Influence of Temperature on MBBR Denitrification for Advanced Nitrogen Removal of Wastewater Treatment Plant Effluent

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Abstract
High total nitrogen content is an urgent issue for the reuse of Wastewater Treatment Plant (WWTP) effluent. Moving Bed Biofilm Reactor (MBBR) was selected for advanced denitrification of WWTP effluent, and the influence of temperature on the nitrate removal performance was studied. Under 12 h hydraulic retention time (HRT) and 13℃, 19℃, 25℃ and 30℃ conditions, the NO_3^--N removal and denitrification rate were not influenced obviously by the temperature variation. The NO_3^--N removal were 82.9±26.7%, 80.1±27.2%, 85.0±22.3% and 82.9±13.3% respectively; and the denitrification rate achieved 35.5±2.4 mg NO_3^--N •$(L•d)^{-1}$), 36.4±1.5mg NO_3^--N •$(L•d)^{-1}$,38.3±16.5 NO_3^--N •$(L•d)^{-1}$ and 39.2±21.2 NO_3^--N •$(L•d)^{-1}$ accordingly. The similar nitrate removal and denitrification rate might have resulted from the long HRT compensation for the denitrification deficiency at low temperature. Three-dimensional fluorescence spectra showed that the dissolved organic matter (DOM) removal were 47.6%, 49.0%, 50.5% and 52.5%, and they were slightly influenced by the temperature. The amount of denitrification narG, nirS and nosZ genes at 13℃ and 19℃ conditions were higher than those under 25℃ and 30℃, and the narG and nirS gene content of 13℃ were the highest, which were 2.04×10^9 and 1.63×10^7copies/g-SS respectively. Considering the pollutant removal efficiency and the denitrification genes abundance as a whole, 25℃ was the optimum choice for nitrogen removal of WWTP effluent by denitrification MBBR.

Keywords
WWTP effluent; denitrification MBBR; temperature; denitrification genes; qPCR

INTRODUCTION

In recent years, the water shortage has becoming a serious problem with the rapid economic development in China, which is more serious in North China, and the water resource per capita is only a quarter of the world average. The amount of municipal wastewater treatment plant (WWTP) effluent was large in China and could reach 3.4×10^{10} m^3 in 2015 (Xu and Chen, 2014), therefore, WWTP effluent is a good resource as reclaimed water. Furthermore, the pollutants of WWTP effluent had the characteristics of low concentration, good stability and easy treatment. So, the recycling urban sewage has becoming one of the potential ways to solve the shortage of water resources, and it had the higher economic and environmental benefits compared with long-distance water desalination and other projects (Jian,1996).The total nitrogen (TN) limitation of WWTP effluent are 15 mg/L according to *Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant* (GB18918-2002), however, the TN limitation of Class IV is not more than 1.5ml/L, which meets the demand of recharge water for water sources, natural wetlands or rivers, in *Environmental Quality Standards for Surface Water* (GB3838-2002). Therefore, the advanced nitrogen removal was necessary for the WWTP effluent reused as supplement water. The organic nitrogen content of WWTP effluent was so low that can be neglected, and the remaining nitrogen was mainly in the form of nitrate, which accounted for 80.8±8.4% (Yuan et al. 2015), so the denitrification was needed for WWTP effluent nitrogen removal.

Moving bed biofilm reactor (MBBR) was developed for nitrogen removal by dosing suspended

carriers in activated sludge system (European Patent no. 0,575,314, US Patent no. 5,458,779) to attain more biomass in 1980s. MBBR has been popularly used for treatment of rural wastewater, urban sewage, industrial wastewater, etc., and it was often operated in aerobic condition with simultaneous nitrification and denitrification for nitrogen removal. There were many factors influencing the MBBR operation efficiency such as temperature, dissolved oxygen (DO), the ratio of carbon to total nitrogen(C/N) , mixing speed, etc.(Hoang, et al., 2014; Li, et al., 2007). The research about the MBBR denitrification was also reported for sewage wastewater (Aspegren et al., 1998; Mases et al. 2010), nitrate contaminated seawater(Labelle, et al., 2005) and landfill leachate (Cortez et al., 2011) nitrogen removal with good denitrification effficiency. The full-scale MBBRs had also been applied since 1997 & 1999 for post denitrification in Sjölunda and Klagshamn WWTPs of Malmö City, Sweden, and the effluent TN could met the WWTP limitation (Mases et al. 2010). For the WWTP effluent reuse, the denitrification MBBR has been developed for full-scale WWTP effluent advanced nitrogen removal, and the influencing of carriers types, hydraulic retention time (HRT) and C/N ratio on its efficiency have been studied extensively in our published study (Yuan et al. 2015; 2016).

