Environmental Science and Pollution Research (2022) 29:49105–49115
https://doi.org/10.1007/s11356-022-19382-w

RESEARCH ARTICLE

Microbial composition and nitrogen removal pathways in a novel sequencing batch reactor integrated with semi-fixed biofilm carrier: evidence from a pilot study for low- and high-strength sewage treatment

Peng-fei Chen[1] · Rui-jian Zhang[1] · Zhi-li Du[1] · Guang-hua Wang[1] · Hao-tao Dong[1] · Bin Cui[2] · Ru-pei Fan[1] · Lu-xin Li[1] · Qian-bin Wang[1] · Ying-shi Liu[1] · Zhi-min Sun[1]

Received: 29 November 2021 / Accepted: 20 February 2022 / Published online: 25 February 2022

Abstract

The sequencing batch reactor (SBR) activated sludge process is a well-established technology for sewage treatment. One of the drawbacks of SBRs, however, total nitrogen (TN) removals is insufficient. By means of introducing four improvements, including semi-fixed biofilm carrier, sludge elevation mixing and change for the mode of influent and effluent, compliant standard for TN discharge was obtained in this novel SBR configuration during low- and high-strength sewage load. To illustrate the microbial compositions and functions of the attached biofilm on semi-fixed carrier and the suspended aggregates, as well as the nitrogen removal pathway, high throughput 16S rRNA gene amplicon sequencing, PICRUSt2 algorithm, and KEGG database were applied. The results revealed that (i) the microbial communities from suspended aggregates and biofilm samples were significantly different from each other; (ii) during low-strength sewage loads, TN removal was mainly by nitrification–denitrification. The suspended aggregates was responsible for denitrification, while the biofilm was focused on ammonium oxidation; (iii) during high-strength sewage loads, function of nitrate reductase from suspended aggregates was faded, and anammox and N assimilation by biofilm became dominant. Meanwhile, TN removal referring to the formation of L-glutamine via assimilation was the main pathway.

Keywords SBR · Semi-fixed biofilm carrier · Bacterial community · Nitrogen removal pathway

Introduction

Nitrogen (N) removal in sewage is one of the main goals of biological sewage treatment. High N emissions can cause accelerated eutrophication in a receiving water body (De Sotto et al. 2018). The sequencing batch reactor (SBR) activated sludge process is commonly used in rural sewage treatment plants, offering advantages in terms of investment, fluctuation of sewage characteristic and volume, energy consumption, as well as good removal of organic matter and phosphate (Maslon et al. 2019). Nevertheless, the effluent on total N (TN) would not meet the first class requirement of the National Municipal Wastewater Discharge Standards of China (NMWDS) (TN < 10 mg L^{-1}) (Yuan et al. 2016; Chen et al. 2016). Reliable TN removal requires adequate and stable denitrifier populations, suitable carbon sources, and adequate duration for anoxic denitrification (Onnis-Hayden et al. 2011). Thus, process modifications were applied that were related to the reaction period of SBR, the way in which the sewage and oxygen interact, and the inclusion of a suspended biofilm carrier (Mihelcic and Zimmerman 2014; Zhang et al. 2019a, b, c, d; Tombola et al. 2019; Tao and Hamouda 2019). Moving bed biofilm reactors (MBBRs) have been shown to be successful for the enhancement of nitrification and denitrification in a WWTP upgrade project (Onnis-Hayden et al. 2011; Makowska and Maciejewska 2016; Sytek-Szmeichel et al. 2016). To obtain

Responsoble editor: Gerald Thouand.

✉ Rui-jian Zhang
zhangrj@gzmedri.com

[1] Research center, Guangzhou Municipal Engineering Design & Research Institute, Guangzhou 510060, China

[2] Graduate School, Guangzhou University, Guangzhou 510060, China

sufficient mixing and interaction between sewage and the suspended biofilm carrier in MBBRs, high intensity aeration is necessary, causing drastic collisions among the carrier and the inner wall of the MBBR. In a full-scale MBBR, it was found that (1) broken carriers and its debris caused clogging of the subsequent clarifier and (2) abrasion of the MBBR inner wall (see images presented in Supplementary Information Fig. S1). Prior to maintenance of the aeration system (ca. every two years), all of the carriers need to be cleared from the system, resulting in great losses in carrier quantity. All these problems mentioned above need to be solved for efficient full-scale application of MBBRs.

To shun problems on carrier loss and operation, the semi-fixed biofilm carrier was introduced in this study, as well as decoupling the growth rate of nitrifying populations and the suspended mixed liquor phase solids retention time. Meanwhile, the sludge elevation mixing and the change on the mode of influent and effluent were also conducted, obtaining reliable TN removal for both low- and high-strength sewage treatment. The performance of our novel SBR would be attributed to the joint effect of microorganisms, including ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), denitrifying bacteria (DNB) (Rud et al. 2017) and other heterotrophic bacteria. Although analysis of the classical bacterial 16S rRNA gene-based marker can reveal community composition, this type of analysis will not provide direct functional analysis of TN removal in this novel SBR. Recently, the microbial community and its hydrolase functional profile in the mainstream upflow nitritation-anammox system with hybrid anaerobic pretreatment (Li et al. 2017b), sewage treatment systems (Cui et al. 2019; Zhang et al. 2019a, b, c, d; Zhao et al. 2020), sewage sludge, and manure composting processes (Jiang et al. 2019; Zhong et al. 2020) were analyzed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)—a widely used algorithm which optimizes genome prediction that enables improved accuracy on functional profiling of complex phenotypes and integration to customary databases (Douglas et al. 2019). Therefore, to understand the microbial community composition from suspended aggregates and semi-fixed biofilm carrier, the change in community structure related to variable organic load, and their role in the N removal pathway, PICRUSt2 (the latest version) was applied in this work.

The aim of this study is to (1) investigate the bacterial community from suspended aggregates and semi-fixed biofilm carrier during low- and high-strength sewage treatment; (2) explore the biomarkers of N removal in the bacterial community; (3) screen the possible functional shifts of the bacterial community driven by changes in sewage load strength; and (4) understand the interactions among microbial species. By means of high-throughput sequencing technology, principal component analysis (PCA), and PICRUSt2, our result will provide insights into the bacterial community from suspended aggregates and semi-fixed biofilm carrier in terms of phylogenetic structure and functional profiles during low- and high-strength sewage treatment in this novel SBR.

