Effects of organic carbon source on the performance and bacterial structure in biofilm processes for source water pretreatment

Ya-lei Liu¹,#, Bin Tu¹,#, Guang-feng Yang¹,²*, Yi-chun Zhao¹, Yi-shu Li¹, Yuan-yuan Fang¹, De-dong Song¹, Jun Mu¹,⁵, Jing-ya Sun¹,*, Liang Zhu³,⁴, Xiang-yang Xu³,⁴

¹Department of Environmental Engineering, Zhejiang Ocean University, No.1 Haida South Road, Zhoushan 316022, P.R. China;
²Zhejiang Provincial Key Laboratory of Petrochemical Environmental Pollution Control, P.R. China;
³Department of Environmental Engineering, Zhejiang University, Hangzhou, 310058, P.R. China;
⁴Key Laboratory of Water Pollution Control and Environmental Safety of Zhejiang Province, P.R. China;
⁵Hainan tropical Ocean University, Yucai Road, Sanya City, Hainan Province, 572022, P.R. China.

* Corresponding author: ygfs@126.com (G.F. Yang); 2558068654@qq.com (J.Y. Sun)
# Co-first authors Ya-lei Liu and Bin Tu contributed equally to this work.

Abstract. The operation performance of biofilm system is limited by the oligotrophic quality of source water, especially the bioavailable organics. In this study, two lab-scale biofilm reactors (R₁ and R₂) feeding different organic carbon sources (OCS) were built up using sediment in drinking water source as bacterial source. Experimental results showed that sediment in biofilm systems enhanced the NH₄⁺-N removal performance. Using ethanol as OCS was more beneficial to the removal of NH₄⁺-N with ammonia removal efficiency (ARE) of 87.0 ± 5.4%, which was higher and more stable than that of glucose with ARE of 83.9 ± 13.3%. Organic carbon source changed the bacterial structure in biofilm systems. The dominant phyla in biofilm under ethanol condition were Proteobacteria, Bacteroidetes and Firmicutes with relative abundances (RA) of 29.1%, 32.7% and 22.0%, respectively. The dominant phyla in biofilm exposure to glucose was Proteobacteria with an RA of 63.4%. At genus level, Nitrospira, Lachnospiraceae, Arcobacter and Hyphomicrobium were dominant under ethanol condition (R₁) with the total relative abundance of 29.1%, 32.7% and 22.0%, respectively. The dominant phyla in biofilm exposure to glucose was Proteobacteria with an RA of 63.4%. At genus level, Nitrospira, Lachnospiraceae, Arcobacter and Hyphomicrobium were dominant under ethanol condition (R₁) with the total relative abundance of 20.5%. Sphaerotilus was the dominant genus under glucose condition (R₂) with RA of 33.7%. These dominant bacteria were basically having the ability for the removal of nitrogen and organic matter.
1. Introduction
In recent years, the deterioration of source water quality has been caused by pollutants from industrial wastewater, urban sewage and agricultural runoff. Many reports showed that source water in developing countries has been widely polluted by nitrogen and organic matter [1-4], which seriously threatened the water security of drinking supply [1,5]. Usually, a series of processing techniques were used to treat polluted source water for obtaining safe drinking water, including coagulation, sedimentation, filtration and disinfection processes [5-6]. However, potential secondary pollutants and disinfection by-products (DBPs) may be introduced in these processes, and the safety of drinking water was difficult to be guaranteed [6-10].

Recently, biofilm processes have been widely studied for the pretreatment of polluted source water due to the advantages of lower operation cost and less secondary pollution [11-12,]. Reports showed that biofilm process effectively reduced the pollutants loading rates for subsequent processes, reduced the DBP precursors and enable the biological stability of source water [6-7]. However, the oligotrophic characteristics of source water with total organic matter (TOC) of much less than 10 mg/L limited the growth of microorganisms in biofilm systems. Especially, the utilisable carbon for growth of heterotrophic microorganisms in source water was 0.5-5% of TOC [13-14].

The bacterial structure in biofilm systems for source water treatment was affected by water quality and operation conditions. In this study, two lab-scale reactors filled with elastic stereo media (ESM) were established and parallely operated under different organics conditions. The objectives were to 1) enhance the startup and operation performance of biofilm reactor, and 2) study the effects of different organic carbon sources on the performance and bacterial characteristics.

