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ORIGINAL PAPER

Effect on simultaneous removal of ammonia, nitrate, and phosphorus via advanced stacked assembly biological filter for rural domestic sewage treatment

Ziyun Fan · Zhiwei Liang · Ancheng Luo · Yunlong Wang · Yuanyuan Ma · Yi Zhao · Xiansheng Lou · Ruijie Jia · Yan Zhang · Shaowei Ping

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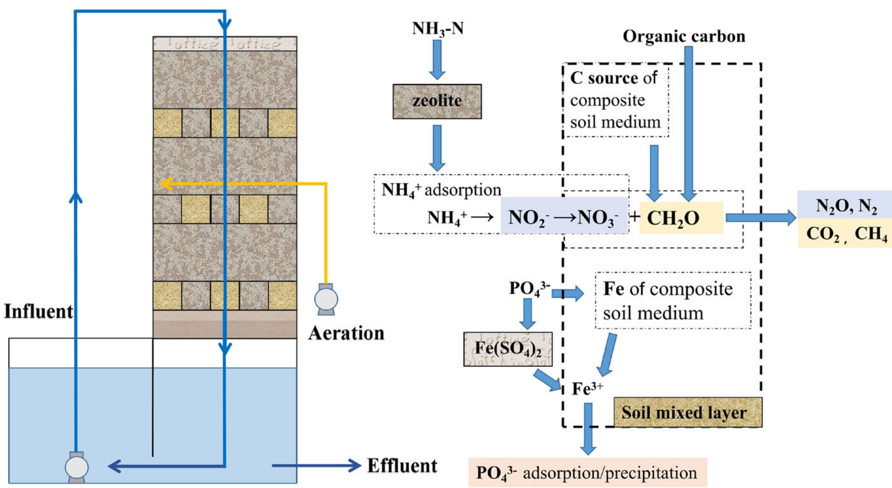
Abstract The discharge of ammonia–nitrogen (NH_3–N), total nitrogen (TN), chemical oxygen demand (COD), and total phosphorus (TP) in rural sewage usually exceeds the Pollutant Discharge Standard for Urban Sewage Treatment Plants (GB18918-2002). Efficient and cost-effective removal of these pollutants cannot be simultaneously realized using conventional rural sewage treatment methods. Thus, an assembled biological filter (D50 × W50 × H113 cm), including a phosphorus removal layer filled with solid polymeric ferric sulfate and alternating aerobic-anaerobic layers, is proposed herein. The aerobic (anerobic) layers were filled with zeolite (zeolite and composite soil) at different intervals. This system was used for the treatment of synthetic sewage having COD: 122.0–227.0 mg/L; NH_3–N: 29.1–47.0 mg/L; TN: 28.0–58.0 mg/L; and TP: 2.0–3.8 mg/L. Based on optimal operation conditions (40 L/h reflow rate, without artificial aeration, and 12-h operation cycle), the system showed NH_3–N, TN, COD, and TP removal efficiencies of 87.1 ± 8.1, 83.4 ± 7.9, 91.0 ± 9.4, and 80.0 ± 6.4%, respectively. Further, in the pilot-scale test, under the same optimal parameters, the removal efficiencies of NH_3–N, TN, COD, and TP were 78.9 ± 8.1, 75.4 ± 7.9, 82 ± 9.4, and 76 ± 6.4%, respectively. Furthermore, in the different functional units of the system, a large number of functional bacteria capable of efficiently facilitating the simultaneous removal of the different pollutants from sewage were identified. Therefore, this proposed system, which complies with current environmental discharge regulations, can be a more sustainable approach for the treatment of unattended rural sewage.

Z. Fan · Z. Liang (✉) · A. Luo · Y. Ma · Y. Zhao · X. Lou · R. Jia · Y. Zhang · S. Ping
College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China
e-mail: zhiweiliangzg@zju.edu.cn

Y. Wang
Environmental Resources and Soil Fertilizer Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China

Graphic abstract



Keywords Assembled biological filter · Nitrogen removal · Phosphorus removal · Rural domestic sewage · Circulating sewage treatment

Introduction

The low efficiency of sewage treatment equipment (Cheng et al. 2020) causes increasingly significant rural water pollution owing to the economic and geographical factors that are characteristic of the Chinese society (Gu et al. 2016). Specifically, rural domestic sewage is characterized by scattered sources (Li et al. 2020), small nighttime discharge (Mahmoud and Lier 2011), and large changes in influent loading rates, as well as the risk of pollution accidents (Igbinosa and Okoh 2009), such as eutrophication, which contribute to an increase in oxygen demand as well as toxin and nutrient loading in water bodies (Igbinosa and Okoh 2009).

The most conventional rural sewage treatment methods consist of an operational sequence of aerobic/anoxic/aerobic processes. For example, the Anaerobic-Anoxic-Oxic (A^2O) is one of the most typical schemes for the treatment of domestic sewage in rural regions in China. However, some researchers have observed that the simultaneous and efficient removal of NH_3-N, TN, COD, and TP is not easy to realize (Gonzalez-Tineo et al. 2020). The presence of COD may restrict the anaerobic removal of ammonium (Yuan et al. 2014). Further, the nitrate present in the returned sludge adversely affects the performance of aerobic microorganisms with respect to the uptake of phosphorus. Furthermore, incomplete denitrification in the anoxic zone can be attributed to the limited number of carbon sources. It has also been reported that long hydraulic retention times, high operating costs, and the requirement for large areas of land are factors that cannot be ignored (Chan et al. 2012). Therefore, it is necessary to explore new technologies for the simultaneous and efficient removal of COD, nitrogen, and phosphorus.

The main functional structure of such an efficient integrated system should consist of a phosphorus removal functional zone as well as aerobic and anaerobic functional zones. The principle of phosphorus removal includes biological, chemical, and physical mechanisms (Eveborn et al. 2012), and in traditional biological treatment (Cieslik and Konieczka 2017), the presence of phosphorus accumulating organisms (PAOs) (Ma et al. 2016) may adversely affect the efficiency of carbon and nitrogen removal. Reportedly, chemical adsorption and physical sedimentation are better pathways for TP removal (Pan et al. 2016); they involve mechanisms such as the precipitation of aluminum, iron, and calcium phosphate (Eveborn et al. 2012). Zeolite has a porous structure that enables it to absorb organic substances. It also has a high affinity for NH_4^+ (Kuronen et al.

2000). Therefore, it can act as a substrate for the degradation and removal of organic substances and NH_4^+ by microorganisms (Luanmanee et al. 2001). It has also been reported that adding carbon powder, gravel, and quartz to soil can improve its hydraulic load and reduce system blockage (Su et al. 2020). Additionally, if a slow-release carbon source is added to soil, it can play a key role in enhancing denitrification efficiency.

The goal for this study was to establish an advanced stacked biological filter assembly for wastewater treatment that is characterized by easy assembly as well as efficient nitrogen and phosphorus removal. Specifically, the objectives of this study were to (1) verify the effectiveness of the designed bioreactor, (2) explore the optimal range of operating conditions, such as reflow rate, aeration mode, and cycle time, and (3) analyze the microbial community structure as well as the roles of the dominant species of the corresponding functional units.