Temperature is one of the key factors influencing the cost of the biological treatment process, and it influences the microorganisms population, composition, pollutants degradation ability, adsorption and the organic, nitritions transformation (Jiu et al., 2004;Calderon et al., 2012). WWTPs were usually built outside and the temperature varied obviously in different seasons in North China, so it was necessary to study the temperature influence. The temperature is also one of the most important factors influencing the denitrification efficiency (Baptiste et al.;Adouania et al., 2015), and the appropriate denitrification temperature is between 20 and 35℃. When the temperature was lower than 15℃, the denitrification rate decreased obviously (Song, et al., 2013).

Denitrification is a biochemical process involving the stepwise reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to produce the gaseous NO, N_2O, and N_2 under anoxic conditions, which is mediated by physiologically diverse groups of microorganisms (Zhou et al.; Zumft, 1997). Molecular genes that encode key enzymes (nitrate-, nitrite-, nitric oxide-, and nitrous oxide reductases) in nitrate reduction processes have been established as molecular markers (Zhou et al.,1997). These functional genes include nitrate reductase genes (narG and napA) (Bru et al.), nitrite reductase genes (nirS and nirK) (Braker et al.), nitric oxide reductase gene (norB) and nitrous oxide reductase gene (nosZ). qPCR has been used to quantify the copy numbers of denitrifying functional genes extracted from various environmental samples for explanation of the denitrification process (Chon et al.).

In this study, the denitrification MBBR was applied for the advanced nitrogen removal of the full-scale WWTP effluent, and the temperature influence on denitrification efficiency, nitrogen and organic removal were extensively studied to obtain the appropriate temperature parameters for engineering application. The change of denitrificans narG, nirS and nosZ genes with different temperatures in denitrification MBBR were also investigated by qPCR molecular approach.

MATERAIALS AND METHODS

Equipments and carriers

MBBR denitrification reactor was made by plexiglass column with 120 mm inner diameter, 500 mm height, 6.0 L volume and 5.7 L effective volume (Yuan et al., 2015), and the carriers were polyethylene particles with 25 mm diameter, 10 mm height, 0.96~0.98 g/L density and 620 m^2/m^3 specific surface area.

Experimental and operation conditions

The experiments were carried out and optimized at four stages, i.e., under the conditions of 13℃, 19℃, 25℃ and 30℃, which were controlled by heating rod and cooling equipment. Secondary sedimentation tank effluent of Beijing Yongfeng WWTP was used as MBBR influent, and methanol was added to adjust C/N ratio to 8. The influent characteristics and operation conditions were shown in Table 1.The MBBR reactor was operated at the conditions of 30% filling rate (volumetric ratio of carriers to reactor), 12h HRT, 80 rpm agitation speed to keep carriers suspended, and peristaltic pump (BT100-1L, Baoding Lange constant pump Ltd.) was used for continuously operation.