2. Materials and methods

2.1 Experimental setup
In this study, two lab-scale upflow biofilm reactors named R1 and R2 were constructed and parallely operated for polluted source water treatment. The effective volume of each reactor was 5.0 L, and ESM used as biofilm carrier was filled into each reactor with filling ratio of 2.71% (v/v). The specific surface area and density of ESM carrier were 732.3 m2 m-3 and 1.041 g mL-1, respectively.

2.2 Polluted source water quality
Synthetic polluted source water was used as influent and pumped into each biofilm reactor. The main components of synthetic polluted source water included NH4⁺-N, organics and inorganic salt. Thereinto, NH4⁺-N with concentrations of 7.52 mg/L was provided by (NH4)2SO4 in both reactors, and organics with organic carbon of 5.16 mg/L are ethanol and glucose in reactors R1 and R2, respectively. The inorganic salts contained many elements including K, P, Mg, Ca, Fe, Zn, Co, Mn, Cu, Mo, Ni and B. The detailed information of inorganic salts was same to the published literature by Yang et al. (2017) [8].

2.3 Experimental procedures and methods
Sediment in drinking water source located in zhejiang province was obtained and used as the bacterial source for biofilm reactors. In the initial operation period, approximately 5.0 L sediment with 20.0 L synthetic polluted source water was mixed and put into a 50.0 L plastic bucket (PB). The ESM carrier was submerged into the mixture of sediment and source water in PB, and then aeration at saturated dissolved oxygen level for the initial biofilm formation. In addition, ethanol and glucose used as organic carbon source were added into PB. Correspondingly, the organic carbon and NH4⁺-N concentrations were 5.16 and 7.52 mg L⁻¹, respectively. The water in PB was replaced by fresh influent in every 3-5 d to increase the feeding substrate levels.

After initial acclimatization for 9 d, ESM carrier with sediments in PB was homogeneously filled into reactors R1 and R2 and continuously feeding polluted source water. The ethanol was used as organic carbon source and added into reactor R1. The organic carbon source added into reactor R2 was glucose.
The average influent NH₄⁺-N and CODₘₙ concentration of reactor R₁ were 6.32 ± 3.10 mg/L and 8.27 ± 2.00 mg/L, respectively. Correspondingly, the influent NH₄⁺-N and CODₘₙ concentrations of reactor R₂ were 6.30 ± 3.37 mg/L and 8.42 ± 2.12 mg/L, respectively. The hydraulic residence time (HRT) of the two reactors was set at 8-12h in the whole operation period. Mechanical aeration was used to provide dissolved oxygen (DO) for both reactors with DO concentrations of 7.8-8.1 mg/L. After operation for 74 d, the sediments in both reactors were removed and further operated approximately 38d with ESM biofilm only.

2.4 Analysis methods

2.4.1 Water quality analysis
Water quality indicators including turbidity, CODₘₙ, NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, UV₂₅₄ and UV₄₁₀ were measured by standard methods [15].

2.4.2 Bacterial community analysis
At the end of this experiment, ESM biofilm samples in reactors R₁ and R₂ were obtained for the analysis of bacterial structure. Total DNA of sediment and ESM biofilm samples was extracted using a soil DNA extraction kit (OMEGA), and V3-V4 variable regions of 16S rRNA genes was analyzed by Illumina Miseq sequencing technology in OE biotech Co. Ltd (Shanghai, China). The bacterium-specific primer for the amplifying of V3-V4 regions of 16S rRNA genes were 343F (5’-TACGGRAGGCAGCAG-3’) and 798R (5’- AGGGTATCTAATCCT-3’). 25 μL reaction system was used for PCR amplification including NEB Q5 DNA high-fidelity polymerase (0.25 μL), DNA template (1 μL, 20 ng), dNTPs (0.5 μL, 10 mM), 5×PCR reaction buffer (5 μL), 5 × high GC buffer (5 μL), forward primer (1 μL), reverse primer (1 μL) and sterilizing ultrapure water (11.25 μL). PCR conditions were same to the reported literature [16-17]. The DNA information of Illumina Miseq sequencing was further analysis after the treatment including trimming barcodes and primers and removing defective reads with incognizable reverse primer, shorter than 150 bp or containing ambiguous bases. The bacterial information was based on operational taxonomic unit (OTU) with sequences similarity of higher than 97% were clustered into one operational taxonomic unit (OTU) [17].