Materials and methods

SBR pilot plant operation and sewage quality monitoring

The pilot-scale SBR integrated with semi-fixed biofilm carrier (Fig. 1) was set up in December 2017 at a sewage treatment plant in Guangzhou (China), and was operated for over 300 days with a capacity of 90 m^3 day^{-1}. As depicted in Fig. 1, four improvements, including semi-fixed biofilm carrier, gravity influent, sludge elevation mixing, and gravity effluent, were introduced. The semi-fixed biofilm carrier (Supplementary Information Fig. S2d) is composed of plastic film clip and vinylon fibers (Jiangsu Dadu plastic CO. LTD, Zhejiang, China), which bundled in steel frame and fixed in the lower half of the reactor (Fig. 1). The other major equipment includes an aeration system, mixer, diffuser, and other components. The seeding sludge was introduced from sewage treatment plant, while SRT was set approximately 25 days. Operation of this reactor was controlled automatically by a system designed for pilot-scale operation and was set on-site. This automatic system controls the electric pumps and air valves that were used for the flow of water and sludge, respectively into and out of the reactor. The total cycle was 8 h, with the main process following the cycle of influent (~ 10 min), first stage return of activated sludge elevation mixing under anaerobic conditions (~ 50 min, DO < 0.2 mg L^{-1}), aeration (~ 120 min with air flow ratio of 1000–3000 L h^{-1}, DO 1.5–3.0 mg L^{-1}), second stage return of activated sludge elevation mixing under anoxic conditions (~ 210 min, DO < 0.5 mg L^{-1}), dosing for the removal of phosphorus (~ 10 min), sedimentation (~ 70 min), and effluent (~ 10 min). The feed water was imported from primary sedimentation effluent (defined as low-strength sewage, 48–224 mg L^{-1} for chemical oxygen demand (COD) and 10–30 mg L^{-1} for TN, respectively), stored in head tank and then flowed into the reactor in the form of gravity flow via wide tube. To test the performance on high-strength sewage (ca. 64–482 mg L^{-1} for COD and 16–57 mg L^{-1} for TN, respectively), condensed wastewater from a septic tank was introduced and mixed with the primary sedimentation effluent between August and October 2018. The main characteristics of the influent sewage applied in this work are described in Supplementary Information Table S1.

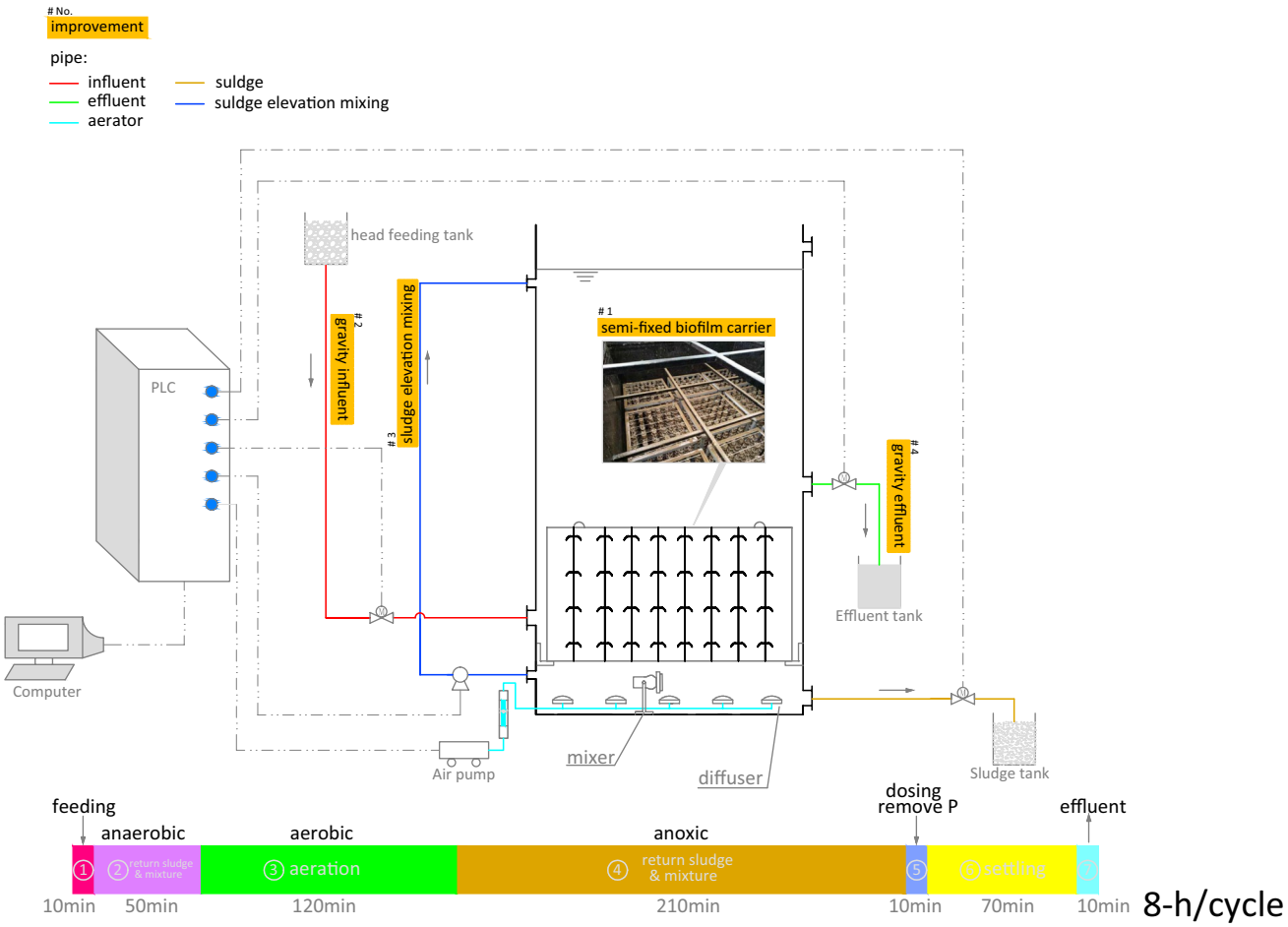

Fig. 1 Diagram of the novel sequencing batch reactor integrated with semi-fixed biofilm carrier reactor

The influent sewage and effluent were collected daily in triplicate. And the COD, ammonia, TN, and total phosphates (TP) tests were conducted using commercial kits from Hach® following the manufacturer's instructions. Briefly, filtered samples were diluted (for TP, TN, and ammonia tests) individually in tubes. COD and TP samples were digested using a heat block at 150 °C for 2 h and 30 min, respectively, while TN was digested for 30 min at 105 °C. The digested samples were cooled down before analysis. Concentrations were identified using the default programs of a DR/2500 spectrophotometer (Hach, Loveland, CO, USA).

Sample preparation

Suspended aggregates (SU) and biofilms (B), named as L_SU, L_B, H_SU, and H_B from low- and high-strength sewage loads in May and October, respectively, were collected independently at the end of the anoxic period (Fig. 1); samples were collected in triplicate using sterile tubes and pooled. To isolate the biofilm biomass from detachable carriers, the carrier was snipped into fragments and then mixed with lysozyme at 35 °C for 20 min. Afterwards, the biofilm was separated from the carrier using a Vortex oscillator (GL-88B, Jiangsu Haimen Kirin Medical Instrument factory). After removing the carrier, the remaining suspension was used as the biofilm sample. Samples L_SU, L_B, H_SU, and H_B were centrifuged at 15,000 × *g* for 15 min and stored – 20 °C until DNA extraction.

DNA extraction and bacterial community analysis

PICRUSt2 was used to predict the metabolic dynamics of the communities (Li et al. 2017b). The protocols for DNA extraction, high-throughput 16S rRNA gene pyrosequencing, and biodiversity analysis are presented in Supplementary Information Text S2.

Data analysis

The COD, ammonia, TN, and TP values from influent and effluent were analyzed using Origin 10.0 software (Origin Lab, Northampton, USA). A high-throughput sequencing

technology, PCA and PICRUSt2, was used to investigate the bacterial community.