Table1 Influent ingredients and operation conditions

Time(d)	Temperature(℃)	CODCr(mg/L)	TN(mg/L)	NO_3^--N (mg/L)
Phase I(1-49d)	13	104.6±86.3	11.1±7.0	4.8±2.6
PhaseII(50-98d)	19	109.7±82.0	12.0±7.6	4.8±2.6
PhaseIII(99-147d)	25	87.8±29.7	10.7±2.7	3.9±2.1
PhaseIV(148-190d)	30	85.6±30.4	10.6±2.7	4.0±2.1

Sequencing batch test

Table 2 Influent ingredients of sequencing batch experiments

Time(d)	Temperature(℃)	CODCr(mg/L)	TN(mg/L)	NO_3^--N (mg/L)
1-3	30	117.8	11.1	4.6
4-6	25	109.0	11.9	4.1
7-9	20	91.4	11.8	4.5
10-12	15	113.4	11.2	4.3

A 2.5L brown jar was used as sequencing batch MBBR reactor with 2.0L effective volume, and it was filled with polyethylene carriers by 30% filling rate. Under the conditions of 12h HRT, 80 r / min stirring speed and 8 C/N ratio, the sequencing batch MBBR was operated under the temperatures of 30 ℃, 25 ℃, 20 ℃, 15 ℃ and 10 ℃ (Table 2). At the steady-state of each temperature, MBBR was sampled once every hour for 8 times in one HRT to study the nitrogen variation at different temperatures.

Analytical methods

Water Quality Index Detection

TN, NO_2^--N, NO_3^--N and the dissolved organic matter (DOM) were analyzed after the water samples filtered by 0.45 μm membrane. TN was determined using the TN unit of TOC-V_{CPH} analyzer. NO_3^--N and NO_2^--N were measured by ion chromatography (Dionex ICS-1000, Dionex Inc., Sunnyvale, CA, USA); NH_4^+-N was determined by Nessler's reagent spectrophotometry method (APHA, AWWA, WEF 1992); COD was determined with a COD speedy testing instrument (Model TCL-12, Hebei Chengde Huatong Environmental Instrument Ltd. Co., Chengde, China); pH was measured with an S-25 pH Analyzer (Shanghai Precision ＆Scientific Instrument Ltd. Co., Shanghai, China).

DOM analysis

The DOM was characterized by three-dimensional excitation-emission matrix (EEM) fluorescence spectrophotometer (Hitachi F-7000 Fluorescence Spectrophotometer, Japan).Excitation source was used for the spectrometer, both excitation (Ex) and emission (Em) were 200-450 nm with 5 nm

bandwidth, and the scanning speed was 12000 nm·min^{-1}.

DNA extraction and qPCR analysis

Some sludge was collected for DNA extraction in stable period. DNA was extracted by UltraClean DNA extraction kit (Mobio Laboratories, Carlsbad, USA).Amplification reactions of qPCR were performed with 20 μL reaction mixture, which contained 10.0 μL SYBR Master Mix (ROCHE), 0.4 μL primer for each type, 1.0 μL template DNA, and 8.2 μL ddH_2O on an ABI 7500 fast real time PCR platform (Life technologies, USA). The fractions were PCR amplified using primers NarG-F (5'-TA(CT)GT(GC)GGGCAGGA(AG)AAA -3') and NarG-R (5'-CGTAGAAGAAGCTGGTGCTGTT-3')(Smith et al., 2015), NirS-F(GTSAACGTSAAGGARACSGG) and NirS-R(GASTTCGGRTGSGTCTTGA) (Kandeler et al., 2006, NosZ-F (5'- CG(C/T)TGTTC(A/C)TCGACAGCCAG -3') and NosZ-R (5'-CG(G/C)ACCTT(G/C)TTGCC(C/G)T(T/C)GCG -3')(Mao et al., 2011). Q-PCR was conducted using a two-step amplification procedure under the following conditions: 95℃ for 10 min; 40 cycles of 95℃ for 15 s, 60℃ for 1 min. The specificity of each PCR assay was confirmed by both melting curve analysis and agar gel electrophoresis methods. All the measurements were carried out in triplicate.

Biomass analysis and SEM observation

At steady-state condition, 4-6 MBBR carriers were taken out for biomass analysis. The carriers were put into 50ml 1mol/L NaOH solution, then water bathed for 30 mins at 80 ℃ and ultrasonic cleaned for 1min at 100W, and finally the solution suspended solids were analyzed by the standard methods(Liu, 1992). Sample preparation for SEM was carried out (Wang et al., 2003) and the microbial morphology of the carriers were examined (HITACHI S-570 SEM, Hatachi Inc., Naka, Japan) at an accelerating voltage of 12 kV.