3. Results and discussion

3.1 Performance of PB system
The NH₄⁺-N concentration was gradually decreased when fresh influent was added into PB systems. NH₄⁺-N removal rate (ARR) was increased from approximately 0.76 mg/L/d ($R^2=0.963$) to 1.12 mg/L/d ($R^2=0.978$). It has been reported that NH₄⁺-N removal performance was enhanced in biofilm systems when sediment was used as the bacteria source [18]. In this study, higher ARR values were observed comparing with that of 0.13-0.46 mg/L/d in sediment-free biofilm systems.

At the initial operation for bacterial acclimatization, NO₂⁻-N accumulation phenomenon was observed due to the non-complete nitrification and further decreased after operation for several days. Reports showed that sediments in biofilm systems would increase the accumulation of NO₂⁻-N accumulation during the initial days in biofilm system treating source water [8]. It is generally believed that extending the treatment time to 5-7d can effectively reduce the accumulation of NO₂⁻-N [14]. Due to shorter operation time, higher NO₂⁻-N concentration of 0.19-0.22 mg/L was observed before fresh polluted source water was added into PB system.

3.2 Performance of continuous-flow biofilm systems

3.2.1 Nitrogen removal performance
On Day 10, continuous-flow reactors R₁ and R₂ were parallely operated with identical ESM carrier and sediments from PB. The nitrogen removal performance is shown in Fig. 1. In the initial several days,
NH$_4^+$-N removal efficiency (ARE) values were more than 50.0% with maximum value of more than 90.0% due to the initial culture in PB. Zhang et al. (2013) considered that ARE of 45.8% after initial operation for 15d could be used as a parameter indicating the successful startup of biofilm reactor for polluted source water treatment. Obviously, the NH$_4^+$-N removal efficiency in biofilm system was strengthened due to the preculture in PB system with sediment addition. However, the NH$_4^+$-N removal performance was unstable due to the release of nitrogen and organic matter from sediments [8]. Days 10-74d, the ARE and ARR of reactor R1 were 75.8 ± 13.3 and 13.0 ± 4.9 mg/L/d, respectively. Corresponding values of reactor R2 were 74.3 ± 11.5% and 12.9 ± 5.0 mg/L/d, respectively. After removing the sediment, the ARE of reactor R1 and R2 reduced from 82.0% and 82.5% to 66.3% and 77.7%, respectively. After 4 days’ operation (Day 78d), the ARE of R1 and R2 recovered quickly to 88.5% and 87.2%, respectively.

Fig. 1 Operation performance of each reactor: (a) R1 and (b) R2

Due to the removal of the sediment from reactors on Day 75, the NH$_4^+$-N removal performance of reactor R1 was improved and stably operated for a long time with ARE of 87.0±5.4%. The NH$_4^+$-N removal performance of reactor R2 was also improved but unstably operated with ARE of 83.9±13.3%. In addition, there were NO$_2^-$-N accumulation during Days 10-48d in both reactors reactor R1 and R2 with effluent NO$_2^-$-N concentrations of 0.25±0.13 and 0.26±0.15 mg/L, respectively. Then NO$_2^-$-N level dropped below 0.10mg/L. From Day 62d, effluent NO$_2^-$-N level was lower than the detection limitation concentration. As shown in Fig. 1, the total nitrogen (the sum of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N) was slightly reduced. This result showed that the nitrification with a certain denitrification effect was occurred. Reactor R1 has the highest total nitrogen removal efficiency. These results indicated that no significant NO$_2^-$-N accumulation was found in biofilm reactors with ethanol and glucose as organic carbon source. However, it was not conducive to the stably removal of NH$_4^+$-N using glucose as an organic carbon source. This may be caused by the fact that ethanol is more easily to be metabolized by microorganisms in oligotrophic conditions [13].
3.2.2 Removal of organic compounds

Fig. 2 shows the removal performance of UV$_{254}$ in two reactors. UV$_{254}$ can be used to describe the humic acid and macromolecular organic matter with benzene ring in the source water [2,8]. In the initial operation days, the effluent UV$_{254}$ concentrations were higher than those of influent due to the releasing of organics from sediment [8]. Even removed the sediment from two reactors, this phenomenon was also observed from Day 75 to 93. Then, the UV$_{254}$ values of reactors were obviously removed with effluent UV$_{254}$ values of 0.306 ± 0.016 and 0.311 ± 0.022/cm. UV$_{410}$ reflects the larger conjugated system organic matter in water, which mainly come from the sediment and biofilm metabolites. In this study, there was no significant difference of the two reactors. As shown in Fig. 3, the influent turbidity was negligible, and the turbidity mainly come from the sediment in the reactor. Due to the presence of sediment during Days 10-74, the effluent turbidity were obviously higher. After removing the sediment, the effluent turbidity of each reactor was obviously reduced and no obvious differences were observed in both reactors.

