. Technol., 100 (2009) 1548–1554.
[4] D.J. Wan, H.J. Liu, J.H. Qu, P.J. Lei, S.H. Xiao, Y.N. Hou, Using the combined bioelectrochemical and sulfur autotrophic denitrification system for groundwater denitrification, Bioresour. Technol., 100 (2008) 142–148.
[5] S. Murat, G. Insel, N. Artan, D. Orhon, Effect of temperature on the nitrogen removal performance of a sequencing batch reactor treating tannery wastewater, Water Sci. Technol., 48 (2003) 319–326.
[6] H. Yuan, Y. Chen, H. Zhang, S. Jiang, Q. Zhou, G. Gu, Improved bioproduction of short-chain fatty acids (SCFAS) from excess sludge under alkaline conditions, Environ. Sci. Technol., 40 (2006) 2025–2029.
[7] Y. Chen, S. Jiang, H. Yuan, Q. Zhou, G. Gu, Hydrolysis and acidification of waste activated sludge at different pHs, Water Res., 41 (2007) 683–689.
[8] F.J. Cervantes, d. V. S. Van, G. Lettinga, J.A. Field, Quinones as terminal electron acceptors for anaerobic microbial oxidation of phenolic compounds, Biodegradation, 11 (2000) 313–321.
[9] D. Lovley, J.C. Woodard, E.J.P. Philips, E.L. Bluntharris, J.D. Coates, Humic substances as electron acceptors for microbial respiration, Nature, 382 (1996) 445–448.
[10] J.A. Field, F.J. Cervantes, Microbial Redox Reactions Mediated by Humus and Structurally Related Quinones, Springer, Netherlands, Vol. 52, 2005, pp. 343–352.
[11] F.P.V.D Zee, F.J. Cervantes, Impact and application of electron shuttles on the redox (bio)transformation of contaminants: a review, Biotechnol. Adv., 27 (2009) 256–277.
[12] C. Aranda-Tamaura, M.I. Estrada-Alvarado, A.C. Texier, F. Cuervo, J. Gómez, F.J. Cervantes, Effects of different quinoid redox mediators on the removal of sulphide and nitrate via denitrification, Chemosphere, 69 (2007) 1722–1727.
[13] J.D. Coates, K.A. Cole, R. Chakraborty, S.M. O'Connor, L.A. Achenbach, Diversity and ubiquity of bacteria capable of utilizing humic substances as electron donors for anaerobic respiration, Appl. Environ. Microbiol., 68 (2002) 2445–2452.
[14] J. Guo, L. Kang, J. Yang, X. Wang, J. Lian, H. Li, Y. Guo, Y. Wang, Study on a novel non-dissolved redox mediator catalyzing biological denitrification (RMBDN) technology, Bioresour. Technol., 101 (2010) 4238–4241.
[15] S. Yao, J. Ni, Q. Chen, A.G. Borthwick, Enrichment and characterization of a bacteria consortium capable of heterotrophic nitrification and aerobic denitrification at low temperature, Bioresour. Technol., 127 (2013) 151–157.
[16] E.W. Rice, American Public Health Association. Standard Methods for the Examination of Water and Wastewater, American Public Health Association, USA, 2012.
[17] B. Li, S. Irvin, The comparison of alkalinity and ORP as indicators for nitrification and denitrification in a sequencing batch reactor (SBR), Biochem. Eng. J., 34 (2007) 248–255.
[18] Y. Wang, J. Chen, S. Zhou, X. Wang, Y. Chen, X. Lin, Y. Yan, X. Ma, M. Wu, H. Han, 16S rRNA gene high-throughput sequencing reveals shift in nitrogen conversion related microorganisms in a CANON system in response to salt stress, Chem. Eng. J., 317 (2017) 512–521.
[19] W. Liu, D. Yang, W. Chen, G. Xiao, High-throughput sequencing-based microbial characterization of size fractionated biomass in an anoxic anammox reactor for low-strength wastewater at low temperatures, Bioresour. Technol., 231 (2017) 45–52.
[20] Y. Chen, Z. Zhao, Y. Peng, J. Li, L. Xiao, L. Yang, Performance of a full-scale modified anaerobic/anoxic/oxic process: high-throughput sequence analysis of its microbial structures and their community functions, Bioresour. Technol., 220 (2016) 225–232.
[21] K. Kujawa, B. Klapwijk, A method to estimate denitrification potential for pre-denitrification systems using NUR batch test, Water Res., 33 (1999) 2291–2300.
[22] H. Liu, J. Guo, J. Qu, J. Lian, W. Jefferson, J. Yang, H. Li, Catalyzing denitrification of *Paracoccus versutus* by immobilized 1,5-dichloroanthraquinone, Biodegradation, 23 (2012) 399–405.
[23] H. Li, J. Guo, J. Lian, L. Zhao, Z. Xi, H. Du, J. Yang, Effective and characteristics of anthraquinone-2,6-disulfonate (AQDS) on denitrification by *Paracoccus versutus* sp.GW1, Environ. Technol., 34 (2013) 2563–2570.
[24] D.R. Lovley, J.L. Frage, E.L. Blunt-Harris, Humic substances as a mediator for microbially catalyzed metal reduction, Acta. Hydroch. Hydrob., 26 (1998) 152–157.
[25] A. Keck, J. Klein, M. Kudlich, A. Stolz, H.J. Knackmuss, R. Mattes, Reduction of azo dyes by redox mediators originating in the naphthalenesulfonic acid degradation pathway of *Sphingomonas* sp. strain BN6, Appl. Environ. Microbiol., 63 (1997) 3684–3690.
[26] Y. Tan, M. Wang, Q. Luo, Factors affecting denitrification by denitrificans coated electrode, J. Hyg. Res., 33 (2004) 527–530.
[27] J. Guo, J. Zhou, D. Wang, J. Yang, Z. Li, The new incorporation bio-treatment technology of bromoamine acid and azo dyes wastewaters under high-salt conditions, Biodegradation, 19 (2008) 93–98.
[28] C. Etchebehere, J. Tiedje, Presence of two different active nirS nitrite reductase genes in a denitrifying *Thauera* sp. From a high-nitrate-removal-rate reactor, Appl. Environ. Microbiol., 71 (2005) 5642–5645.
[29] B. Kraft, M. Strous, H.E. Tegetmeyer, Microbial nitrate respiration--genes, enzymes and environmental distribution, J. Biotechnol., 155 (2011) 104–117.
[30] A. Chakraborty, F. Picardal, Neutrophilic, nitrate-dependent, Fe(II) oxidation by a *Dechloromonas* species, World J. Microbiol. Biotechnol., 29 (2013) 617–623.