Materials and methods

Experimental system

The modified system that was used to realize the sewage treatment in this study consisted of alternating aerobic or anaerobic zones, which significantly improve the efficiency of the removal of pollutants from water bodies (Su et al. 2020). Therefore, exploring the efficiency of such a system so as to synchronize the removal of NH_3–N, TN, COD, and TP is of significance.

The experiment was performed at Zhejiang University, Hangzhou City, China, using a laboratory-scale bioreactor that was established for the purpose of this study. The treatment system consisted of a stainless-steel box with dimensions D50 × W50 × H113 cm. The box also included a water distribution layer for the distribution of sewage and the supply of oxygen via air (Liu et al. 2018), a 5 cm phosphorus removal layer, three groups of alternating 20 cm aerobic and 10 cm anaerobic layers to prevent clogging and improve sewage infiltration (Latrach et al. 2018), and a 10 cm catchment layer. The effective volume of the tank was 200 L, which represents the volume of sewage that can be treated in the tank during a single operation. The water distribution layer was empty, and consisted of four perforated pipes with spray heads for uniform sewage distribution. The phosphorus removal layer, which was filled with solid polymer ferric sulfate, was located at the top of the tank (Di Capua et al. 2020). Coupled with the iron filings that were added to the soil, the iron hydroxide formed owing to its oxidation also had an adsorption effect on phosphorus; thus, the reactor could realize TP removal. According to our previous experimental results, three groups of alternating aerobic and anaerobic modules were adopted in the system for the efficient and cost-effective removal of pollutants. For the aerobic layers, the filler was zeolite (diameter = 3–5 mm), and an aeration pipe (1.5 cm in diameter) that was connected to a blower (dimensions, D350 × W250 × H250 mm; power, 90 W) was installed in the second aerobic layer. For the anaerobic layer, which was divided into 5 × 5 = 25 grids, zeolite particles with diameters in the range 3–5 mm and a composite soil medium were placed in the grids at regular intervals (Guo et al. 2019). The soil mixture layers consisted of 70.0% soil (humus-rich volcanic ash soil) (Sato et al. 2005), 10.5% wood dust, 8.5% iron filings, 5% bamboo charcoal, and 6.0% anaerobic microbial agents. The catchment layer was also filled with zeolite. Additionally, there were 5-mm diameter water distribution holes that were evenly distributed between each packing layer. The holes were used for sewage flow, and rubber seals were used at the edges to prevent leakage. The above structure ensured that the sewage inside the biological filter was in full contact with the functional filler.

The sewage was lifted from the tank of the bioreactor to the water distribution branch pipe using a submersible pump (dimensions, D191 × W119 × H129 mm; power, 150 W) and then sprayed uniformly on the water distribution layer. The initially treated sewage returned to the tank after treatment in the phosphorus removal layer and in the three groups of alternating aerobic and anaerobic layers in sequence. Finally, the sewage was discharged from the treatment system after the circulation treatment. Dissolved oxygen concentrations in the aerobic and anaerobic zones were maintained above 4.0 mg/L and below 0.1 mg/L, respectively.

Experimental operation

The synthetic sewage used in this study was prepared such that it contained sucrose, glucose, NH_4Cl, KH_2PO_4, $MgCl_2$, $CaCl_2$, $MgSO_4$, K_2SO_4, and $Fe_2(SO_4)_3$ in proportions as shown in Table 1. Additionally, as shown in Table 2, the synthetic sewage also consisted of COD, NH_3–N, TN, and TP with concentrations in the ranges 122.0–227.0, 29.1–47.0, 28.0–58.0, and 2.0–3.8 mg/L, respectively. There was no need to inoculate the sludge with special microorganisms, and in this study, the natural start-up method was adopted during the start-up process of the bioreactor. The system achieved the initial colonization and accumulation of microorganisms in batch mode after three weeks (Li et al. 2020). This resulted in the compact biofilm that was seen on the filler. It was also important to investigate the relationship between the mechanism of pollutant removal and the distribution of the biological community in the bioreactor.

Effect of different reflow rates on the operation of the system

In the bioreactor, sequential batch circulation was adopted for the treatment of the domestic sewage. The cycle of the biological filter batch treatment process was 24 h without aeration. The number of cycles of sewage treatment in the system were 2.4, 3.6, 4.8, and 6 times at reflow rates of 20, 30, 40, and 50 L/h, respectively, when the single treatment volume was 200 L. Additionally, the surface load of the filling tower layer was 1.92, 2.88, 3.84, and 4.8 $m^3/m^2/d$ at reflow rates of 20, 30, 40, and 50 L/h, respectively (Table 3).

Table 2 Characteristics of the synthetic sewage in the operation

Parameter	Range dimension	Mean ± S.D
NH_3-N (mg/L)	29.1–47.0	37.6 ± 4.3
TN (mg/L)	28.0–58.0	40.1 ± 5.8
COD (mg/L)	122.0–227.0	171.9 ± 23.3
TP (mg/L)	2.0–3.8	2.7 ± 0.4
pH	6.5 –8.9	7.7 ± 1.2

Effect of different aeration methods on the operation of the system

The best reflow rate obtained from the above experiment was adopted for subsequent treatment processes using the bioreactor. Thus, the sewage treatment performance of the system with and without aeration (100 L/min) were further compared (Table 4).

Effect of different cycle times (hydraulic load) on the operation of the system

To determine the effect of different cycle times on the operation of the system, reflow rate and aeration approach obtained from the above two experiments were adopted. In this stacked biological filter assembly, a sequential batch method was adopted for sewage treatment. The sewage capacity of the tank was 200 L, the processing time of a single batch of sewage was 24 h, and sampling was performed at 10:40, 10:50, 11:00, 11:10, 11:20, 11:30, 11:50, 12:50, 13:50, 14:50, 15:50, 16:50, 17:50, 18:50, 20:20, and 21:50, and at 12:50 and 18:50 on the next day. This experiment was conducted for 30 d, and the average optimal cycle time was obtained (Table 5).

Table 1 Translations of simulated sewage

Ingredients	Concentration (mg/L)	Ingredients	Concentration (mg/L)
Glucose	100–300	$CaCl_2$	0.8
NH_4Cl	30–50	K_2SO_4	0.05
KH_2PO_4	2–6	$Fe_2(SO_4)_3$	0.05
$MgCl_2$	1	$MgSO_4$	0.03

Table 3 The removal rate of pollutant at different reflow rate

phase(d)	reflow rate(L/h)	NH_3–N removal rate (%)	TN removal rate (%)	COD removal rate (%)	TP removal rate (%)
1–36	20	10.5 ± 3.3[c]	9.4 ± 3.5[c]	57.9 ± 5.3[c]	82.0 ± 1.6[a]
37–72	30	73.6 ± 9.4[b]	63 ± 8.9[b]	66.3 ± 8.4[b]	83.01 ± 3.6[b]
72–107	40	88.0 ± 4.5[a]	86.5 ± 5.0[a]	86.3 ± 8.6[a]	82.6 ± 3.2[a]
108–143	50	91.1 ± 4.7[a]	84.5 ± 5.0[a]	85.2 ± 4.7[a]	81.9 ± 3.0[a]