Fig. 2 Changes of UV254 and UV410 in two reactors: (a) R1 and (b) R2
Fig. 3 Changes of turbidity in two reactors

Fig. 4 CODMn removal performance in reactors R1 and R2

Fig. 4 shows the variation of influent and effluent CODMn concentrations in reactors R1 and R2. Before removing the sediment, the average CODMn removal efficiency of R1 and R2 was 12.0 ± 8.4% and 12.1 ± 8.3%, respectively. After removing the sediment, the average CODMn removal of R1 and R2 was improved with CODMn removal efficiency of 18.4 ± 8.7% and 16.4 ± 7.2%, respectively. These values were obviously higher than 13.5±9.7% observed in biofilm reactor with elastic carrier [16]. Experimental results showed that the CODMn removal had no obviously different in both reactors feeding with organics of glucose or ethanol, respectively. Obviously, the presence of sediment impaired the removal of CODMn due to the releasing of organics from sediments.

3.3 Kinetics characteristics analysis

The modified Stover-Kincannon model (Eqs. 1, 2 and 3 can be used to describe the kinetics characteristics of the attached growth microorganism system[16,19].

\[
\frac{dS}{dt} = \frac{Q}{V} (S_a - S)
\]  

\[
\frac{dS}{dt} = \frac{U}{K_s + (QS)}
\]  

\[
\left(\frac{dS}{dt}\right)^{-1} = \frac{V}{Q(S_a - S)} \left(\frac{K_a}{U_{max}}\right) = \frac{V}{U_{max}} \frac{1}{Q(S_a - S)}
\]
Where, $dS/dt$ is the substrate removal rate, mg/L/d; $U_{\text{max}}$ is the maximum substrate removal rate constant, mg/L/d; $K_B$ is the saturation constant, mg/L/d; $Q$ is influent flow rate, L/d; $V$ is the volume of the reactor, L (5.0L in this study); $S_0$ and $S$ is the influent and effluent substrate concentration, respectively, mg/L.

Before removing sediments from reactors, the fitting equations for NH$_4^+$-N removal of R$_1$ and R$_2$ were $S = S_0 - S_0/(1.3532 - 0.006QS_0/V)$ ($R^2=0.7343$) (Fig. 5a) and $S = S_0 - S_0/(1.4682 + 0.0005QS_0/V)$ ($R^2=0.7406$) (Fig. 5b), respectively. After the removal of sediments, corresponding equations were $S = S_0 - S_0/(1.1916 - 0.0026QS_0/V)$ ($R^2=0.9103$) (Fig. 5c) and $S = S_0 - S_0/(1.3056 - 0.0111QS_0/V)$ ($R^2=0.9271$) (Fig. 5d). This result showed that ARR was positively correlated with ALR. Because the operation performance was unstable due to the existence of sediment, lower correlation coefficient $R^2$ was observed. After removing the sediments, the operation was stable and higher correlation coefficient $R^2$ was observed. At this time, the $U_{\text{max}}$ values of R$_1$ and R$_2$ were -384.6 and -90.1 mg/L/d, respectively. These results indicated that the ARR had a decreasing trend, which may be caused by the decrease in NH$_4^+$-N concentration and ALR levels [16]. The two reactors have different nitrogen removal characteristics due to the different organic carbon sources. Due to the low correlation coefficient ($R^2=0.0263-0.1556$), the modified Stover-Kincannon model was difficult to be used for the quantitative description and prediction of COD$_{\text{bio}}$ removal.

Fig. 5 Fitting curves of nitrogen removal performance of the modified Stover-Kincannon model.

