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Abstract: Black water is highly concentrated human waste water but represents only a minor portion of domestic sewage. A modified type of anaerobic baffled reactor (ABR) was studied to assess its potential for pretreating black water in rural China. The classification of microbial structure was also investigated to confirm its potential in application. The structure of the ABR was modified according to demand for application in practice. A hydraulic retention time (HRT) of 48 h was chosen as the optimal HRT after comparison among 24 h, 36 h, 48 h, and 72 h. Under the 48 h HRT, the ABR achieved average removal efficiencies of 94.05% of chemical oxygen demand (COD), 28.78% of total nitrogen (TN), 14.21% of ammonium nitrogen (NH_4^{+}), and 32.54% of total phosphorus (TP) during 112 days of continuous operation. Samples from three different compartments were collected after 60-day continuous operation for bacterial and archaeal community investigation by 16S rRNA. Abundant degradation-related bacteria and methanogenic archaea were found in the ABR. The three samples had similar bacterial compositions at phylum, class, and genus levels, but the percentages of bacteria differed among the compartments. The distribution of archaea showed succession with the flow direction. In general, the ABR shows good performance under an HRT of 48 h and shows good potential for practical application.

Evaluation of an anaerobic baffled reactor for pretreating black water: potential application in rural China

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Dear Editor,

Please find enclosed our manuscript entitled “Evaluation of an anaerobic baffled reactor for pretreating black water for potential application in rural China,” which we request you to consider for publication in the *Journal of Environmental Management*.

Black water is highly concentrated human waste but represents only a minor portion of domestic sewage. In the submitted paper, we studied a type of anaerobic baffled reactor (ABR) to assess its potential for pretreating black water in rural China. The classification of microbial structure is also investigated to confirm its potential in application. The structure of the ABR was modified according to demand for application in practice. A hydraulic retention time (HRT) of 48 h was chosen as the optimal HRT after comparison among 24 h, 36 h, 48 h, and 72 h. Under the 48 h HRT, the ABR achieved average removal efficiencies of 94.05% of COD, 28.78% of TN, 14.21% of ammonia, and 32.54% of TP during 112 days of continuous operation. Sludge samples from three different compartments were collected after 60 days for bacterial and archaeal community investigation by 16S rRNA. Abundant degradation-related bacteria and methanogenic archaea were found in the ABR. The three samples had similar bacterial compositions at phylum, class, and genus levels, but the percentages of bacteria differed among the compartments. The distribution of archaea showed succession with the flow direction. In general, the ABR shows good performance under an HRT of 48 h and shows good potential for practical application. The findings of this research are timely and of significant potential impact both theoretically and practically. We feel sure that they would be of considerable interest both to your immediate readership and to a wider audience through subsequent citations, for which your journal is rightly renowned. We hope very much that you will consider this manuscript for publication.

This manuscript has not been published elsewhere and is not under consideration by another journal. We have approved the manuscript and agree with its submission to the *Journal of Environmental Management*. There are no conflicts of interest to declare.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership. The manuscript has been carefully reviewed by an experienced editor whose first language is English and who specializes in editing papers written by scientists whose native language is not English.

We look forward to hearing from you at your earliest convenience.

Sincerely,

Xiao Zha

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Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript title "Evaluation of an anaerobic baffled reactor for pretreating black water: potential application in rural China".

We're now mainly responding to Reviewer's comment:

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Response:

We regret that the arrangement of former manuscript is not good enough to show the novelty and not attractive enough. Now, we try to improve the expression to make the novelty clear. Some sentences were revised and you can see them in red. In the meanwhile, some sentences were deleted to make the manuscript more concise. Hope we show this study in a better way now. Looking forward to your opinion.

Your sincerely,
Xiwu Lu

Highlights

1. A modified anaerobic baffled reactor was established for pretreating black water.
2. The optimal hydraulic retention time was investigated.
3. Performance of the anaerobic baffled reactor was evaluated under continuous mode.
4. The microbial structure in the different compartments was investigated.
5. The main aim of this study is the potential application in rural China.