Results and discussion

Nutrient removal efficiency

After startup (January to March 2018), this novel SBR exhibited reliable treatment performance for the removal of TN (Fig. 2), COD, and ammonia (Supplementary Information Text. S3) at COD/TN influent ratio < 7.5. The effluent met the first class requirement of the NMWDS. Even the sewage strength load soar, minor differences from sewage parameters between low- and high-strength sewage load at aerobic, anoxic, and effluent stage (Supplementary Information Fig. S9) would show that this novel SBR is available to load impact. Moreover, the reactor presented excellent TN removal during high-strength sewage load, with average values of 11.66 mg L^{-1} for effluent, comparing to that of 7.33 mg L^{-1} during low-strength sewage load. It was assumed that semi-fixed biofilm carrier and the sludge elevation mixing offer abundant C and biomass to cope with the augment of sewage strength for denitrification. On the other hand, the hydraulic retention time for denitrification (~ 330 min), which occupied 67.3% of the total operation duration, would also favor TN removal. Herein, gravity influent was applied to replace common pressure flow by pump. The total duration for influent in this novel SBR was merely 10 min (Fig. 1), which is far less than that of pressure flow (30 min ~ 60 min). Similarly, gravity effluent via wide tube was another improvement to save reaction time. The improvements on influent and effluent mode would free up ca. 40 min ~ 100 min for anoxic denitrification. However, the poor TP removal from July to September (some plots > 0.5 mg L^{-1} in Supplementary Information Fig. S8) was because of the absence of dosing poly aluminum chloride.

Microbial communities in suspended aggregates and biofilms

The microbial communities in suspended aggregates and in attached biofilm on carrier within this novel SBR were investigated. After quality filtering, the diversity of OTUs, calculated by estimating the number of total OTUs at the 97% similarity level by the Shannon diversity index, was about 5.98, 5.95, 5.73, and 5.76 in L_SU, L_B, H_SU, and H_B, respectively (Supplementary Information Table S2), indicating that community diversity was higher in low-strength sewage than in high-strength sewage. Analysis of the shared OTUs among samples showed that there were 852 OTUs observed in both samples while more OTUs were present in low-strength sewage than in high-strength sewage (Supplementary Information Fig. S10). This could be explained by the leap of some strains, which is fond of high-strength sewage and become dominant. Besides, the different bacterial composition was also analyzed by PCA based on the abundance of OTUs. The first axis (F1) and the second axis (F2) accounted for 81.4% and 12.1% of the variability, respectively (Supplementary Information Fig. S11). PCA revealed the difference in community composition between

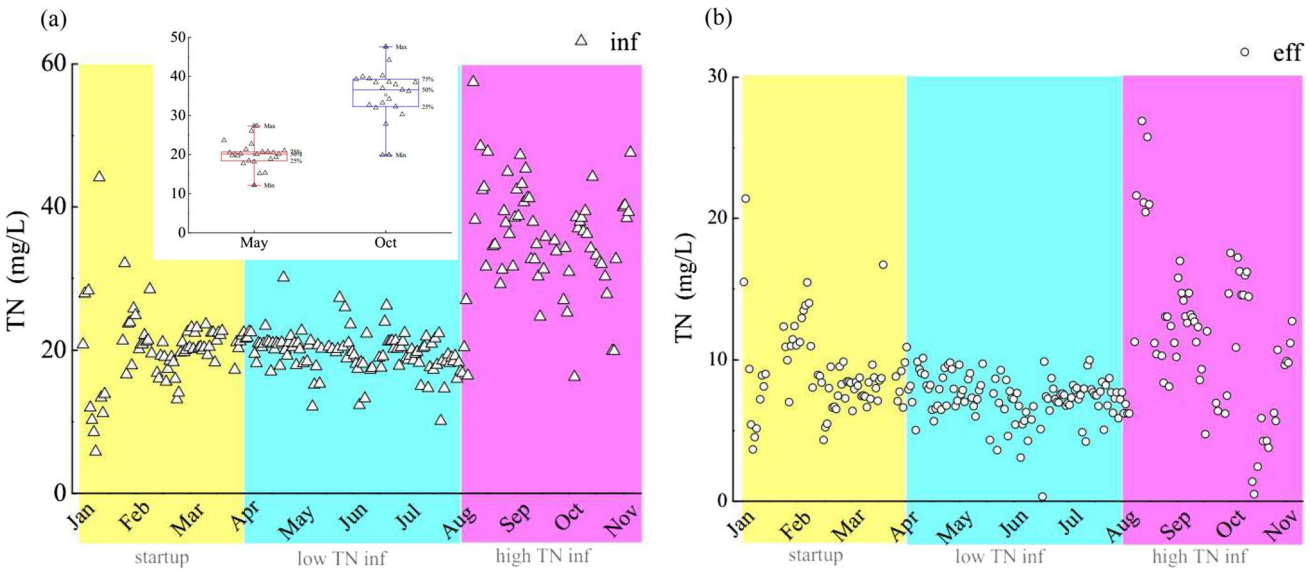

Fig. 2 Total nitrogen (TN) concentrations in **a** influent (and the values of May and October in the insert box chart, respectively represented for low- and high-strength sewage load); **b** effluent. This novel SBR had been operated with three stages, including startup (from January to April), low TN influent (from April to August), and high TN influent (from August to November)

suspended aggregates and attached biofilm. H_SU and H_B communities were more distant from each other than those of L_SU and L_B communities. These results suggest that the bacterial compositions of H_SU and H_B were more dissimilar thus facilitating cooperation during treatment of high-strength sewage.

Taking a closer look at the microbial community constituting the suspended and attached growth is necessary to understand the association between these two forms of growth and the influence of microorganisms in suspended aggregates on the formation of attached biofilm on carriers, and to eventually elaborate the evolution of microorganisms during the change in sewage strength. A total of nine phyla were identified in the samples (Fig. 3). The abundance of the OTUs in different phyla was listed in order as follows: *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Planctomycetes*, and *Chloroflexi*. Phyla *Acidobacteria* and *Chloroflexi* are known to contribute to carbon and N cycles (Eichorst et al. 2018; Nguyen, et al. 2019). Meanwhile, previous studies (Chen et al. 2018; Lage et al. 2019) have linked the presence of *Proteobacteria*, *Bacteroidetes*, and *Planctomycetes* to nitrite denitrification.

To get a better understanding of the N removal pathway, the group diversity was measured to discriminate between communities in suspended aggregates and attached biofilm during low- and high-strength sewage load treatment. Figure 4 is a hierarchical clustering analysis of communities in L_SU, L_B, H_SU, and H_B. A positive z-score (red) denotes that the value in that sample is above the mean of the group while negative z-scores (blue) are for those values that are below the mean. The predominant bacterial community consisted of 50 genera (Fig. 4a) mainly from phylum *Proteobacteria* (20 out of 50) followed by *Bacteroidetes* (9 out of 50) and *Acidobacteria* (5 out of 50). The group diversities observed during low- and high-strength sewage treatment were diverse and were present at different abundances as well as in suspended aggregates and attached biofilm for the same sewage load (Fig. 4a). The different communities could exert different contributions to the removal/biotransformation of compounds containing N, P, and pollutants like recalcitrant hydrocarbons because of their metabolic and biochemical capabilities. In detail, L_SU was mainly characterized by *Nitrospira*, *Rhodocyclaceae*, and *Anaerolineaceae*. The genus *Nitrospira* are prevalent NOB in most sewage treatment systems (Shu et al. 2015), while *Rhodocyclaceae* and *Anaerolineaceae* are capable of autotrophic denitrification (Guo et al. 2016) and carbohydrate decomposition under anaerobic conditions (Narihiro et al. 2012), respectively. When compared with L_SU, the predominant strains in L_B were mainly those capable of ammonium oxidation (*Pirellulaceae* (Miao et al. 2019) and *Ellin6067* (Xia et al. 2005)) and organic contaminants degradation (*Bacteroidetes* (Chen et al. 2007) and *Sphingomonadaceae* (Thelusmond et al. 2016)). Besides *Rhodocyclaceae*, some denitrifying strains (including *Haliangium* (McIlroy et al. 2014) and *Dechloromonas* (Zhang et al. 2019a, b, c, d)) were present at higher abundance in L_SU than in L_B. The processes of denitrification (represented by DNB) and nitrite oxidation (represented by NOB) were more prevalent in L_SU than in L_B, whereas the opposite was the case for the process of ammonia oxidation (represented by AOB; Fig. 4b). From these results, it was concluded that the microbial community of L_SU played a more important role in denitrification than that of L_B during low-strength sewage treatment. With the increase in sewage strength, some strains associated with organic contaminants degradation (including *Fimbriimonadaceae* (Feng et al. 2016) and *Novosphingobium* (Liu et al. 2005)) became dominant in H_SU (Fig. 4a), while strains with unknown function became dominant in H_B, such as *Chitinophagales*, *AKYH767*, *SBR1031*,