Values are means of triplicates ± Standard deviations (SD); Means with the same letter are not significantly different ($p > 0.005$)

Table 4 Pollutant removal rates under different aeration methods

Phase (d)	Aeration method	NH_3N removal rate (%)	TN removal rate (%)	COD removal rate (%)	TP removal rate (%)
1–35	Natural ventilation	88.0 ± 4.5[a]	86.5 ± 5.0[a]	86.3 ± 8.6[a]	82.6 ± 3.2[a]
36–70	Artificial aeration	90 ± 5.7[a]	65 ± 7.5[b]	92 ± 7.1[a]	82.1 ± 7.5[a]

Values are means of triplicates ± Standard deviations (SD); Means with the same letter are not significantly different ($p > 0.005$)

Effect of system on pollutant removal from rural domestic sewage

The lab-scale and pilot-scale tests were both set up at an outdoor test site, with an acrylic top layer to isolate rainwater, to prevent its mixing with the sewage. The environment around the system was ventilated. The operational temperature was in the range 8–29 °C, and the temperature difference between day and night was $5 \sim 10$ °C (Table 6).

The pilot-scale test was used for the treatment of rural domestic sewage in Maojiawan Village, Changxing County, Huzhou, Zhejiang Province. The system consisted of 304 stainless steel tanks with the dimensions D1.5 × W2.0 × H1.4 m. The structure and water inlet method adopted in this pilot-scale test were both consistent with the previous laboratory design conditions. As shown in Table 6, the concentrations of NH_3–N, TN, COD, and TP in the rural sewage were in the ranges of 37.0–92.0, 40.0–96.0, 48.0–143.0, and 1.23–4.32 mg/L, respectively. Additionally, during the pilot-scale test, the sewage flow rate was determined taking into account the population of the associated area. The composition of the influent and effluent were further monitored during treatment.

Table 5 Boltzmann curve fitting parameter table

Parameters	Removal rate (%)	Stable time (h)	R^2
NH_3–N	87.1 ± 8.1	8	0.9604
TN	83.4 ± 7.9	8	0.9638
COD	91 ± 9.4	12	0.9823
TP	80 ± 6.4	12	0.9718

Table 6 Concentrations of different pollutants in rural sewage influent concentration

Parameters	Range dimension	Mean ± S.D
NH_3–N	37.0–92.0	64 ± 4.3
TN	40.0–96.0	68 ± 4.9
COD	48.0–143.0	100.0 ± 8.5
TP	1.23–4.32	2.79 ± 1.5
pH	6.60–8.52	7.21 ± 2.5

Sampling and analyses

Sewage sample collection and analysis

The bioreactor was run in the sequential batch mode, that is, the circulating pump began to run cyclically after the tank was filled. Sewage samples were collected at 3-day intervals from the influent and effluent of the bioreactor and stored in a refrigerator at 4 °C. The NH_3–N, TN, COD, and TP contents of the influents and effluents were determined using a HACH prefabricated reagent and the corresponding spectrophotometer (DR2800, HACH, USA).

Filler collection and analysis

The different types of microorganisms present in the fillers play different roles in the pollutant removal process. The main phytobacteria involved were detected using DNA sequencing technology, and sequence analyses were performed using Uparse software. Sequences with similarity > = 97% were assigned to the same operational taxonomic unit (OTU). The representative sequence of each OTU was screened for further annotations. Further, for each representative sequence, the Silva Database (Quast et al. 2013) was used to annotate the taxonomic information based on the Mothur algorithm. Furthermore, the differences in the microorganisms within the same type of filler in the biological filter at different heights were analyzed to determine the phylogenetic relationships of the different OTUs. Differences in the dominant species in different samples (groups) as well as multiple sequence alignments were determined using the MUSCLE software (Edgar 2013).

Results

Performance of system under different reflow rates

The removal efficiencies of NH_3–N, TN, COD, and TP under different reflow rates are shown in Fig. 1, which shows that higher reflow rates resulted in better NH_3–N, TN, and COD removal from sewage. At reflow rates of 20, 30, 40, and 50 L/h, the average NH_3–N removal efficiencies were 10.5 ± 3.3, 73.6 ± 9.4, 88.0 ± 4.5, and 91.1 ± 4.7%, respectively (Fig. 1a). TN removal efficiencies were 9.4 ± 3.5, 63 ± 8.9, 86.5 ± 5.0, and 84.5 ± 5.0% on average at reflow rates of 20, 30, 40, and 50 L/h, respectively (Fig. 1b), and average COD removal rates were 57.9 ± 5.3, 66.3 ± 8.4, 86.0 ± 8.6, and 85.2 ± 4.7% at reflow rates of 20, 30, 40, and 50 L/h, respectively (Fig. 1c). The fluctuations in the reflow rates had almost no effect on the removal rate of TP. Under reflow rates of 20, 30, 40, and 50 L/h, respectively, the average TP removal rates of the system were 82.0 ± 1.6, 83.01 ± 3.6, 82.6 ± 3.2, and 81.9 ± 3.0%, respectively (Fig. 1d). The removal efficiencies at reflow rates of 40 and 50 L/h were similar, and from an energy conservation perspective, coupled with the above mentioned conclusions, 40 L/h was considered as the optimal rate.

As shown in Table 7, the reflow rate was positively correlated to NH_3–N, TN, and COD removal efficiencies, and the corresponding correlation coefficients were 0.858 ($p < 0.01$), 0.588 ($p < 0.01$), and 0.611 ($p < 0.01$), respectively. Interestingly, the correlation coefficient between reflow rates and TP removal was 0.035 ($p = 0.886$).

Performance of system under different aeration methods

We studied the performance of the biological filter with respect to the removal of pollutants under two aeration conditions, namely, natural oxygenation and artificial aeration at a reflow rate of 40 L/h. From Fig. 2a and c, it is evident that the average removal rates of NH_3–N and COD were 90 ± 5.7 and 92 ± 7.1%, respectively, under artificial aeration, and 88.0 ± 5.0 and 86.0 ± 8.6, respectively, under natural ventilation. Figure 2b shows that the removal rate of TN was 65.0 ± 7.5% under artificial aeration and 86.5 ± 5.0% under natural ventilation. Regarding TP removal, the removal efficiencies were 82.1 ± 7.5 and 83.01 ± 3.6% under artificial and natural ventilation, respectively. The comprehensive pollutant removal efficiency realized using this bioreactor without aeration (natural ventilation) was better than that realized using conventional methods. In sewage treatment, aerobic processes usually require intensive energy; thus, the high cost of investment, operation, and maintenance cannot be ignored (Bhowmick et al. 2019b). Traditional combined systems such as the MBR system require the maintenance of high energy to meet the aeration requirement of the system during operation (Bhowmick et al. 2019a).