RESULTS AND DISCUSSION

Influence of temperature on Nitrogen Removal

Influence of temperature on NO_3^--N Removal

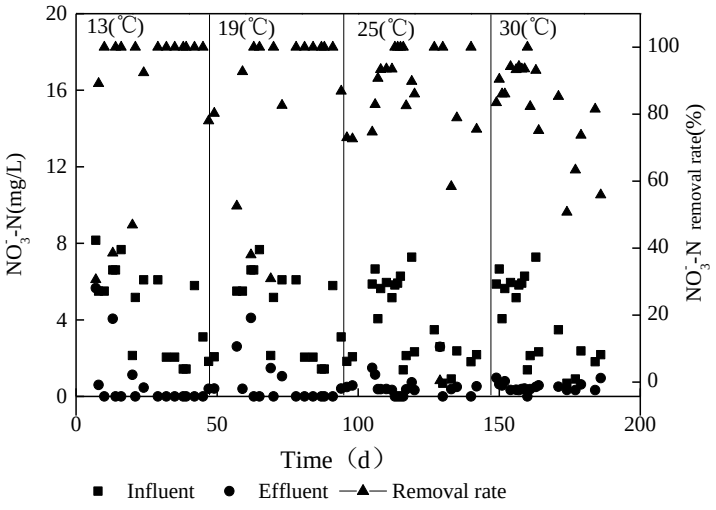


Figure 1. NO_3^--N removal of MBBR under different temperature

Under the conditions of 13℃, 19℃, 25℃ and 30℃, 12h HRT and 4.77±2.71mg/L, 4.18±2.14mg/L, 3.93±2.1 mg/L and 4.0±2.1mg/L influent NO_3^--N concentrations, the MBBR effluent NO_3^--N removal rate were 82.9±26.7 %, 80.1±27.2 %, 85.0±22.3 % and 82.9±13.3 respectively (Figure 1), and the denitrification rate were also not influenced obviously by temperature, which achieves 35.5±2.4 mg NO_3^--N (L d)$^{-1}$, 36.4±1.5mg NO_3^--N (L d)$^{-1}$), 38.3±16.5 mg NO_3^--N (L d)$^{-1}$ and 39.2±21.2 mg NO_3^--N (L d)$^{-1}$accordingly. The removal rate and denitrification rate changed little with the temperature variation, and it indicated the temperature has little effect on the nitrate removal at

12h HRT, which might be because the long HRT (12h) of the MBBR could compensate for the low denitrification rate at low temperature. At low temperature, the microorganisms could achieve the same nitrate removal efficiency as that at high temperature by extending the HRT.

The nitrate removal for 6 h reaction at 10 ℃ could be close to that for 2h reaction at 30 ℃(Ma et al., 2008). The denitrification rate showed only a rather weak dependence on the temperature, the rate at 3℃ being approximately 55% of that at 15℃, which resulted from the psychrotrophic bacteria activity and their operation under diffusional restrictions (Welander &Mattiasson, 2003).

The TN removal efficiency usually decreased in winter according to the low temperature, and the microorganisms amount and their enzyme catalytical rate would decrease, which inhibited microbial activity, affected the substrate diffusion rate, thereby affecting the microbial sewage treatment efficiency. The popular solution was to extend the HRT, to reduce F / M (OLR) and adding carriers into the treatment systems (Hu et al., 2012).