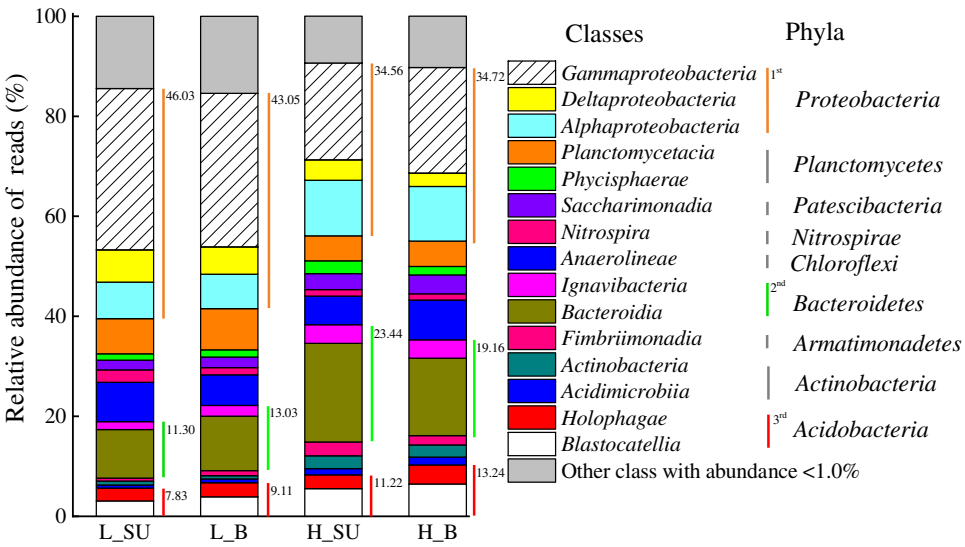

Fig. 3 Relative abundance of classes (and phyla) in suspended aggregates during low-strength sewage load stage (L_SU), biofilms during low-strength sewage load stage (L_B), suspended aggregates during high-strength sewage load stage (H_SU), and biofilms during high-strength sewage load stage (H_B)

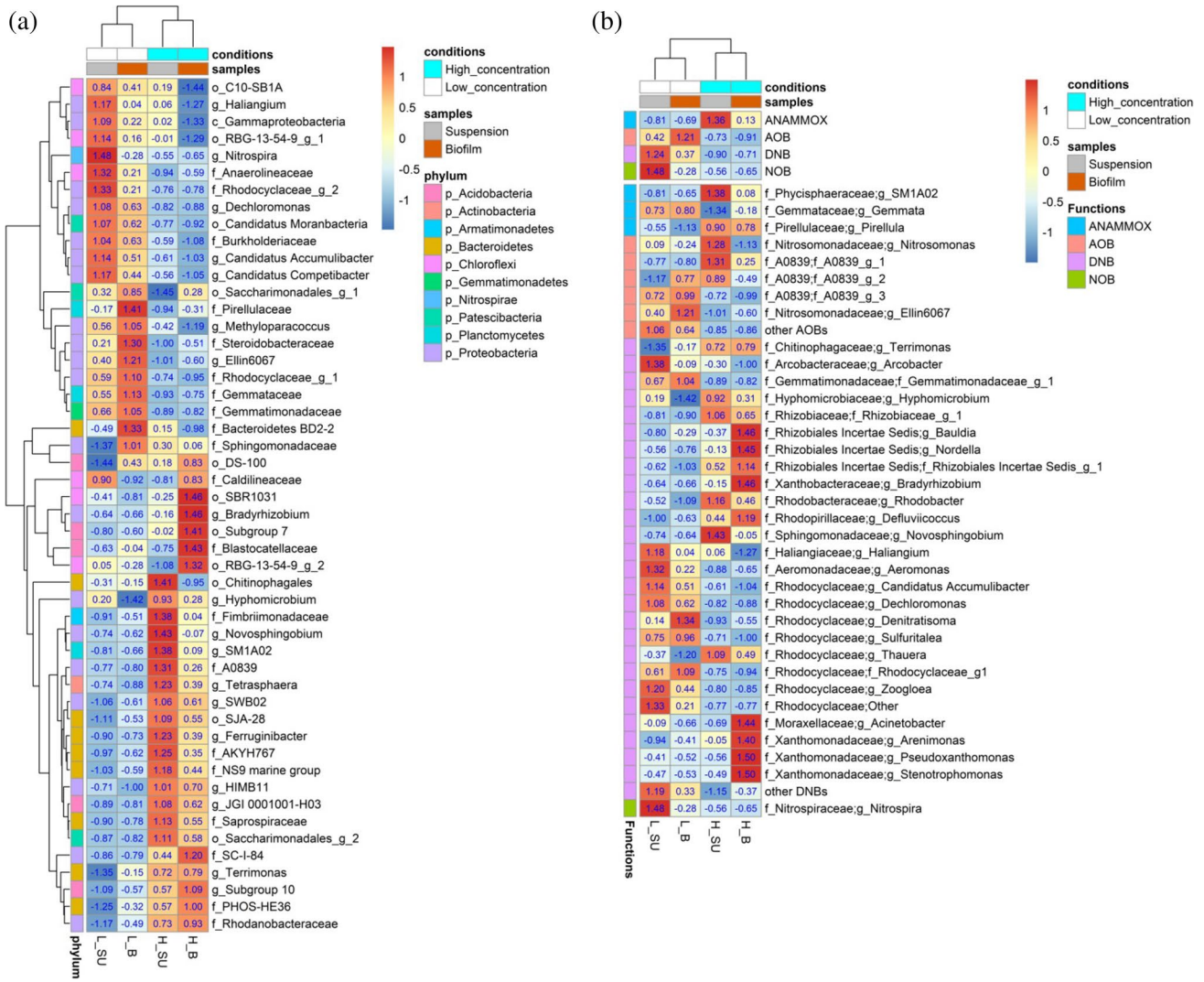

Fig. 4 Hierarchical cluster analysis of bacterial communities. The relative abundance of **a** 50 most abundant operational taxonomic units (OTUs) and **b** functional community of anaerobic ammonia oxidation (ANAMMOX), ammonia-oxidizing bacteria (AOB), denitrifying bacteria (DNB) and nitrite-oxidizing bacteria (NOB)

RBG-13–54-9_g_2. The presence of SM1A02 strains has been widely detected in anammox systems (Tian et al. 2017; Zhang et al. 2018; Meng et al. 2019; Gao et al. 2020), indicating that anammox process potential was a crucial link in the N metabolism pathway. Anammox is a biologically mediated process in which ammonium is oxidized to N_2 gas under anaerobic conditions with nitrite serving as the electron acceptor (Mulder et al. 1995; Strous et al. 2006). Li et al. (2020) investigated two anammox granular sludge reactors operating under different N loading rates and found that high TN removal was observed under high N loading. Herein, the average TN removal during high-strength sewage treatment was as high as 73.47%, whereas lower average TN removal rate (63.41%) was recorded during low-strength sewage treatment (calculated from Fig. 2). Besides, ampler nitrite at anaerobic stage during high-strength load (Supplementary Information Fig. S9) would provide favorable substrate for anammox.