### 3.4 Analysis of bacterial structure

#### 3.4.1 $\alpha$-diversity analysis

The $\alpha$-diversity of ESM biofilm bacteria in reactor R$_1$ and R$_2$ was analyzed, and the related indexes are shown in Table 1. The Good’s coverage indexes of R$_1$ and R$_2$ were close to 1, indicating that the sequencing depth had basically covered all species of ESM biofilm in reactors. As shown in Table 1, the Chao index, Shannon index, Simpson index and observed species index of R$_1$ were all higher than those in R$_2$, indicating a higher biodiversity in R$_1$. The PD whole tree indices of R$_1$ and R$_2$ were similar, indicating that the species in the two reactors had little difference in their evolutionary history.
preservation. Obviously, the abundance of biofilm with ethanol as the organic carbon source was higher than that using glucose as the organic carbon source.

Table 1. Alpha diversity index of ESM biofilm samples

| Index  | Chao1  | Goods coverage | Observed species | PD whole tree | shannon | simpson |
|--------|--------|----------------|------------------|---------------|---------|---------|
| ESM.R1 | 603.1  | 0.9990         | 584.0            | 25.9          | 6.92    | 0.9785  |
| ESM.R2 | 586.0  | 0.9985         | 558.0            | 25.1          | 5.52    | 0.8769  |

3.4.2 Bacterial structure

The ESM biofilm in two reactors mainly contained 30 bacterial phyla. Especially, all known phyla with relative abundance (RA) of over 1% were belonged to five phyla, i.e., Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Nitrospirae (Fig. 6a). In the ESM biofilm in reactor R1 with ethanol, the top three phyla were Proteobacteria, Bacteroidetes and Firmicutes with RA values of 29.1%, 32.7% and 22.0%, respectively. In reactor R2 with glucose as the organic carbon source, the main phyla included Proteobacteria, Bacteroidetes and Actinobacteria with RA values of 63.4%, 10.4% and 9.3%, respectively. Obviously, Proteobacteria was absolutely dominant in R2 with RA of 63.4%, which was 6.10 and 6.82 times of that of Bacteroidetes and Actinobacteria. Published reports showed that Proteobacteria was the most common bacteria in oligotrophic source water and drinking water treatment systems, and played an important role in the removal of nitrogen and organic matter [21-22]. Proteobacteria bacteria were dominant in the two reactors, the removal of nitrogen and organics and the long-term stable operation may be attributed to these bacteria. Reported literature showed that Bacteroidetes can metabolize carbohydrates under anaerobic conditions and hydrolyze complex organic matter into small molecules [16,24]. At the same time, some bacteria of Proteobacteria and Bacteroidetes were the main bacteria for COD degradation [26]. Firmicutes is mainly composed of Bacillus, and organic macromolecules in sewage were decomposed by proteases and amylases produced by Bacillus. In addition, some species of Bacillus has a nitrifying ability, which could oxide NH$_4^+$-N in wastewater [23-24].
Fig. 6 Biofilm structure of the biofilm sample (phyllum level) Distribution of microorganisms in different biofilm samples: (a) phylum level and (b) genus level.

Fig. 6 (b) shows the main known bacterial composition of ESM biofilm samples in two reactors at genus level (Top 15). The main genera in ESM biofilm in reactor R1 with ethanol as the organic carbon source were Nitrospira, Lachnospiraceae, Arcobacter and Hyphomicrobiurn with RAs of 5.9%, 5.0%, 5.8% and 3.8%, respectively. Nitrospira was reported as a nitrifying bacterium belonging to phylum Nitrospirae, which oxidizes nitrite to nitrate. Lachnospiraceae, Arcobacter and Hyphomicrobiurn belonging to Proteobacteria phylum participated in the removal of nitrogen and organic matter in the reactor [21,25]. In ESM biofilm samples in reactor R2 with glucose, genus Sphaerotilus became the absolute dominant with RA of 33.72%. Sphaerotilus was reported as a common filamentous bacterium in wastewater, which was an important genus in biofilm for the removal of organic matters [26].

As shown in Figs. 8, the different carbon sources caused a significant changes in the bacterial structure in the two biofilm reactors. At phylum level, the top three RAs of biofilms with ethanol as the organic carbon source were more balanced distribution, while Proteobacteria was absolute dominant in biofilm with glucose as the organic carbon source, and the RA is much larger than the sum of all other phyla. At genus level, the biofilm bacteria using ethanol as the organic carbon source also appeared to have more balanced RA among different genera, and Sphaerotilus was dominant in biofilm with glucose as the organic carbon source.