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Abstract

Black water is highly concentrated human waste water but represents only a minor portion of domestic sewage. A modified type of anaerobic baffled reactor (ABR) was studied to assess its potential for pretreating black water in rural China. **The classification of microbial structure was also investigated to confirm its potential in application.** The structure of the ABR was modified according to demand for application in practice. A hydraulic retention time (HRT) of 48 h was chosen as the optimal HRT after comparison among 24 h, 36 h, 48 h, and 72 h. Under the 48 h HRT, the ABR achieved average removal efficiencies of 94.05% of chemical oxygen demand (COD), 28.78% of total nitrogen (TN), 14.21% of ammonium nitrogen (NH_4^+), and 32.54% of total phosphorus (TP) during 112 days of continuous operation. Samples from three different compartments were collected after 60-day continuous operation for bacterial and archaeal community investigation by 16S rRNA. Abundant degradation-related bacteria and methanogenic archaea were found in the ABR. The three samples had similar bacterial compositions at phylum, class, and genus levels, but the percentages of bacteria differed among the compartments. The distribution of archaea showed succession with the flow direction. In general, the ABR shows

good performance under an HRT of 48 h and shows good potential for practical application.

Key words: black water; anaerobic baffled reactor; microbial structure

1. Introduction

Black water (BW) is wastewater from toilets, but it accounts for only a small portion of domestic sewage (Gao et al., 2019a). The literature indicates that around 12–33% of the volume of domestic sewage is BW (Thibodeau et al., 2014; Jin et al., 2018a). Though the portion of BW is such small, it contains large portions of chemical oxygen demand (COD), Nitrogen (N), phosphorus (P), etc. The composition of BW fluctuates with the shifting proportions of urine, feces, and flushed water, however it always possesses higher concentrations of COD, total nitrogen (TN), and total phosphorus (TP) compared to grey water and mixed domestic wastewater (Xu et al., 2019). Therefore, there is potential in treating that small portion of highly concentrated wastewater separately which can help to reduce the energy consumption of sewage treatment (Gao et al., 2019b). it particularly means a big step for decentralized domestic waste water treatment. BW could be subjected to pretreatment before the further treatment of decentralized domestic sewage. Pretreating BW would help to relieve the operating load and avoid excessive shock for the subsequent processes. Besides, it would also help to relieve the pressure of sewer network.

In China, how to treat domestic sewage properly in rural areas is an ongoing problem that urgently needs to be solved. Investment costs need to be considered in parallel with processing efficiency. Small-scale combined processes for treating mixed domestic sewage are common choices nowadays (Guo et al., 2014). Pretreating BW would reduce both the load of the entire process and the cost of sewer construction. Regarding the domestic wastewater treatment facilities

in rural China, a considerable number of the treatment units are open to the air. Pretreating BW would help to mitigate the spread of such microorganisms as BW contains large amounts of pathogenic microorganisms (Nordin et al., 2018).

As means of treating BW, recent studies have considered septic-tank underground infiltration, anaerobic treatments, aerobic biological treatments, constructed wetlands, and electrochemical systems (Jin et al., 2018b; Rogers et al., 2018). Of these, anaerobic digestion systems show particular promise by providing good capacity for COD degradation and killing pathogenic microorganisms. It has also been reported that treating BW using anaerobic reactors might even allow the recovery of energy and nutrients(Yee et al., 2019). However, almost all previous studies selected up-flow anaerobic sludge blankets (UASBs) for the anaerobic digestion of BW(Butkovskyi et al., 2016; J. R. Cunha et al., 2018; Jorge Ricardo Cunha et al., 2018a). In fact, the main characteristics needed for rural areas are that (i) it is with good capacity of degradation (ii) it is cheap to construct locally and (iii) it is easy to operate and maintain. Nevertheless, applying UASBs is a huge challenge in rural China due to the construction and maintain.