The aeration method was positively correlated with NH_3-N and COD removal efficiencies, with correlation coefficients of 0.501 ($p < 0.05$) and 0.493 ($p < 0.05$), respectively. However, the aeration method was negatively correlated with TN removal efficiency, the correlation coefficient being −0.607 ($p < 0.01$). Further, the correlation coefficient

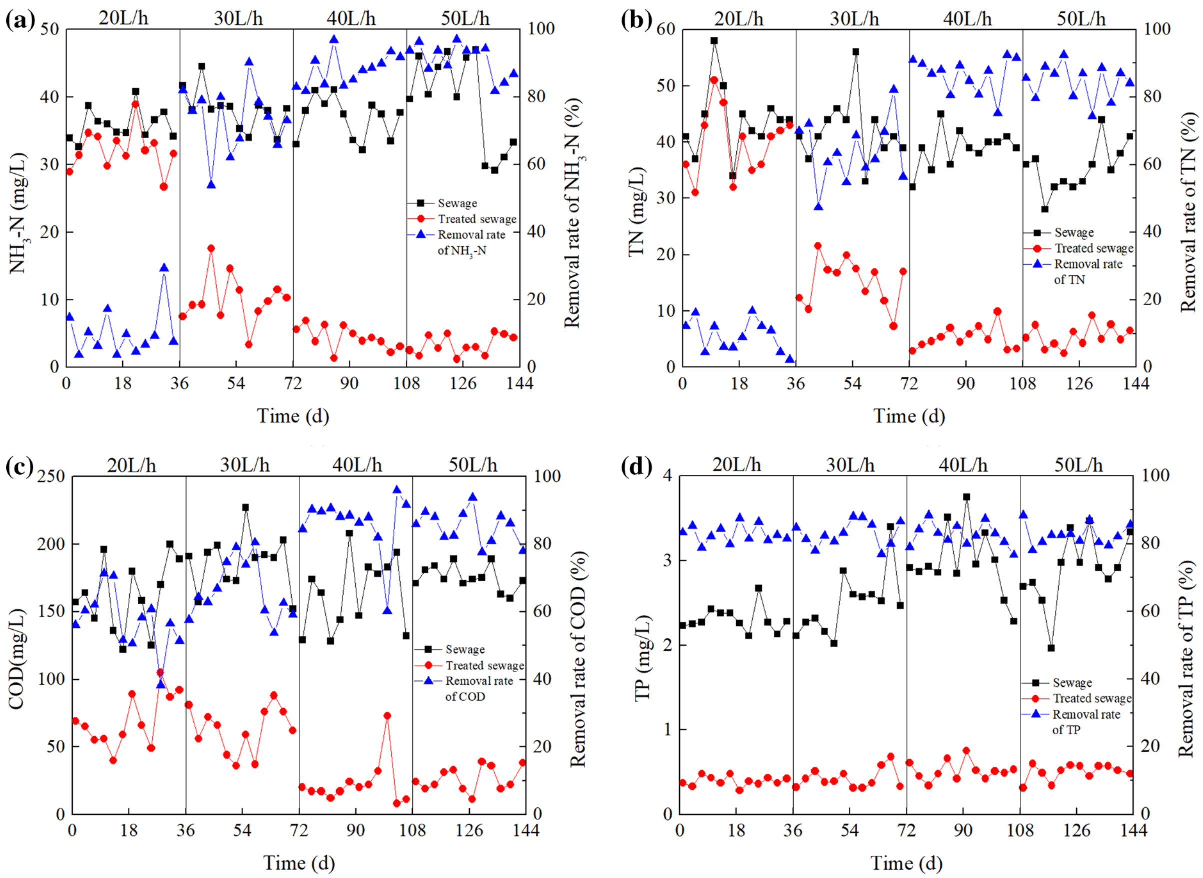


Fig. 1 Concentrations and removal rates of NH_3-N, TN, COD, and TP under different reflow rates

Table 7 Spearman correlation coefficients between environmental conditions and pollutant removal efficiency

Parameters	Reflow rate	Aeration method	Cycle time
NH_3–N	0.858**	0.501*	0.717**
TN	0.588*	−0.607**	0.766**
COD	0.611**	0.493*	0.764**
TP	0.035	0.043	0.645**

*$p < 0.05$ and **$p < 0.01$

between aeration and TP removal efficiency was 0.043 (p = 0.753).

Performance of system under different cycle times (hydraulic load)

A reflow rate of 40 L/h and natural ventilation were adopted for further study. Figure 3a shows that the concentrations of effluent pollutants gradually decreased and then stabilized. Further, the Boltzmann curve was fitted to the curve shown in Fig. 3b and the parameters obtained are as shown in Table 2. The NH_3–N, TN, COD, and TP removal efficiencies of the bioreactor were close to the maximum values after the cycle times of 8, 8, 12, and 12 h, respectively. The sewage contained 32.2 ± 5.1, 44.0 ± 6.4, 234.0 ± 9.8, and 3.9 ± 3.1 mg/L of NH_3–N, TN, COD, and TP, respectively, and after treatment, the stable treated effluent contained 1.8 ± 1.1, 10.0 ± 1.8, 23.0 ± 2.5, and 0.8 ± 0.5 mg/L of NH_3–N, TN, COD, and TP, respectively. Further, their final removal rates during this cycle were 87.1 ± 8.1, 83.4 ± 7.9, 91 ± 9.4, and 80 ± 6.4%, respectively. These results show that the pollutant removal efficiency of the biological filter was optimal after an operation cycle of 12 h.

Additionally, the cycle time was positively correlated with NH_3-N, TN, COD, and TP removal

