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

Bin Cui (✉ cuibin@gzhu.edu.cn)

Guangzhou University

Research Article

Keywords: SBR, semi-fixed biofilm carrier, bacterial community, nitrogen removal pathway

Posted Date: April 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-271306/v1>

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Abstract

The sequencing batch reactor (SBR) activated sludge process is a well-established technology for sewage treatment. One of the drawbacks of SBRs, however, is the failure to conform to the standard for total nitrogen (TN) discharge. 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.

1. 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 sewage treatment plants, offering advantages in terms of footprint, investment, 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) ($\text{TN} < 10 \text{ mg L}^{-1}$) (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 *et al.*, 2014; Zhang *et al.*, 2019; Tombola *et al.*, 2019; Tao *et al.*, 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 *et al.*, 2016; Sytek-Szmeichel *et al.*, 2016). To obtain 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; 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, software such as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) and Functional Annotation of Prokaryotic Taxa (FAPROTAX) has been successfully used in predicting metagenome functional contents (Langille *et al.*, 2013; Louca *et al.*, 2016). In particular, PICRUSt is widely used and is appropriate for predicting function in various environmental samples, such as sewage treatment systems (Cui *et al.*, 2019; Zhang *et al.*, 2019; Zhao *et al.*, 2020), sewage sludge and manure composting processes (Jiang *et al.*, 2019; Zhong *et al.*, 2020), surface water and sediment samples (Wang *et al.*, 2018; Su *et al.*, 2018). 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, it is necessary to explore the functional profile by means of PICRUSt.

In this study, a high-throughput sequencing technology was used to investigate the bacterial community from suspended aggregates and semi-fixed biofilm carrier during low- and high-strength sewage treatment. Multivariate and bioinformatic analysis methods, such as principal component analysis (PCA) and PICRUSt2 (the latest version) (Douglas *et al.*, 2019), were applied to explore the biomarkers of N removal in the bacterial community, to screen the possible functional shifts of the bacterial community driven by changes in sewage load strength, and to understand the interactions among microbial species. This basic information 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.

2. Materials And Methods

2.1 SBR pilot plant operation and water 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 m³ d⁻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 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. 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), aeration (~120 min with air flow ratio of 1000–3000 L h⁻¹), second stage return of activated sludge elevation mixing under anoxic conditions (~210 min), 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 feed water applied in this work is described in Supplementary Information Table S1.

The feeding 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).

2.2 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.

2.3 DNA extraction and bacterial community analysis

The protocols for DNA extraction, high-throughput 16S rRNA gene pyrosequencing and biodiversity analysis are presented in Supplementary Information Text S2.

2.4 Data analysis

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

3. Results And Discussion

3.1 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) when compared with other pilot scale SBRs (Chen *et al.*, 2015) 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 water 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, 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 ($\sim 330 \text{ min}$), 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 (30min $\sim$ 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. 40min $\sim$ 100 min for anoxic denitrification. However, the poor TP removal from July to September (some plots $> 0.5 \text{ mg L}^{-1}$ in Supplementary Information Figure. S8) was because of the absence of dosing poly aluminum chloride.

3.2 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 Figure. 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 Figure. S11). PCA revealed the difference in community composition between 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 were 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. Fig. 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. 4 a) 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. 4 a). 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.*, 2019)) 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. 4 b). 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. 4 a), while strains with unknown function became dominant in H_B, such as *Chitinophagales*, *AKYH767*, *SBR1031*, *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 N₂ 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, ample nitrite at anaerobic stage during high-strength load (Supplementary Information Fig. S9) would provide favorable substrate for anammox.

3.3 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.*, 2015), the N metabolism pathways were mapped as Supplementary Information Figure. S12, and then subdivided as the core (Supplementary Information Figure. S13) and the subordinate (Supplementary Information Figure. S14) pathways. Referring to Kanehisa *et al.* (2015), 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 section 3.2). These results were similar to those of Qiu *et al.* (2019), 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.*, 2018; 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 section 3.2) 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 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.* (2019) 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 Figure. 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 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.*, 2017). 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 need more data to support this postulation.

4. 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 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 was 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.

Declarations

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).

Ethics approval

Not applicable.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Consent to Participate

Not applicable

Consent to Publish

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

Authors Contributions

This manuscript was carried out by Bin Cui, including investigation, writing, methodology, data analyse, etc.

Competing Interests

The author do not have any possible conflicts of interest in this manuscript.

Availability of data and materials statement

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

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Figures

Figure 1

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

Figure 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).

Figure 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).

Figure 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).

Figure 5

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

Figure 6

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

Supplementary Files

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