A possible alternative to the UASBs is the anaerobic baffled reactors (ABRs), which is easy to construct both aboveground and underground. ABRs were also identified as an appropriate sanitation technology (Bwapwa, 2013). The most important advantage of ABRs is commonly regarded as being the ability to separate acidogenesis and methanogenesis as a two-phase system longitudinally down the reactor (Barber and Stuckey, 1999), and the usual aim when designing an ABR structure is to enhance the capacity for retaining solids. However, it is difficult to construct some conventional structural details in a scaled-up application; for example, slanted edges on baffles are easily damaged (Barber and Stuckey, 1999). Therefore, the actual situation should also

be taken into account for practical application. ABR associated with membrane reactor was studied for treating blackwater in South Africa (Bwapwa, 2010). However, the membrane reactors are not widely accepted due to the expensive cost for both construction and maintain. Thus, a simpler alternative is needed.

In the present study, an ABR was established for pretreating BW under mesophilic conditions. The structure of the ABR was designed with reference to its potential application in rural China, considering both practicality and decontamination capacity. The optimum hydraulic retention time (HRT) was explored according to evaluation of contaminant-removal efficiency. Then performance of the ABR in continuous mode was investigated under optimal HRT. The microbial communities in different compartments were also investigated to confirm its feasibility in application.

2. Materials and methods

2.1. ABR system

Fig. 1. Schematic of modified anaerobic baffled reactor (ABR) system.

The modified ABR is shown schematically in Fig. 1. It has a working volume of 60 L

(material: PVC; length: 0.65 m; width: 0.2 m; height: 0.6 m). Considering the convenience of construction and the flow pattern, it was divided into three compartments (R1, R2, R3), each of which has two parts, namely a down-flow part and an up-flow part. **The absence of any slanted edges should be noted, which reduce the difficulty for construction.** The down-flow part of R1 is smaller (width: 0.05 m) than the other parts (width: 0.12 m). The reactor is filled with vertical combined packing (around 20% of total volume), except for the down-flow part in R1 to prevent clogging. Mesophilic condition was selected for this system. Water heating is in place around the ABR to keep the temperature of the reactor at $36\pm1^{\circ}\text{C}$.

2.2. Inoculum and start up

Developing a high and stable contaminant-removal efficiency in the shortest possible time is a characteristic of anaerobic reactors and reflects their competitiveness (Zwain et al., 2013). Many factors influence the startup of anaerobic systems, including their composition, strength of influent, volume of inoculum, and reactor configuration. Based on some studies of ABR start-up strategies, a longer HRT, a lower COD concentration, and more inoculum were selected. This start-up strategy helps to ensure growing microorganisms are not over loaded and encourage better solids accumulation (Bodkhe, 2009). Because no mesophilic digester was available, the ABR was inoculated with activated sludge from an ambient UASB operating in our laboratory (Wuxi, Jiangsu, China). Nevertheless, the ABR started to run at mesophilic temperature directly and was then filled with synthetic wastewater at the start. The ABR was allowed to rest for 3 d, after which the mixture was looped at low speed inside the reactor for 10 d using a peristaltic pump. Then synthetic wastewater with ranged concentration (from low concentration to high concentration, shown in supplementary material Fig. S1) was introduced into the ABR. The characteristics of the

wastewater during the set-up period are given in the supplementary material (table S1.). The synthetic wastewater was fed into the ABR at an HRT of 72 h with a speed of 0.833 L/h controlled stably by a peristaltic pump. The COD concentration of the influent was adjusted according to the COD removal efficiency. When the COD removal efficiency reached a high level (> 80%) for more than 5 d, the COD load was changed to a higher level until the COD concentration approached that in actual BW. From day 45 onward, actual BW was fed as the influent, and high removal efficiency was achieved. The production rate of biogas from the ABR was 5.37–6.21 L/d during days 45–50. Both the COD removal and biogas production indicated that the reactor started up successfully and quickly. The active sludge was expected to adapt well from ambient conditions to mesophilic conditions.

2.3. Operational mode

Actual BW was introduced into the ABR after the latter was set up. Referring to former studies (Bwapwa, 2013; Gao et al., 2019a) and based on expectation for practical application, four different HRTs (72 h, 48 h, 36 h, and 24 h) were selected to determine which one was optimal for the pre-treatment process. Each HRT was controlled by feeding the influent at a constant rate that was maintained with a peristaltic pump. The ABR was operated at each HRT for 20 d and the contaminant-removal efficiency was measured from day 6 onward. After five consecutive readings, the HRT was changed to the next value.