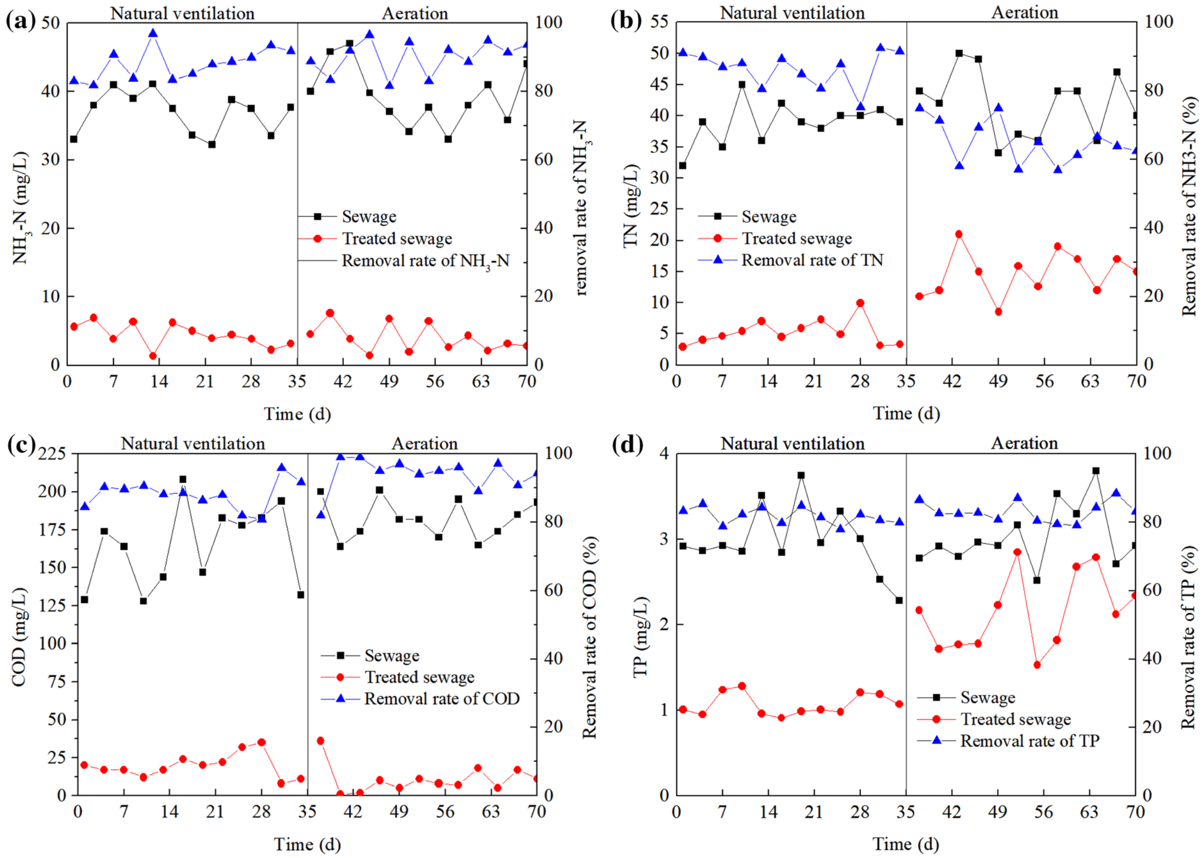


Fig. 2 Concentrations and removal rates of NH_3-N, TN, COD, and TP under different aeration methods

efficiencies, and the corresponding correlation coefficients were 0.717 ($p < 0.01$), 0.766 ($p < 0.01$), 0.764 ($p < 0.01$), and 0.645 ($p < 0.01$), respectively.

Performance of system in rural domestic sewage treatment

Under the optimal operation conditions, namely, natural oxygenation, a reflow rate of 40 L/h, a cycle time of 12 h, the pilot-scale test has run for 100 days, and the average NH_3–N, TN, COD, and TP removal rates were 78.9 $\pm$ 8.1, 75.4 $\pm$ 7.9, 82 $\pm$ 9.4, and 76 $\pm$ 6.4%, respectively.

Analysis of the microbial diversity in the biological filter during operation

The structure of the advanced stacked biological filter assembly is shown in Fig. 4; in which fs1 and tr1 represent untreated zeolite and soil samples, respectively. The zeolite samples fs2, fs3, and fs4 were collected from aerobic layers 1, 2, and 3, respectively. Zeolite sample fs5 was collected from the catchment layer, and the zeolite samples fs6, fs7, and fs8 were collected from anaerobic layers 1, 2, and 3, respectively. Soil samples tr2, tr3, and tr4 were collected from anaerobic layers 1, 2, and 3, respectively.

The zeolite and soil could both serve as a filter and as a habitat for microorganisms. Zeolite, clay particles, and humic substances all function as ion exchanger substances inside the soil. It is apparent that they all showed the capability of mechanically filtering and adsorbing the pollutant. Additionally, in all the fillers in the biological filter, a total of 45 phyla and 204 genera were detected using the DNA sequencing technology.

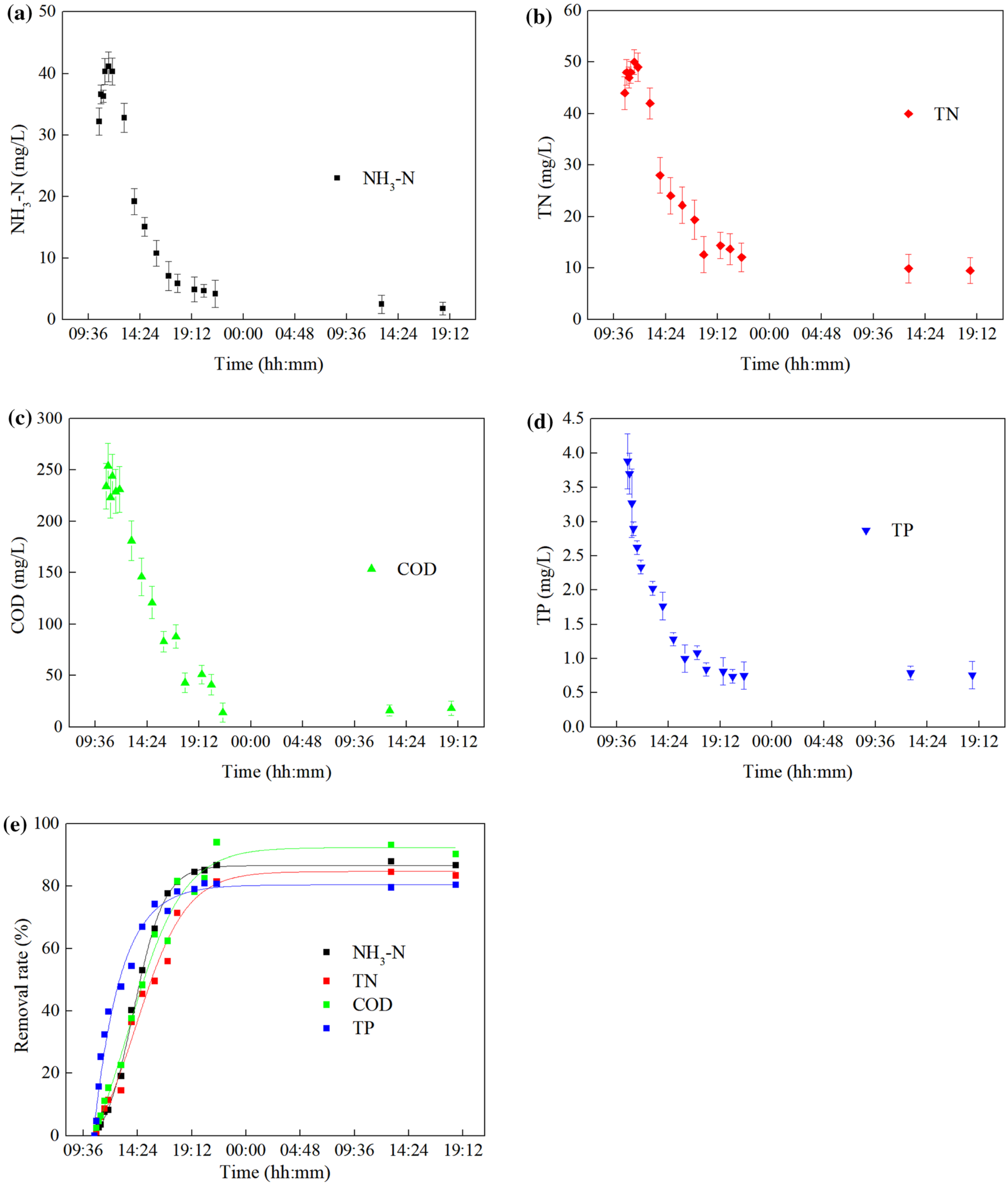


Fig. 3 Concentrations and removal rates of NH_3-N, TN, COD, and TP at different cycle times. The error bars represent standard deviation