4. Conclusion
The preculture of biofilm using sediment as bacterial source could quickly start up the biofilm reactors for source water pretreatment and enhance the removal of NH4+-N and organics. Glucose was not conducive to the stable removal of NH4+-N, but ethanol enhanced the nitrogen removal. However, glucose and ethanol as organic carbon source has little effects on the removal of CODMn, but the presence of sediment impaired its removal. Organic matters affected the bacterial structure at both phylum and genus levels. The dominant phyla in biofilms using ethanol as the organic carbon source were Proteobacteria (29.1%), Bacteroidetes (32.7%) and Firmicutes (22.0%) with total RA of 83.8%, and Proteobacteria of R2 using glucose as the organic carbon source became the absolute dominant species with RA of 63.4%. At genus level, nitrogen and organic removal related bacteria Nitrospira, Lachnospiraceae, Arcobacter and Hyphomicrobiurn were dominant in R1 and genus Sphaerotilus became absolute dominant in R2.

Acknowledgments
This work was financially supported by National Natural Science Foundation of China (51808498), Natural Science Foundation of Zhejiang Province of China (No. LQ17E090002), Fundamental Research
Funds for the Provisional Universities (2019J00050), and Key Laboratory of Water Pollution Control and Environmental Safety of Zhejiang Province, China (No. 2017ZJSHKF02).

Reference

[1] L.J. Feng, L. Zhu, Q. Yang, G.F. Yang, J. Xu, X.Y. Xu, Simultaneous enhancement of organics and nitrogen removal in drinking water biofilm pretreatment system with reed addition, Bioresour. Technol. 129: 274-280 (2013).
[2] Z.X. Yu, H.Q. Chu, D.W. Cao, Y.Q. Ma, B.Z. Dong, Y. Wei. Pilot-scale hybrid biotrickling filter reactor for slightly polluted source water purification. Desalination 285: 73-82 (2012).
[3] J.A. Camargo, A. Alonso. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ. Int. 32 (6): 831-849 (2006).
[4] J.Q. Sang, X.H. Zhang, L.Z. Li, Z.S. Wang. Improvement of organics removal by bio-ceramic filtration of source water with addition of phosphorus. Water Res. 37: 4711-4718 (2003).
[5] J. Benner, D.E. Helbling, H.P. Kohler, J. Wittebol, E. Kaiser, C. Prasse, T.A. Ternes, C.N. Albers, J. Aamand, B. Horemans, D. Springael, E. Walravens, N. Boon. Is biological treatment a viable alternative for micropollutant removal in drinking water treatment processes? Water Res. 47 (16): 5955-5976 (2013).
[6] G.F. Yang, L.J. Feng, Q. Yang, L. Zhu, J. Xu, X.Y. Xu, Startup pattern and performance enhancement of pilot-scale biofilm process for raw water pretreatment. Bioresour. Technol. 172: 22-31 (2014).
[7] J.W.A Charrois, S.E. Hrudey. Breakpoint chlorination and free-chlorine contact time: implication for drinking water N-nitrosodimethylamine concentrations. Water Res. 41: 674-682 (2007).
[8] G.F. Yang, L.J. Feng, C.R. Guo, T. Xia, X.Y. Xu, L. Zhu. Performance improvement of source water pretreatment process with pre-inoculation biofilm: feasibility and limiting factors. Bio degradation 28 (1): 1-13 (2017).
[9] E. Carraro, E.H. Bugliosi, L. Meucci, C. Baiocchi, G. Gilli. Biological drinking water treatment processes, with special reference to mutagenicity. Water Res. 34 (11): 3042-3054 (2000).
[10] H.L. Yang, S.J. Ye, J.J. Wang, H. Wang, Z.W. Wang, Q. Chen, W.J. Wang, L. Xiang, G.M. Zeng, X.F. Tan. The approaches and prospects for natural organic matter-derived disinfection byproducts control by carbon-based materials in water disinfection progresses. J. Clean. Prod. 311: 127799 (2021).
[11] S. Zhang, Y. Wang, W. He, M. Wu, M. Xing, J. Yang, N. Gao, D. Yin. Responses of biofilm characteristics to variations in temperature and NH4+-N loading in a moving-bed biofilm reactor treating micro-polluted source water. Bioresour. Technol. 131 (3): 365-373 (2013).
[12] S. Xiang, Y.T. Han, C. Jiang, M.Y. Li, L.C. Wei, J.S. Fu, L. Zhu. Composite biologically active filter (BAF) with zeolite, granular activated carbon, and suspended biological carrier for treating algae-laden raw water. J. Water Process Eng. 42: 102188 (2021).
[13] T. Egli. How to live at very low substrate concentration. Water Res. 44 (17): 4826-4837 (2010).
[14] G.F. Yang, L.J. Feng, S.F. Wang, Q. Yang, X.Y. Xu, L. Zhu. Performance and enhanced mechanism of a novel bio-diatomite biofilm pretreatment process treating polluted raw water. Bioresour. Technol. 191:271-280 (2015).
[15] APHA, Standard methods for the examination of water and wastewater, 21th edn. American Public Health Association, Washington, DC, USA (2005)
[16] L.J. Feng, J. Mu, J.Y. Sun, Y. Kong, J. Wang, Z.H. Lv, L. Zhu, X.Y. Xu, G.F. Yang. Kinetic characteristics and bacterial structures in biofilm reactors with pre-cultured biofilm for source water pretreatment. Int. Biodeter. Biodegr. 121: 26-34 (2017).
[17] L.J. Feng, K. Chen, D.D. Han, J. Zhao, Y. Lu, G.F. Yang, J. Mu, X.J. Zhao. Comparison of nitrogen removal and microbial properties in solid-phase denitrification systems for water purification with various pretreated lignocellulosic carriers, Bioresour. Technol. 224: 236-245 (2017).
[18] Z.H. Lv, J. Wang, G.F. Yang, L.J. Feng, X.Y. Xu. Underestimated effects of sediments on enhanced startup performance of biofilm systems for polluted source water pretreatment. Biodegradation 29 (16):1-15 (2018).