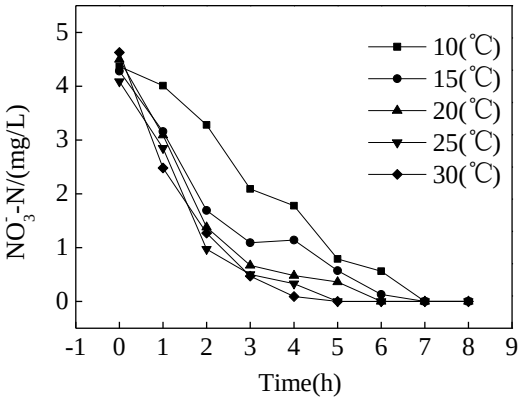


Figure 2. NO_3^--N removal in one HRT under different temperature

Within one HRT(12h) for sequencing batch tests, 5h and 7h operation were required for complete nitrate removal at 30 and 10 ℃ respectively (Figure 2), and the same nitrate removal could be obtained at different temperatures with the prolonged operation time. The denitrification rate increased with the temperature increase, and they were 0.64mg/L ħ, 0.58mg/L ħ, 1.12mg/L ħ, 1.29mg/L ħ and 1.14mg/L ħ under 10℃, 15℃, 20℃, 25℃ and 30℃ respectively. The microbiological activity was higher under the higher temperature, which resulted the higher nitrate removal (Cho et al., 2015).With the HRT increase, the microorganisms at low temperature could arrive the same removal efficiency as that of the high temperature for the time compensation (Zinatizadeh & Ghaytooli, 2015). The batch tests also explained why the similar nitrate removal were obtained at long HRT (12h) at 13℃, 19℃, 25℃ and 30℃.

Influence of temperature on the Removal of Nitrogen in Other Form

Table 3 The removal of other forms of nitrogen

Temperature (℃)	NH_4^+-N			NO_2^--N			TN		
	Influent (mg/L)	Effluent (mg/L)	Removal	Influent (mg/L)	Effluent (mg/L)	Removal	Influent (mg/L)	Effluent (mg/L)	Removal
13	0.8±0.7	0.5±0.4	27.2±46.3	2.0±1.2	1.8±0.8	4.3±3.6	11.1±6.9	5.5±4.1	53.1±28.4
19	0.9±0.8	0.7±0.4	23.5±51.1	2.0±1.2	1.9±0.7	4.1±3.3	12.0±6.9	6.2±4.2	49.0±28.2
25	0.8±0.3	0.6±0.4	30.5±24.9	2.3±0.7	2.3±0.6	4.2±7.1	10.7±2.7	4.9±2.2	52.8±16.8
30	0.7±0.4	0.5±0.3	31.3±11.1	2.5±0.6	2.4±0.7	6.1±7.3	10.5±2.8	4.8±3.0	54.6±23.9

The influent low NH_4^+-N and NO_2^--N were 0.7±0.4~0.9±0.8 mg/L and 2.0±1.2~2.5±0.7 mg/L， and their removal efficiency were 23.5±51.1~ 31.3±11.1% and 4.1±3.3~6.1±7.3% respectively (Table 3), which varied little with the temperature. In the MBBR denitrification process, NH_4^+-N and NO_2^--N removal had little contribution to the WWTP effluent nitrogen removal. The NH_4^+-N could be transformed into NO_2^--N and NO_3^--N by nitrification bacteria under aerobic circumstances.

However, the denitrification MBBR had the anoxic condition and the NH_4^+-N had little change. The NH_4^+-N of the WWTP effluent was only about 0.5mg/L, which could not influence the TN removal obviously. The NH_4^+-N perhaps could also be removed with NO_2^- through ANAMMOX process (Zhang et al., 2011): $NH_4^+ + NO_2^- \rightarrow N_2 + H_2O$, which was limited under anoxic condition. The NH_4^+-N and NO_2^--N also could be accumulated, and so they had little change during the denitrification process (Ciudad et al., 2005). Under the temperatures of 13℃, 19℃, 25℃ and 30℃, the TN removal were 53.1±28.4%, 49.0±28.2%, 52.8±16.8% and 54.6±23.9% accordingly, which were also not affected by temperature obviously and similar to the nitrate removal for long HRT compensation.

Under the conditions of 10℃, 15℃, 20℃, 25℃ and 30℃, the NH_4^+-N, NO_2^--N removal were not influenced obviously by the temperature in the batch tests. However, the TN removal was increased with the temperature, and the TN removal rate were 0.58mg/L ħ, 0.63mg/L ħ, 1.12mg/L ħ, 1.29mg/L ħ and 1.39mg/L, respectively. The TN removal could arrive at 39.62%, 43.10%, 47.45%, 48.99% and 46.38% for 8h operation in one HRT, which were similar and explained why the temperature had no obvious influence on TN removal efficiency on the long HRT (12h).