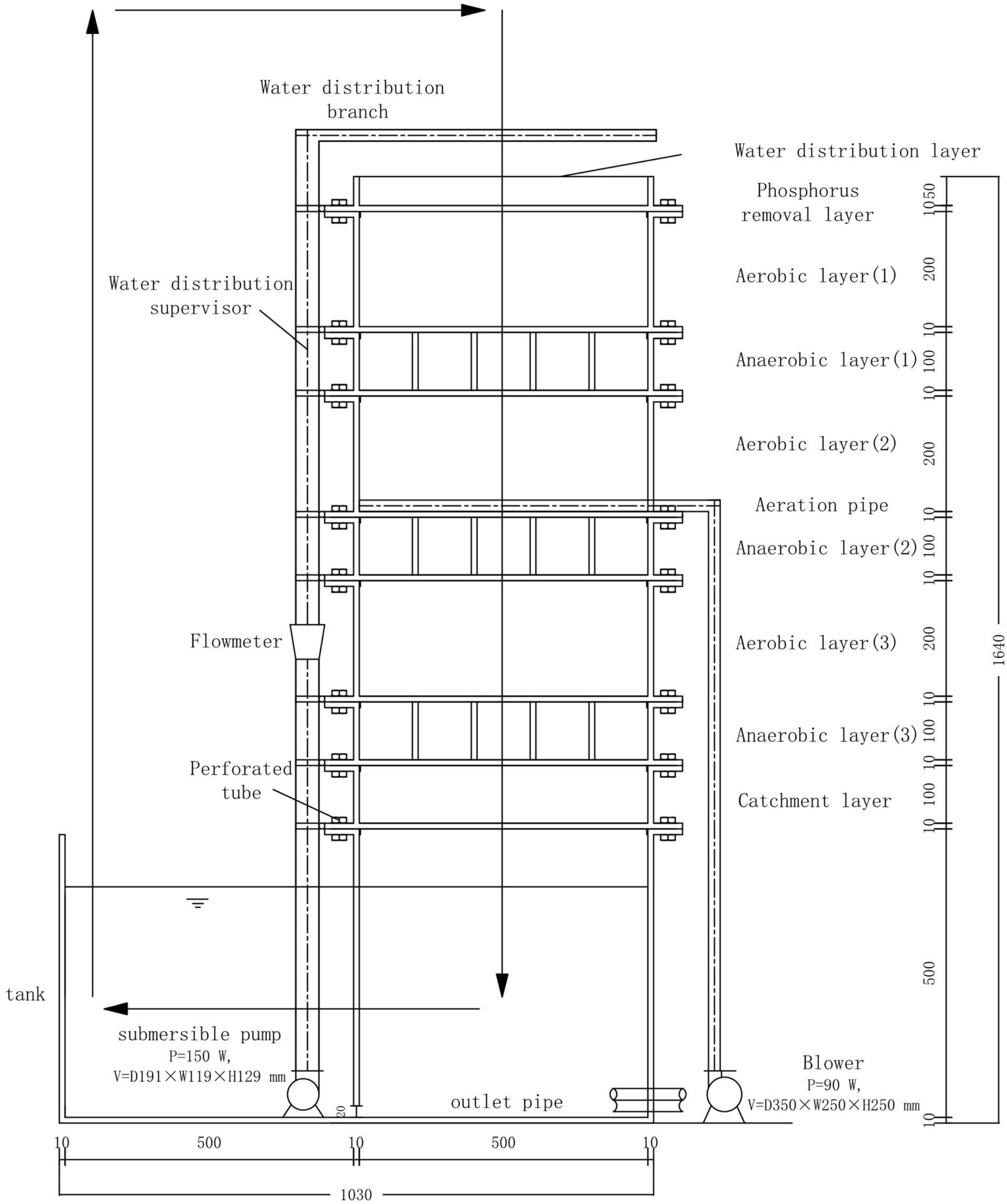


Fig. 4 Structure of the advanced stacked biological filter assembly for sewage treatment

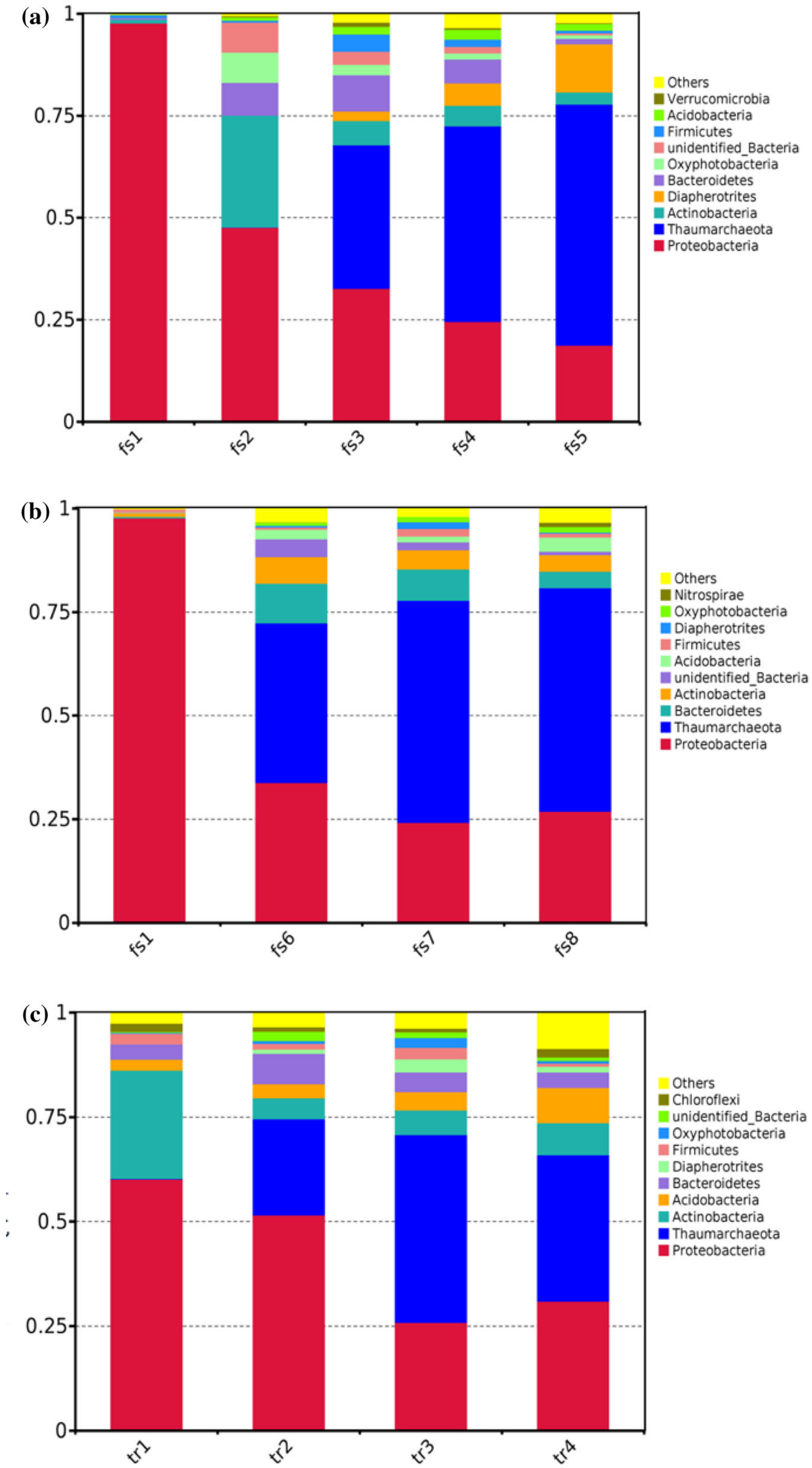


Fig. 5 **a** Top ten bacteria and their proportions in the zeolite filler in the aerobic layer and the corresponding zeolite petals figure. **b** Top ten bacteria and their proportions in the zeolite filler in the anaerobic layer and the corresponding zeolite Venn figure of zeolite. **c** Top ten bacteria and their proportions in the composite soil media in the anaerobic layer and the corresponding composite soil media Venn figure