Mechanism of N removal

To illustrate the N metabolism during low- and high-strength sewage treatment, the PICRUSt2 algorithm and KEGG database (Douglas et al. 2019) were applied to categorize the functional genes distribution and the enzyme families' abundance, respectively. Based on the reference pathway (KO) of N metabolism (map00910) (Kanehisa et al. 2016), the N metabolism pathways were mapped as Supplementary Information Fig. S12, and then subdivided as the core (Supplementary Information Fig. S13) and the subordinate

(Supplementary Information Fig. S14) pathways. Referring to Kanehisa et al. (2016), four reduction pathways (M00175 for N fixation, M00531 for assimilatory nitrate reduction, M00530 for dissimilatory nitrate reduction, and M00529 for denitrification) and one oxidation pathway (M00528 for nitrification) were involved in the core N metabolism pathway (Supplementary Information Fig. S15). The modules of M00528 and M00531 showed higher abundance during high-strength than low-strength sewage treatment, while M00175, M00530, and M00529 were the opposite. The increase in sewage loading reduced the contribution of autotrophic nitrate reducing bacteria and weakened their dominant role, even though the TN removal rate was high during high-strength sewage treatment (see "Microbial communities in suspended aggregates and biofilms" section). These results were similar to those of Qiu et al. (2020), which demonstrated that the relative abundance of denitrifiers decreased with increasing organic load during long-term operation in a sulfur-based denitrification reactor. In our study, the increase in sewage strength boosted the total microbial abundance, provided favorable habitat for microbial communities that were not related to autotrophic denitrification, and then chopped the total relative abundance of denitrifier and functional genes (Miao et al. 2019; Gu et al. 2019; Sun et al. 2020). These conclusions were supported by the high abundance of M00531 (assimilatory nitrate reduction pathway) and the low abundance of M00804 (complete nitrification pathway) during high-strength sewage treatment (Supplementary Information Fig. S15). The lower M00804 during high-strength sewage load would result in an increase in nitrite formation, which is one of the substances required for anammox (Liu et al. 2020). This data would support the potential for anammox during high-strength (see "Microbial communities in suspended aggregates and biofilms" section) rather than low-strength sewage load. However, because of the lack of key enzyme genes in the KEGG database, the predictive functional profiling for anammox could not be concluded in this study. In brief, the difference in N metabolism between low- and high-strength sewage loads was explained by the prevalence of nitrification–denitrification for TN removal during low-strength sewage load, whereas assimilation was prevalent during high-strength sewage load.

In combination with the key enzyme coding (Li et al. 2019; Yang et al. 2020) presented in this study, the main N metabolism processes were shown in Fig. 5. Several key enzymes were involved in the nitrification–denitrification

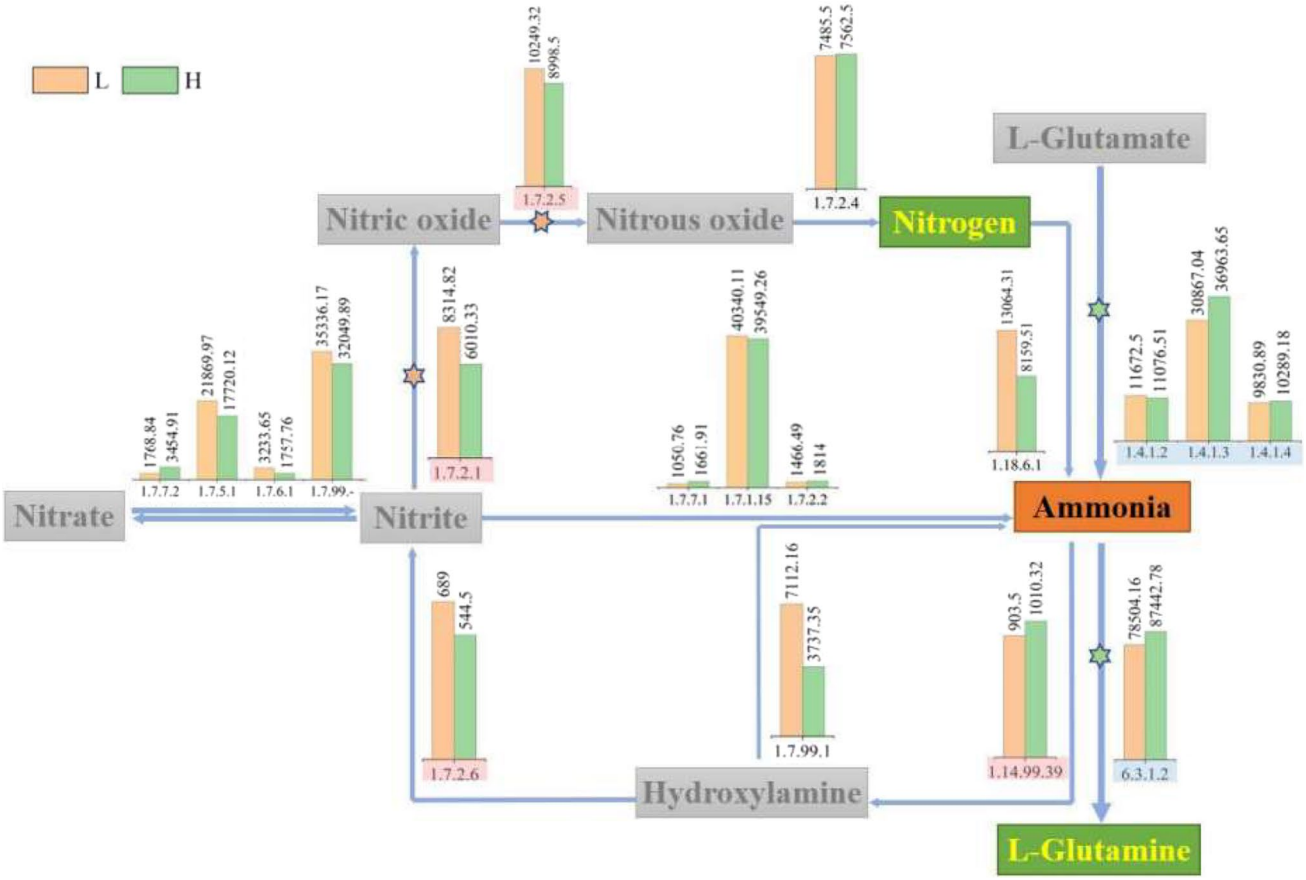

Fig. 5 Abundances of key enzyme classifications (identified by their EC number) for each treatment in the nitrogen metabolism pathway