Influence of temperature on Organic Pollutant Removal

COD Removal

Under 12h HRT and 13℃, 19℃, 25℃ and 30℃ conditions, the COD_{Cr} removal were 53.3±44.9%, 52.5±30.6%, 65.5±18.6% and 63.8±19.5%, which indicated that the temperature had little effect on the COD_{Cr} removal. The effluent COD concentrations could meet the Class IV limitation of *Environmental Quality Standards for Surface Water* (GB 3838-2002). The CODcr removal were steady after 5h operation, and the COD_{Cr} removal rate increased gradually with the temperatures of 10℃, 15℃, 20℃, 25℃ and 30℃ in batch tests, which showed the similar phenomenon as the nitrate removal.

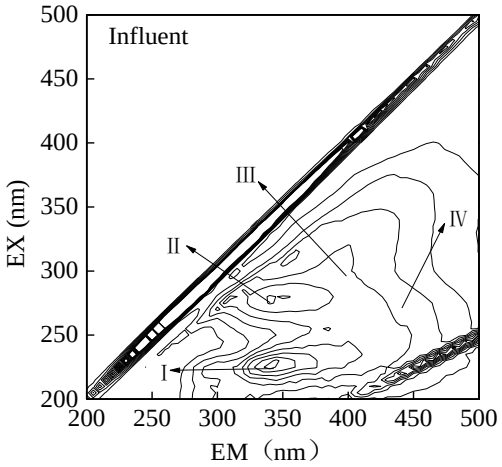


Figure.3 Three-dimensional fluorescence spectrum of MBBR influent

DOM Removal

COD was one of the traditional wastewater pollutant index, could not characterize the detailed organic ingredients. Howerver, the three-dimensional fluorescence spectroscopy was an effective way to characterize the readily biodegradable, refractory and biodegradable organics of the municipal wastewater, and it could display the DOM fingerprint features (Wu et et al., 2011; Song et al., 2013).The constituents of DOM in municipal wastewater were complicated, and they were humic acid, fulvic acid, hydrophilic organic acid, amino acid, nucleic acid, etc., wherein the amino acid and other protein-like substance were the main organics (Yang &Yang, 2015).

The fluorescence in different spectral regions are related with different organic functional groups in DOM. Figure 3 showed the DOM fluorescence spectra of the MBBR influent, and there were 4 major fluorescence peaks: Peak I (Ex/Em: 260-280nm/330-370nm) and Peak II (Ex/Em: 270-320nm/380-420nm) represented the tryptophan-like substances, Peak III (Ex/Em: 305-315/395-400) represented the visible fulvic acid-like substance, and Peak IV (Ex/Em: 240-260/410-450) represented the UV fulvic acid-like substance (Zhu et al., 2015; Patels et al., 2002; Chen et al., 2003;

Wang et al., 2013).

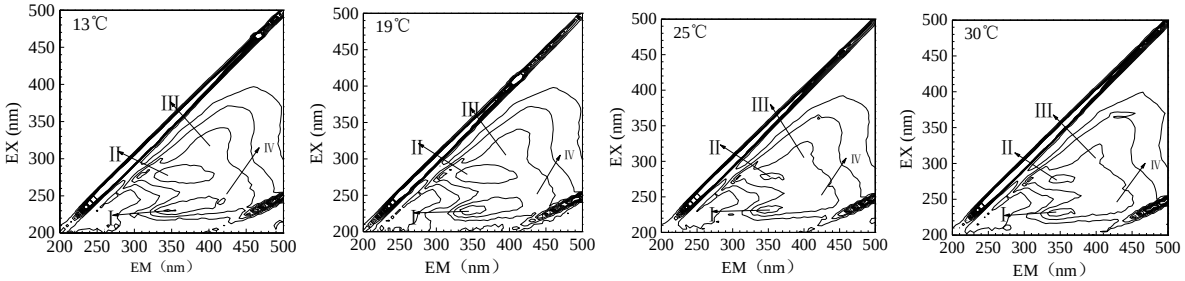


Figure. 4 Influence of the different temperature on the DOM removal of MBBR

Table 4. The fluorescence intensity change of MBBR effluents under different temperature