The ABR was run stably at the optimal HRT for 112 d, and samples were collected after 60-day stable operation to explore the microbial communities in each compartment. The contaminant-removal efficiency during the stably continuous period was measured in triplicate every two days.

2.4. DNA extraction and PCR amplification

Deoxyribonucleic acid (DNA) from the samples was extracted using an E.Z.N.ATM Mag-Bind Soil DNA kit (OMEGA Bio Tec, China) following the manufacturer's instructions. Then the extracted DNA was checked by agarose gel electrophoresis. The genomic DNA was quantified accurately by means of a Qubit 3.0 DNA assay kit to calculate the amount of DNA that should be added to the polymerase chain reaction (PCR). The primers used in the PCR were merged to the V3–V4 universal primers of the Miseq sequencing platform, namely BAR341F/805R (CCCTACACGACGCTCTTCCGATCTG (barcode) CCTACGGGNGGCWGCAG; GACTGGAGTTCCTTGGCACCCGAGAATTCCA GACTACHVGGGTATCTAATCC) and ARC349F/806R (GYGCASCAGKCGMGAAW; GGACTACVSGGGTATCTAAT).

The 30 μ L PCR reaction admixture contained 2 $\times$ Taq master mix (15 μ L), Bar-PCR primer F (10 μ M; 1 μ L), primer R (10 μ M; 1 μ L), genomic DNA (10–20 ng), and H₂O. The PCR was performed under the following conditions: 94°C for 3 min; five cycles of 94°C for 30 s; 45°C for 20 s and 65°C for 30 s; then 20 cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s; and finishing with a final extension at 72°C. Illumina bridge-type compatible PCR primers were introduced for the second round of PCR. The second-round 30 μ L PCR reaction mixture contained 2 $\times$ Taq master mix (15 μ L), primer F (10 μ M; 1 μ L), primer R (10 μ M; 1 μ L), genomic DNA (20 ng), and H₂O. The second-round PCR was performed under the following conditions: 95°C for 3 min followed by five cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s; and concluding with a final extension at 72°C for 5 min. The recovered DNA was then quantified accurately using a Qubit 3.0 DNA detection kit (Life, USA) to facilitate sequencing in equal amounts of 1:1. When

mixed in equal amounts, the amount of DNA per sample was 10 ng and the final sequencing concentration was 20 pmol.

2.5. Analytical methods

The water quality of the samples (COD, NH_4^+ -N, TN, and TP) was examined according to standard methods (APHA/AWWA/WEF, 2012). Different forms of COD, N and P were examined from samples pre-treated by a 4.4 μm paper filter and a 0.45 μm membrane filter. The calculation of the different forms of contaminant is shown in the supplementary material.

The operational taxonomic units (OTUs) were classified by Mothur (version 1.30.1). The sequences that were obtained were allocated phylogenetically down to their different levels at 97% similarity for taxonomic analysis. For community composition analysis, representative sequences from each OTU were subjected to the classifier (version 2.12) of the Ribosomal Database Project (RDP) and the National Center for Biotechnology Information (NCBI) Blast+ (version 2.28). The community structure component map was drawn using the R code, and the phylogenetic tree was constructed with the “vegan” package of the R code.

3. Results

3.1. ABR performance under different HRTs

72, 48, 36, and 24 h were selected as the possible optimal HRT. As shown in Fig. 2(a), the average COD removal efficiency under 72 h, 48 h, 36 h, and 24 h was $97.67 \pm 1.22\%$, $95.24 \pm 1.20\%$, $82.95 \pm 0.45\%$, and $75.06 \pm 3.56\%$, respectively. In general, the COD removal efficiency decreased with the decrease of HRT, but it remained roughly the same from 72 h HRT to 48 h HRT. The COD concentration of the effluent was 59.33 ± 37.98 mg/L, 101.82 ± 26.89 mg/L, 373.77 ± 99.49 mg/L, and 563.05 ± 142.57 mg/L when the HRT was 72 h, 48 h, 36 h, and 24 h,