pathway, such as ammonia monooxygenase (EC:1.14.99.39) and hydroxylamine dehydrogenase (EC:1.7.2.6) responsible for nitrosation (Fig. 5), nitrate reductase EC:1.7.7.2, EC:1.7.5.1, EC:1.7.6.1 and EC:1.7.99, involved in the redox between nitrate and nitrite, nitrite reductase (EC:1.7.2.1) related to the reduction of nitrite to nitric oxide, nitric oxide reductase (EC:1.7.2.5) associated with the reduction of nitric oxide to nitrous oxide, and nitrous oxide reductase (EC:1.7.2.4) responsible for the reduction of nitrous oxide to N_2. With the increase in sewage strength, EC:1.7.2.1 and EC:1.7.2.5 showed an obvious decrease; meanwhile, glutamate dehydrogenase (EC:1.4.1.3 and EC:1.4.1.4) and glutamate-ammonia ligase (EC:6.3.1.2) were both increased. These indicated that during high-strength sewage load, first, there was a whittle for denitrification pathway; second, TN removal was mainly through the formation of L-glutamine via assimilation. These results are similar to those of Zhang et al. (2019a, b, c, d) and Wang et al. (2020), who documented that organic supplementation enhance the assimilation efficiency of ammonia-N and boosted the conversion of ammonia-N to protein. In contrast, because the enzymes involved in nitrosation (EC:1.14.99.39 and EC:1.7.2.6) had far lower abundance than other enzymes (Fig. 5), it seems that the reaction dynamic was constrained. These results were not consistent with the long, reliable, and compliant standard for TN discharge during low- and high-strength sewage loads, respectively. This might be because the samples were collected at the end of the anoxic period (Fig. 1), when nitrosation microorganisms struggled to survive and showed weak endurance (Guo et al. 2009; Cui et al. 2020).

To illustrate the role of suspended aggregates and biofilm carrier involved in TN removal, the proportions of the key functional genes in suspension and biofilm samples related to the main N metabolism pathways were itemized in Fig. 6 and Supplementary Information Fig. S16. The nitrate reductase genes in L_SU (i.e., *nirS*, *norB*, *nosZ*, *narG*, *narH*, *narI*, *napA*, *nirB*, and *nirD*) were present at high abundance, indicating the dominant role of L_SU in TN removal, while these genes were not prominent in L_B. With increasing sewage strength load, the abundance of nitrate reductase genes in H_SU decreased. Meanwhile, the genes (including *nasB*, *nasA*, *nirA*, and *glnA*) related

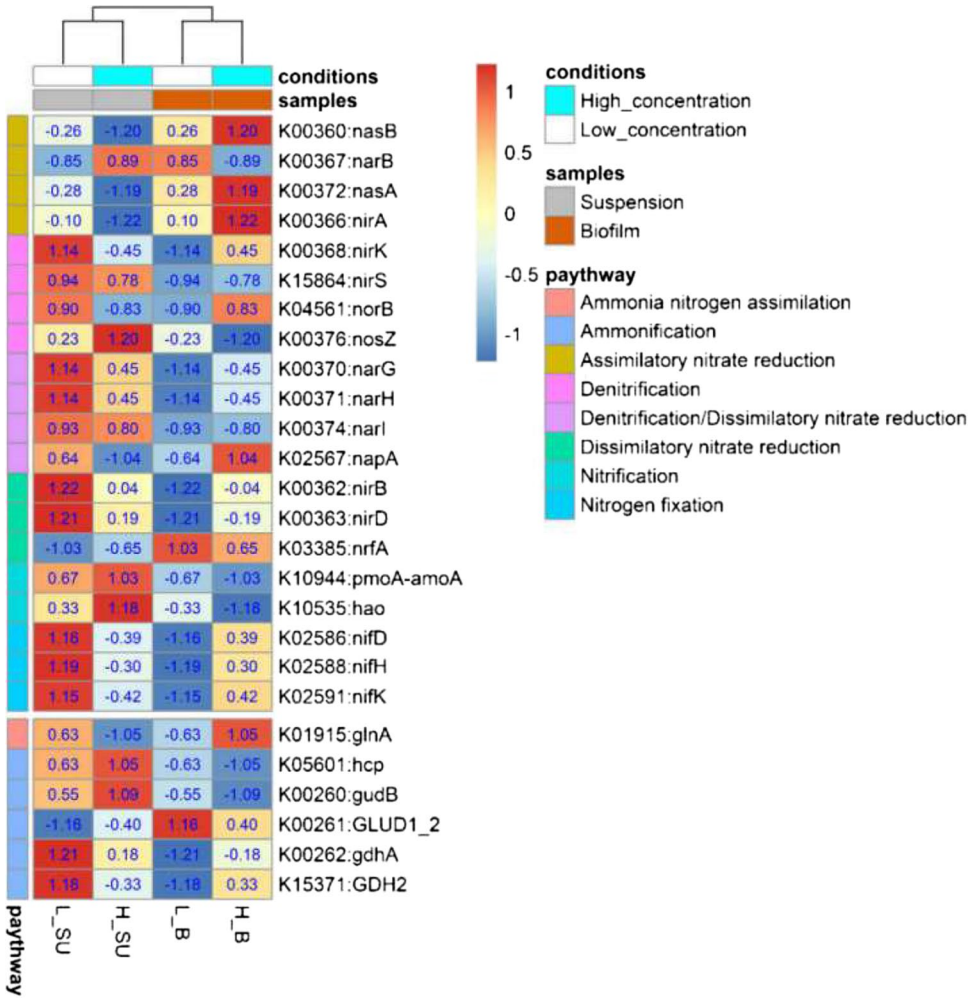

Fig. 6 Heatmap of proportions of the key functional genes in suspension and biofilm samples related to the main nitrogen metabolism pathways

to N assimilation in H_B became dominant. The *glnA* gene is the key gene encoding the enzyme for glutamate synthesis during N uptake via microbial growth and protein synthesis (Matassa et al. 2016; Li et al. 2017a, b). The change in the key genes associated with TN removal was related to the condition of heterogeneous strains, which were less competitive than autotrophic bacteria during low-strength sewage load but tended to increase in abundance with increased nutrient content to impel ammonia assimilation and assimilatory nitrate reduction in this study (Qiu et al. 2020). The biofilm carrier provided the habitat for heterogeneous strains and then enhanced the assimilatory nitrate reduction pathway for protein synthesis during high-strength sewage load. Moreover, with the presence of *Limnodrilus* only during high-strength sewage load (Supplementary Information Fig. S2), predation would be the subsequent pathway after the assimilatory nitrate reduction in this work. But it needs more data to support this postulation.

Conclusion

This study investigated the microbial community and the N removal pathway in the suspended aggregates and biofilm carrier of a novel SBR configuration, during treatment of low- and high-strength sewage loads. The value of weighted NSTI for L_SU, L_B, H_SU, and H_B were less than 0.35, indicating PICRUSt2 in well predicting a high correlation to the metagenome sequencing data on our microbial samples. The reliable TN removal attributed to the introduction of semi-fixed biofilm carrier, the change on the mode of influent and effluent to free up extra reaction time for denitrification, and the sludge elevation mixing during anaerobic and anoxia stage. The suspended aggregates and biofilm carrier played different roles in N pathway. During low-strength sewage loads, the suspended aggregates were responsible for denitrification, while the biofilm took charge of ammonium oxidation. With the augment on sewage strength loads, the role of nitrate reductase from suspended aggregates faded, and anammox and N assimilation by biofilm became dominant. The TN removal during low-strength sewage loads was mainly by nitrification–denitrification, while the formation of L-glutamine via assimilation was prevalent during high-strength sewage loads. These analyses classified the microorganisms that thrive in the different compartments of this novel SBR and highlighted possible relationships between microbial community structure during TN removal.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-19382-w.