Tempera -ture (℃)	Effluent fluorescence intensity					Removal（%）				
	PeakI	PeakII	PeakIII	PeakIV	Total peak	PeakI	PeakII	Peak Ⅲ	Peak Ⅳ	Total peak
13	1026	551.1	370.1	543.3	2490.5	16.0	66.9	54.8	48.2	47.6
19	832.6	916.7	747.4	760.6	2424.7	31.9	45.0	8.8	27.4	49.0
25	1033	525.3	259.3	535	2352.6	15.5	68.5	68.3	48.9	50.5
30	963.6	566.9	369.7	358.9	2259.1	21.1	66.0	54.9	65.7	52.5

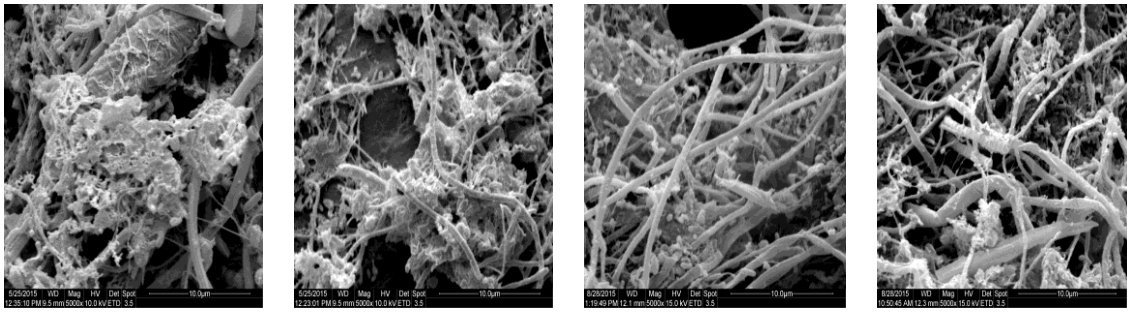
Under the conditions of 13℃, 19℃, 25℃ and 30℃, the total fluorescence intensity of effluents were all decreased compared with influent, and their removal were 47.6%, 49.0%, 50.5% and 52.5% respectively (Table 4). The tryptophan-like substance removal were 45.4%, 39.4%, 46.0% and 47.2%, and the fulvic acid substance removal were 68.4%, 47.8%, 72.5% and 74.8% accordingly. Moreover, the fulvic acid substance removal was higher than that of the tryptophan-like substance (Table 4 & Figure 4). All the results showed that the DOM removal was not obviously affected by the temperature change.

Microbiology characteristics at different temperature

Biomass analysis and SEM observation

At steady-state operation, the biomass of the carriers were 6.9, 9.4, 11.0 and 14.1mg/g respectively under the conditions of 13℃, 19℃, 25℃ and 30℃. The biomass increased with the temperature for the high temperature was in favour of the microorganism growth and proliferation. 25℃ and 30℃ were the optimum temperature for denitrification, so the biomass were relatively higher than at other temperature. Polyethylene carriers had the advantages of good mechanical strength, lower density than water, floating ability on the liquid surface, hydrophilic group and roughness of surface, facilitated microorganism attatchment, etc. When the surface of the carriers was modified, the microorganism would adhere to them more easily (Isaka et al.,2012;Tao et al., 2001).

The SEM results of carriers under the steady-state operation conditions of 13℃, 19℃, 25℃ and 30℃ were illustrated in Figure 5. The microorganisms on the carriers were similar and mainly in bacilliform, filiform and spheroidal form, which might be denitrificans by judging from their morphology. There were large amounts of filamentous microorganisms, which could grow on the carriers surface easily and be helpful for other microorganisms to grow around them for denitrification (Fernandez et al., 2008). Temperature had little influence on the carriers surface morphology (Figure 5), which consisted with the biomass analysis.