Analysis of the biodiversity of the zeolite filler in the aerobic layer

The microbial composition of the zeolite fillers in aerobic layers at different heights are shown in Fig. 5a, which indicates that the top ten dominant bacteria phyla in the zeolite fillers were *Chloroflexi*, *Oxyphotobacteria*, *Acidobacteria*, *Diapherotrites*, *Bacteroidetes*, *Actinomycetes*, *Actinobacteria*, *Firmicutes*, *Thaumarchaeota*, and *Proteobacteria*.

The relative abundance of *Proteobacteria* in samples fs1 to fs5 gradually decreased, and the relative abundance of *Thaumarchaeota* increased significantly from samples fs1 to fs5. The most dominant phylum in samples fs1 and fs2 was *Proteobacteria* (97.7% and 47.6%, respectively). In samples fs3, fs4, and fs5, the most dominant phylum was *Thaumarchaeota* (35.2, 48.0, and 59.2%, respectively). Further, the relative abundance of *Oxyphotobacteria* in sample fs2 was 7.5%, which was higher than that in the other samples.

Analysis of the biodiversity of the zeolite filler in the anaerobic layer

As shown in Fig. 5b, the microbial community of the zeolite fillers in the anaerobic layers at different heights, which indicates the dominant bacteria in the zeolite fillers, included *Nitrospirae*, *xyphotobacteria*, *Acidobacteria*, *Diapherotrites*, *Bacteroidetes*, *Actinobacteria*, *Probacteria*, *Firmicutes*, and *Thaumbacteria*.

One of the dominant phyla in these zeolite fillers in the anaerobic layers was *Nitrospira* compared with the zeolite filter in the aerobic layers. The most dominant bacteria phylum in samples fs6, fs7, and fs8 was *Thaumarchaeota*, and from samples fs6 to fs8, there was an obvious increase in the relative abundance of this phylum, accounting for 38.6, 53.6, and 54.0% of the microbial communities in samples fs6, fs7, and fs8, respectively. However, the relative abundance of *Bacteroidetes* and *Actinobacteria* decreased slightly from fs6 to fs8, with *Bacteroidetes* accounting for 10.21, 7.82, and 3.35%, of the microbial communities in these samples, respectively. Further, *Actinobacteria* accounted for 6.81, 4.47, and 3.38% of the microbial communities in samples fs6, fs7, and fs8, respectively.

Analysis of the biodiversity of the composite soil medium in the anaerobic layer

The microbial composition of the composite soil medium within the anaerobic layer at different heights is shown in Fig. 5c, which indicates that the dominant bacteria in the composite soil medium were *Chloroflexi*, *Oxyphotobacteria*, *Acidobacteria*, *Diapherotrites*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Thaumarchaeota* and *Proteobacteria*.

The dominant bacteria in sample tr1 were *Proteobacteria* and *Actinobacteria* (60.2% and 26.0%, respectively). Further, in samples tr2, tr3, and tr4, the dominant bacteria were *Proteobacteria* and *Thaumarchaeota*; the relative abundance of *Acidobacteria* and *Actinobacteria* increased, while that of *Bacteroidetes* decreased in samples tr2 to tr4. The proportions of *Oxyphotobacteria* and *Thaumarchaeota* in sample tr3 (44.9% and 2.4%, respectively) were significantly higher than those in the other samples. In the same anaerobic layer, there were fewer *Nitrospira* and more *Chloroflexi* in the top ten dominant bacteria composites of the soil medium compared with the zeolite filler.

Data on the alpha indices of the system are shown in Table 8. The Chao1, Shannon, and Simpson values corresponding to the composite soil of the anaerobic units were obviously higher than those corresponding to the zeolite fillers in the aerobic and anaerobic layers. The soil with the composite medium was richer in

Table 8 Data on alpha indices

Sample	Shannon	Simpson	Chao1
fs1	2.862	0.343	725
fs2	6.912	0.814	1720
fs3	7.946	0.905	2042
fs4	8.277	0.898	1937
fs5	7.308	0.877	1819
fs6	8.240	0.952	2087
fs7	8.672	0.926	2247
fs8	9.224	0.944	2402
tr1	7.250	0.890	1878
tr2	9.051	0.946	2345
tr3	9.302	0.972	2410
tr4	9.614	0.996	2548

carbon and inorganic substances. This could provide nutrients, such as carbon and the inorganic salts necessary for the growth of microorganisms. It is obvious that the anaerobic units showed higher species richness and community diversity than the aerobic units. Chao1, Shannon, and Simpson values gradually increased with the direction of water distribution. Besides, *Oxyphotobacteria, Acidobacteria*, *Diapherotrites*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Thaumarchaeota*, and *Proteobacteria* were among the top ten dominant bacteria in the fillers in the system.

Discussion

The advanced stacked biological filter assembly represents an efficient and compact technology for on-site sewage treatment. The alternating arrangement of aerobic and anaerobic layers in the bioreactor filler improved treatment efficiency. The bioreactor could simultaneously guarantee long-term service times at low operational costs. Additionally, based on its design, the bioreactor could handle different load situations flexibly. The decomposition of microorganisms inside the fillers was the main pollutant removal strategy (Latrach et al. 2015) during its operation.

The performance of the biological filter under different reflow rates indicated that lower reflow rates resulted in lower pollutant removal efficiencies, except for TP removal when the bioreactor sequence batch process sewage treatment cycle was 24 h, with 200 L of the single treatment water volume. Under reflow rates of 20, 30, 40, and 50 L/h, the corresponding cycle numbers were 2.4, 3.6, 4.8, and 6, respectively. The contact time between the filler and the pollutants in the water was constant, and it was also observed that the larger the cycle number, the longer the actual sewage treatment time was. Therefore, the removal efficiency of pollutants obviously increased. Regarding the effects of the aeration methods on the operation performance of the biological filter, natural ventilation enhanced the microbial decomposition of organic matter. Additionally, it helped to decrease potential clogging in the biological filter due to organic matter accumulation under aeration (Ho and Wang 2015). Further, with natural ventilation, the removal rates of COD and NH_3–N were higher than those observed without aeration, while the TN removal rate observed without natural ventilation was higher. The removal of COD and NH_3–N in the sewage was more likely to be completed owing to the activity of aerobic microorganisms in the surrounding aerobic environment (Mehrani et al. 2020). Conversely, the denitrification reaction is inclined to be completed by anaerobic microorganisms. However, aeration is not conducive for the growth of anaerobic microorganisms and the formation of the anaerobic zone. The phosphorus removal mechanism principally results from chemical precipitation, which is mainly determined by the contact time between ferric ion and orthophosphate (Zhang et al. 2015). No obvious correlation was observed between TP removal efficiency and reflow rate or the aeration method. When cycle time increased, more sewage in the same phase could be treated by the system (Guan et al. 2012). The cycle time of a single operation cycle was found to be correlated with the pollutants removal efficiencies.