[19] H.Q. Yu, F. Wilson, J.H. Tay. Kinetic analysis of an anaerobic filter treating soybean wastewater. Water Res. 32: 3341-3352 (1998).

[20] G.F. Yang, L.J. Feng, S.F. Wang, J.H. Zhou, C.R. Guo, T. Xia, W.X. Sun, Y.J. Jiang, X.Y. Sun, L. Cao, X.Y. Xu, L. Zhu. Potential risk and control strategy of biofilm pretreatment process treating source water. Bioresour. Technol. 198: 456-463 (2015).

[21] L. Vaz-Moreira, O.C. Nunes, C.M. Manaia. Ubiquitous and persistent Proteobacteria and other Gram-negative bacteria in drinking water. Sci. Total Environ. 586: 1141-1149 (2017).

[22] Zhou, C.L. Chen, S.X. Zhou, K.Y. Bu, P.Y. Li, X.Y. Lin, L.J. Jiang, C.F. Zhang. Performance and microbial community analysis of a bio-contact oxidation reactor during the treatment of low-COD and high-salinity oilfield produced water. Bioresour. Technol. 335: 125267 (2021).

[23] P. Kowal, S. Ciesielski, M. Godzieba, K. Fitobór, M. Gajewska, K. Kolecka. Assessment of diversity and composition of bacterial community in sludge treatment reed bed systems. Sci. Total Environ. 756: 144060 (2020).

[24] Y.N. Wang, R. Xu, H.W. Wang, H. Shi, Y. Kai, Y.J. Sun, W.H. Li, R.X. Bian, M.L. Zhan. Insights into the stabilization of landfill by assessing the diversity and dynamic succession of bacterial community and its associated bio-metabolic process. Sci. Total Environ. 768: 145466 (2021).

[25] G.F. Yang, L.J. Feng, J. Mu, J.Y. Sun, X.Y. Xu. Performance and Spatial Distribution of Functional Bacteria under Low-Temperature Stress in Biofilm Systems for Polluted Source Water Pretreatment[J]. Int. J. Environ. Res. 13: 769-780 (2019).

[26] Y. Li, W. Chen, X.Y. Zheng, Q. Liu, W. Xiang, J.X. Qu, C.F Yang. Microbial community structure analysis in a hybrid membrane bioreactor via high-throughput sequencing. Chemosphere 282: 130989 (2021).