13℃ 19℃ 25℃ 30℃

Figure. 5 Microbiological morphology of MBBR carriers under 13℃, 19℃, 25℃ and 30℃

NarG- ,nirS and nosZ-based qPCR

SYBR green-based qPCR assays were used to determine the copy numbers of narG, nirS and nosZ genes in the carrier biofilm under different temperatures. The results showed that when temperatures were 13℃, 19℃, 25℃ and 30℃, the narG gene number of the biofilms were 2.04×10^9, 1.59×10^9, 1.65×10^9 and 7.19×10^8 copies/g-SS; the nirS gene number of the biofilms were1.76×10^9, 1.95×10^9, 1.20×10^9 and 8.43×10^8copies/g-SS; the nosZ gene number of the biofilms were 1.63×10^7, 4.45×10^6, 2.56×10^6 and 2.38×10^6 copies/g-SS, from which it could be concluded that the sequence abundance of the nitrate-reducing genes and nitrite-reducing genes were both higher than the nitrous oxide reducing genes. The content of narG, nirS and nosZ genes under 13℃ and 19℃ were higher than those under 25℃ and 30℃， and the highest abundance of narG and nosZ genes, i.e. 2.04×10^9 and 1.63×10^7copies/g-SS were achieved at 13℃, which were consistent with the long 12h HRT compensation as shown in section 3.1. Chon et al. (2011) reported the functional narG, nirS and nosZ genes ranged from 1.0×10^6 to 1.0×10^9 copies/g-soil in a wastewater effluent-fed wetland using qPCR method. The functional narG, nirS and nosZ genes amount in denitrification MBBR was higher than the reported wetland due to more microorganisms attatched on the carriers. In the bioretention system characterized by low infiltration rates and long drainage times, the denitrification nirS, nosZ genes varied from 10^5 to 10^8 copies/gram (Chen et al., 2013). The gene abundance in these literatures were similar with that in our study.

The content of narG, nirS and nosZ genes at 13℃ and 19℃ conditions were higher than those under 25℃ and 30℃. The highest abundance of narG, nirS and nosZ genes, i.e. 2.04×10^9 , 1.95×10^9 and 1.63×10^7copies/g-SS, were achieved at 13℃, 19 ℃ and 13℃ respectively, which were consistent with the long 12h HRT compensation.

CONCLUSIONS

1. The NO_3^--N removal and denitrification rate were not influenced by the temperature of 13, 19, 25 and 30℃ at 12h HRT for polyethylene MBBR, the NO_3^--N removal rate were 82.9±26.7 %, 80.1±27.2 %, 85.0±22.3 % and 82.9±13.3, and the denitrification rate achieved 35.5±2.4 mg NO_3^--N $(L\ d)^{-1}$, 36.4±1.5mg NO_3^--N $(L\ d)^{-1}$) ,38.3±16.5 mg NO_3^--N $(L\ d)^{-1}$ and 39.2±21.2 mg NO_3^--N $(L\ d)^{-1}$ respectively. The long HRT of 12h may compensate for the denitrification ability of low temperature, which resulted the similar nitrate removal and denitrification rate at different temperature.

2. The effluent COD could meet the Class IV limitation of *Environmental Quality Standards for Surface Water* (GB 3838-2002). Three-dimensional fluorescence spectra showed that both of the MBBR influent and effluent contained DOM, such as fulvic acid-like and tryptophan-like substance, etc., and the total fluorescence intensity removal were 47.6%, 49.0%, 50.5% and 52.5%, respectively.

3. The abundance of narG, nirS and nosZ gene at 13℃ and 19℃ conditions were higher than those under 25℃ and 30℃. The highest abundance of narG and nosZ genes, i.e. 2.04×10^9 and

1.63×10^7copies/g-SS were achieved at 13℃, which were consistent with the long 12h HRT compensation. The abundance of narG nitrate-reducing gene and nirS nitrite-reducing gene were both greater than that of nosZ nitrous oxide reducing gene.

4. Considering the nitrogen, organic pollutants removal efficiency and denitrification genes abundance as a whole, 25℃ was the optimum temperature for nitrogen removal of WWTP effluent by denitrification MBBR.

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