In aerobic layers, oxygen-producing photosynthetic bacteria showed a strong ability to decompose and transform organic substances. They can perform photosynthesis and release oxygen under anaerobic conditions to degrade nitrite in sewage. This explains the internal oxygen-generating ability of the system. NH_3–N was adsorbed by zeolite and soil first via a physicochemical mechanism (Sklenickova et al. 2020). Ammonia-oxidizing archaea (AOA) strains have good ammonia oxidation ability. As shown in Fig. 5, the most dominant phylum in the zeolite filler was *Thaumarchaeota*, which is a type of AOA strain, belonging to non-thermophilic bacteria in soil, and is adapted to survive at temperatures in the range 25–35 °C (Tourna et al. 2011). Organic matter was absorbed by zeolite, and *Bacteroidetes*, which also played a role in the decomposition of sludge flocs, could metabolize the absorbed organic matter (Larsen et al. 2008). *Proteobacteria* do not only degrade organic matter, but also remove nitrogen. Regarding the removal of phosphorus, it was primarily taken up by bacterial cells and removed from the residual sludge. The relative abundance of *Proteobacteria* gradually decreased from samples fs1 to fs5. *Proteobacteria* are eutrophic, and their relative abundance decreases with decreasing nitrogen content (Fierer et al. 2012). Therefore, the decrease in their relative abundance from fs2 to fs5 indicated that the system could efficiently remove nitrogen from sewage.

In the anaerobic layers, the analysis of the biodiversity of the zeolite fillers showed that *Nitrospira*, a common nitrite oxidizing bacteria in the biological treatment process of sewage (Xing et al. 2016), accounted for a larger proportion of the microbial community in the zeolite filler in the anaerobic layer than in the aerobic layer. It could be inferred that some nitrification reactions occurred in the zeolite filler in the anaerobic layers during the actual sewage treatment process in the system, and the roles of *Bacteroides* and *Actinomyces* in sewage treatment were mainly obligate anaerobic and chemical energy nitrogen fixation. The relative abundance of *Thaumarchaeota* (Konneke et al. 2005) from samples fs6 to fs8 showed an obvious increase and the proportion of *Bacteroidetes* and *Actinobacteria* (Ramirez et al. 2012) decreased slightly from samples fs6 to fs8, both demonstrating that the greater the number of functional components in the system, the stronger the nitrogen removal ability of the system. Regarding the analysis of the biodiversity of the composite soil media in the anaerobic layer, it was observed that the nitrification activity in this media played an important role in NH_3–N removal. The addition of wood dust, which is a slow-release carbon source, to the composite soil medium can provide organic matter for the denitrification reaction (Luanmanee et al. 2002), which also benefited from the enhanced hydrophobic properties resulting from the addition of bamboo charcoal. In the same anaerobic layer, fewer *Nitrospira* in the composite soil medium compared with those in the zeolite filler indicated that the nitrification reaction mainly depended on the microorganisms in the zeolite filler. The presence of *Chloroflexi*, a facultative anaerobic organism, demonstrated that the oxygen content of the composite soil medium is less than that of the zeolite filler. More *Chloroflexi* inside the composite soil medium than in the zeolite filler for the same anaerobic layer demonstrated that the nitrification reaction mainly depended on the microorganisms in the zeolite filler. Additionally, *Chloroflexi* shows good biological phosphorus removal ability (Zhang et al. 2012). The abundance of *Acidobacteria*, an oligotrophic bacteria, decreases as the nitrogen and other nutrient contents in the environment increase (Ramirez et al. 2012). As the nitrogen content in the environment decreases, pH increases, and this may lead to an increase in the relative abundance of *Acidobacteria* as seen from samples tr2 to tr4.

Both phosphorus removal layers and the composite soil medium provided locations for the adsorption of TP. The mechanism of TP removal is based on the adsorption effect of the solid polymer ferric sulfate that was placed on the phosphorus removal layer of the biological filter, coupled with the chemical adsorption of the iron hydroxide formed by the oxidation of the iron filings present in the composite soil medium (Zhang et al. 2015).

Based on our results, the novel bioreactor designed for water treatment was equally efficient in COD removal from sewage as conventional anaerobic/anoxic/aerobic processes. Furthermore, it was more efficient in both nitrogen and phosphorus removal. Conventional biological filters may clog, hindering NO_3^- mass transfer to the biofilm in the fillers in anaerobic layers. However, the continuous liquid-phase circulation adopted in this novel bioreactor enhanced substrate and biofilm distribution in the bioreactor. Thus, sloughed biomass inside the reactor was removed and N_2 supersaturation was reduced. Therefore, this process significantly guarantees a longer lasting and more efficient operation compared with the use of conventional treatment technologies.

Conclusion

Based on the investigation of the novel advanced stacked biological filter assembly developed for water treatment, the results of this study indicated the unique features of the biological filter assembly, which consisted of alternating anaerobic and aerobic layers, integrated with material for dephosphorization. This study successfully demonstrated optimum removal efficiencies of 86, 88, 87, and 83% for COD, NH_3-N, TN, and TP, respectively, obtained under the optimal operation conditions (a reflow rate of 40 L/h, without artificial aeration, and a cycle time of 12 h). This study illustrated that the dominant bacteria in the bioreactor fillers were *Chloroflexi*, *Oxyphotobacteria*, *Acidobacteria*, *Diapherotrites*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Thaumarchaeota*, *Proteobacteria*, and *Nitrospira*. This study also showed the distribution of the microbial community in the bioreactor and revealed the different roles of the above-mentioned dominant species in the different functional stages in the integrated system. Therefore, the results could enhance understanding regarding the pollutant removal mechanisms that occur inside the biological filter.

Considering the lab-scale test, pilot-scale test, and characteristics of existing operating equipment, the specific improvements of the system proposed here include increasing the number of alternating aerobic-anaerobic components, and the change in the water inlet mode from cyclic sequential batch to continuous flow transmission, which could contribute to reducing energy consumption.

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Data availability I certify that this manuscript is original and has not been published and will not be submitted elsewhere for publication while being considered by Biodegradation. Additionally, the study has not split up into several parts to increase the number of submissions and submitted to various journals or to one journal over time. No data have been fabricated or manipulated (including images) to support your conclusions. No data, text, or theories by others are presented as if they were our own.

Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval Approval for submission has been received explicitly from all co-authors, and authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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

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