Acknowledgements The authors would like to acknowledge the financial support for this work provided by the National Natural Science Foundation of China (Grants No.51778164 and Grants No.51608140) and the Basic and Applied Basic Research Program of Guangdong, China (Grants No. 2021A1515010499).

Author contribution Peng-fei Chen: investigation, writing, reviewing, and editing.

Rui-jian Zhang: conceptualization, methodology, software, supervision.

Zhi-li Du and Guang-hua Wang: data analysis, validation, reviewing, and editing.

Hao-tao Dong, Bin Cui, and Ru-pei Fan: visualization, investigation.
Lu-xin Li and Qian-bin Wang: writing—original draft preparation.
Ying-shi Liu and Zhi-min Sun: Software, data analysis.

Funding Major Research Plan,51778164,Zhi-min Sun,Young Scientists Fund,51608140,Rui-jian Zhang,Basic and Applied Basic Research Program of Guangdong,2021A1515010499,Rui-jian Zhang

Data availability All data generated or analyzed during this study are included in this manuscript and its supplementary information file.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication The participant has consented to the submission of the case report to the journal.

Competing interests The authors declare no competing interests.

References

Chen S, Sun DZ, Chung JS (2007) Anaerobic treatment of highly concentrated aniline wastewater using packed-bed biofilm reactor. Process Biochem 42(12):1666–1670

Chen YS, Zhao Z, Peng YK, Li J, Xiao L, Yang LY (2016) Performance of a full-scale modified anaerobic/anoxic/oxic process: High-throughput sequence analysis of its microbial structures and their community functions. Biores Technol 220:225–232

Chen ZG, Wang XJ, Chen XZ, Chen J, Feng XH, Peng XX (2018) Nitrogen removal via nitritation pathway for low-strength ammonium wastewater by adsorption, biological desorption and denitrification. Biores Technol 267:541–549

Cui B, Yang Q, Liu XH, Huang ST, Yang YB, Liu ZB (2020) The effect of dissolved oxygen concentration on long-term stability of partial nitrification process. J Environ Sci 90:343–351

Cui YX, Biswal BK, van Loosdrecht MCM, Chen GH, Wu D (2019) Long term performance and dynamics of microbial biofilm communities performing sulfur-oxidizing autotrophic denitrification in a moving-bed biofilm reactor. Water Res 166:115038

De Sotto R, Ho J, Lee W, Bae S (2018) Discriminating activated sludge flocs from biofilm microbial communities in a novel pilot-scale reciprocation MBR using high-throughput 16S rRNA gene sequencing. J Environ Manage 217:268–277

Douglas GM, Maffei VJ, Zaneveld J, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MG (2019) PICRUSt2: an improved and extensible approach for metagenome inference. BioRxiv 672295

Eichorst SA, Trojan D, Roux S, Herbold CW, Rattei T, Woebken D (2018) Genomic insights into the Acidobacteria reveal strategies for their success in terrestrial environments. Environ Microbiol 20(3):1041–1063

Feng Q, Song YC, Bae BU (2016) Influence of applied voltage on the performance of bioelectrochemical anaerobic digestion of sewage sludge and planktonic microbial communities at ambient temperature. Biores Technol 220:500–508

Gao YJ, Kong X, Zhou AJ, Yue XP, Luo YH, Defemur Z (2020) Enhanced degradation of quinoline by coupling microbial electrolysis cell with anaerobic digestion simultaneous. Bioresour Technol 306:123077

Gu YF, Wei Y, Xiang QJ, Zhao K, Yu XM, Zhang XP, Li CN, Chen Q, Xiao H, Zhang XH (2019) C: N ratio shaped both taxonomic and functional structure of microbial communities in livestock and poultry breeding wastewater treatment reactor. Sci Total Environ 651(1):625–633

Guo JH, Peng YZ, Wang SY, Zheng YN, Huang HJ, Wang ZW (2009) Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. Biores Technol 100(11):2796–2802

Guo Q, Hu HY, Shi ZJ, Yang CC, Li P, Huang M, Ni WM, Shi ML, Jin RC (2016) Towards simultaneously removing nitrogen and sulfur by a novel process: Anammox and autotrophic desulfurization-denitrification (AADD). Chem Eng J 297(297):207–216

Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:457–462

Lage OM,van Niftrik L, Jogler C, Devos DP (2019) Planctomycetes, encyclopedia of microbiology (Fourth Edition). Academic Press, pp 614–626

Jiang JS, Wang Y, Liu J, Yang XL, Ren YQ, Miao HH, Pan YW, Lv JH, Yan GX, Ding LJ, Li YB (2019) Exploring the mechanisms of organic matter degradation and methane emission during sewage sludge composting with added vesuvianite: insights into the prediction of microbial metabolic function and enzymatic activity. Bioresour Technol 286:121397

Li W, Zhuang JL, Zhou YY, Meng FG, Kang D, Zheng P, Shapleigh JP, Liu YD (2020) Metagenomics reveals microbial community differences lead to differential nitrate production in anammox reactors with differing nitrogen loading rates. Water Res 169:115279

Li YT, Wang Y, Fu L, Gao YZ, Zhao HX, Zhou WZ (2017a) Aerobic-heterotrophic nitrogen removal through nitrate reduction and ammonium assimilation by marine bacterium Vibrio sp Y1–5. Biores Technol 230:103–111

Li X, Sun S, Yuan H, Badgley DB, He Z (2017b) Mainstream upflow nitritation-anammox system with hybrid anaerobic pretreatment: Long-term performance and microbial community dynamics. Water Res 125:298–308

Liu CC, Yu DS, Wang YY, Chen GH, Tang P, Huang S (2020) A novel control strategy for the partial nitrification and anammox process (PN/A) of immobilized particles: Using salinity as a factor. Bioresour Technol 302:122864

Li H, Chi ZF, Li JL, Wu HT, Yan BX (2019) Bacterial community structure and function in soils from tidal freshwater wetlands in a Chinese delta: potential impacts of salinity and nutrient. Sci Total Environ 696:134029

Liu ZP, Wang BJ, Liu YH, Liu SJ (2005) Novosphingobium taihuense sp. nov., a novel aromatic-compound-degrading bacterium isolated from Taihu Lake, China. Int J Syst Evol Microbiol 55(3):1229–1232

Makowska M, Maciejewska E (2016) Effect of aeration time on the operation of SBR and SBBR reactor. Acta Scientiarum Polonorum Formatio Circumiectus 15(2):105–116

Maslon A, Tomaszek JA, Zamorska J, Zdeb M, Piech A, Opalinski I, Jurczyk Ł (2019) The impact of powdered keramsite on activated sludge and wastewater treatment in a sequencing batch reactor. J Environ Manage 237:305–312

Matassa S, Verstraete W, Pikaar I, Boon N (2016) Autotrophic nitrogen assimilation and carbon capture for microbial protein production by a novel enrichment of hydrogen-oxidizing bacteria. Water Res 101:137–146

McIlroy SJ, Albertsen M, Andresen EK, Saunders AM, Kristiansen R, StokholmBjerregaard M, Nielsen KL, Nielsen PH (2014) 'Candidatus Competibacter'-lineage genomes retrieved from metagenomes reveal functional metabolic diversity. ISME J 8(3):613–624

Meng YB, Zhou ZB, Meng FG (2019) Impacts of diel temperature variations on nitrogen removal and metacommunity of anammox biofilm reactors. Water Res 160:1–9

Miao LZ, Wang PF, Hou J, Yao Y, Liu ZL, Liu SQ, Li TF (2019) Distinct community structure and microbial functions of biofilms colonizing microplastics. Sci Total Environ 650(2):2395–2402

Mihelcic JR, Zimmerman JB (2014) Environmental engineering: fundamentals, sustainability, design, 2nd edn. Wiley Global Education, Hoboken, New jersey

Mulder A, van de Graaf AA, Robertson LA, Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol 16(3):177–183

Narihiro T, Terada T, Ohashi A, Kamagata Y, Nakamura K, Sekiguchi Y (2012) Quantitative detection of previously characterized syntrophic bacteria in anaerobic wastewater treatment systems by sequence-specific rRNA cleavage method. Water Res 46(7):0–2175

Nguyen LN, Commault AS, Johir AH, Bustamante H, Aurisch R, Lowrie R, Nghiem LD (2019) Application of a novel molecular technique to characterise the effect of settling on microbial community composition of activated sludge. J Environ Manag 251:109594

Onnis-Hayden A, Majed N, Schramm A, Gu AZ (2011) Process optimization by decoupled control of key microbial populations: distribution of activity and abundance of polyphosphate-accumulating organisms and nitrifying populations in a full-scale IFAS-EBPR plant. Water Res 45(13):0–3854

Qiu YY, Zhang L, Mu XT, Li GB, Guan XQ, Hong JY, Jiang F (2020) Overlooked pathways of denitrification in a sulfur-based denitrification system with organic supplementation. Water Res 169:115084

Rud I, Kolarevic J, Holan AB, Berget I, Calabrese S, Terjesen BF (2017) Deepsequencing of the bacterial microbiota in commercial-scale recirculating and semiclosed aquaculture systems for Atlantic salmon post-smolt production. Aquacult Eng 78:50–62

Shu DT, He YL, Yue H, Wang QY (2015) Microbial structures and community functions of anaerobic sludge in six full-scale wastewater treatment plants as revealed by 454 high-throughput pyrosequencing. Biores Technol 186:163–172

Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, Taylor MW, Horn M, Daims H, Bartol-Mavel D, Wincker P, Barbe V, Fonknechten N, Vallenet D, Segurens B, Schenowitz-Truong C, Medigue C, Collingro A, Snel B, Dutilh BE, Op den Camp HJM, van der Drift C, Cirpus I, van de Pas-Schoonen KT, Harhangi HR, van Niftrik L, Schmid M, Keltjens J, van de Vossenberg J, Kartal B, Meier H, Frishman D, Huynen MA, Mewes HW, Weissenbach J, Jetten MSM, Wagner M, Le Paslier D (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440(7085):790–794

Sun HW, Shi WY, Cai CJ, Ge SJ, Ma B, Li XQ, Ding J (2020) Responses of microbial structures, functions, metabolic pathways and community interactions to different C/N ratios in aerobic nitrification. Bioresour Technol 123422

Sytek-Szmeichel K, Podedworna J, Zubrowska-Sudol M (2016) Efficiency of wastewater treatment in SBR and IFAS-MBSBBR

systems in specified technological conditions. Water Sci Technol 73(6):1349–1356

Tao C, Hamouda MA (2019) Steady-state modeling and evaluation of partial nitrification-anammox (PNA) for moving bed biofilm reactor and integrated fixed-film activated sludge processes treating municipal wastewater. J Water Process Eng 100854

Thelusmond JR, Strathmann TJ, Cupples AM (2016) The identification of carbamazepine biodegrading phylotypes and phylotypes sensitive to carbamazepine exposure in two soil microbial communities. Sci Total Environ 571:1241–1252

Tian SN, Tian Z, Yang H, Yang M, Zhang Y (2017) Detection of viable bacteria during sludge ozonation by the combination of ATP Assay with PMA-Miseq Sequencing. Water 9(3):166

Tombola R, Buttiglieri G, Auset M, Gonzalez-Olmos R (2019) Recycled corrugated wire hose cover as biological carriers for greywater treatment in a sequential batch biofilm reactor. J Environ Manag 240:475–484

Wang S, Chen MF, Zheng KK, Wan CL, Li J (2020) Promising carbon utilization for nitrogen recovery in low strength wastewater treatment: ammonia nitrogen assimilation, protein production and microbial community structure. Sci Total Environ 710:136306

Xia SQ, Shi Y, Fu YG, Ma XM (2005) DGGE analysis of 16S rDNA of ammonia-oxidizing bacteria in chemical–biological flocculation and chemical coagulation systems. Appl Microbiol Biotechnol 69(1):99–105

Yang XY, Chen Y, Guo FC, Liu XB, Su XX, He Q (2020) Metagenomic analysis of the biotoxicity of titanium dioxide nanoparticles to microbial nitrogen transformation in constructed wetlands. J Hazard Mater 384:121376

Yuan Y, Liu JJ, Ma B, Liu Y, Wang B, Peng YZ (2016) Improving municipal wastewater nitrogen and phosphorous removal by feeding sludge fermentation products to sequencing batch reactor (SBR). Biores Technol 222:326–334

Zhang DC, Su H, Antwi P, Xiao LW, Liu ZW, Li JZ (2019) High-rate partial-nitritation and efficient nitrifying bacteria enrichment/out-selection via pH-DO controls: efficiency, kinetics, and microbial community dynamics. Sci Total Environ 692:741–755

Zhang JH, Zhang L, Miao YY, Sun YW, Zhang Q, Wu L, Peng YZ (2019) Enhancing sewage nitrogen removal via anammox and endogenous denitrification: Significance of anaerobic/oxic/anoxic operation mode. Bioresour Technol 289:121665

Zhang K, Yang B, Ma YG, Lyu LT, Pan Y, Wang YZ, Li H, Zhu T (2018) A novel anammox process combined with vibration technology. Bioresour Technol 256:277–284

Zhang L, Shen Z, Fang WK, Gao G (2019) Composition of bacterial communities in municipal wastewater treatment plant. Sci Total Environ 689:1181–1191

Zhang ZW, Xu CY, Zhong D, Han YX, Han HJ, Zheng MQ, Zhu H (2019) Enhanced nitrogen removal of coal pyrolysis wastewater with low COD to nitrogen ratio by partial nitrification-denitrification bioprocess assisted with polycaprolactone. Environ Sci Pollut Res 26:21655–21667

Zhao L, Liu YW, Li N, Fan XY, Li X (2020) Response of bacterial regrowth, abundant and rare bacteria and potential pathogens to secondary chlorination in secondary water supply system. Sci Total Environ 719:137499

Zhong XZ, Li XX, Zeng Y, Wang SP, Sun ZY, Tang YQ (2020) Dynamic change of bacterial community during dairy manure composting process revealed by high-throughput sequencing and advanced bioinformatics tools. Bioresour Technol 306:123091