respectively. The fluctuations of both the COD removal efficiency and the concentration in the
 effluent were smooth when HRT exceeded 48 h. According to the investigation of the
 concentration of normal domestic sewage in the Wuxi Campus of Southeast University, it was
 136 ± 47.23 mg/L. The COD degradation ability of the ABR is good enough for BW treatment
 when the HRT exceeds 36 h and can be adjusted according to the required emission standards and
 the treating performance of subsequent processing units. Note that the COD removal efficiency at
 24 h HRT was not as sustainable as the removal efficiency at longer HRT. Therefore, HRT was
 suggested to not be shorter than 24 h.

(a) COD concentration and removal efficiency under different HRTs

(b) TN concentration and removal efficiency under different HRTs

(c) ammonia concentration and increasing ratio under different HRTs

Fig. 2. Pollutant removal performance under different hydraulic retention times (HRTs).

The TN removal performance under different HRTs is shown in Fig. 2(b). An average TN removal efficiency of $23.65 \pm 3.57\%$, $19.33 \pm 2.14\%$, $14.33 \pm 1.99\%$, and $10.54 \pm 1.85\%$ was achieved for an HRT of 72 h, 48 h, 36 h, and 24 h, respectively. Compared to COD removal, TN removal in

the ABR shows larger fluctuations in removal efficiency under each particular HRT. Besides, unlike the high COD removal efficiency, the TN removal efficiency in the whole reactor was quite low, especially with lower HRT. Although the TN concentration in the BW was much higher than that in normal mixed domestic sewage, the conventional anaerobic processes did not show superior capacity in nitrogen removal. As can also be found in Fig. 2(c), the ammonia concentration in the effluent of ABR was even higher than that in the influent. However, it was found that the ammonia concentration decreased in the first one or two compartments but sometimes increased in the final compartment.

3.2. ABR performance under 48 h stable HRT

Considering the demand of future units for decentralized sewage treatment (Zha et al., 2018), 48 h was selected as a possible optimal HRT. The HRT was set at 48 h for 112 d to check its performance under stable conditions. The concentrations of COD, TN, $\text{NH}_4^+\text{-N}$, and TP are shown in Fig. 3 along with the removal efficiency.

(a) COD concentrations of influent and effluent from each compartment, and COD removal efficiency of total reactor under 48 h HRT

(b) TN concentrations of influent and effluent from each compartment, and TN removal efficiency of total reactor under 48 h HRT

(c) $\text{NH}_4^+\text{-N}$ concentrations of influent and effluent from each compartment, and ammonia removal efficiency of total reactor under 48 h HRT

(d) TP concentrations of influent and effluent from each compartment, and TP removal efficiency of total reactor under 48 h HRT

Fig. 3. Pollutant removal performance under 48 h HRT.

The average COD concentration of the influent during the 112-day period was 1929.19 ± 252.94 mg/L, and the average COD concentration of the effluent from R1, R2 and R3 was 791.98 ± 73.71 mg/L, 337.03 ± 47.56 mg/L, and 113.59 ± 21.55 mg/L, respectively. The standard deviation of the COD concentration in the effluent from each compartment decreased from compartment 1 to compartment 3. An average of $94.05 \pm 1.21\%$ of the COD was removed. Around 60% of COD was removed by compartment 1, around 25% was removed by compartment 2, but only around 10% was removed by compartment 3. Using three compartments guarantees the stability of COD removal. It was also found in the experiment that R1 removed $55.32 \pm 3.54\%$ of COD_{ss}, $62.27 \pm 5.99\%$ of COD_{col}, and $68.90 \pm 4.23\%$ of COD_{dis}; R2 removed $23.57 \pm 3.74\%$ of COD_{ss}, $30.39 \pm 4.50\%$ of COD_{col}, and $8.52 \pm 4.89\%$ of COD_{dis}; R3 removed $17.66 \pm 7.83\%$ of COD_{ss}, $6.82 \pm 5.41\%$ of COD_{col}, and $10.03 \pm 3.79\%$ of COD_{dis}. This indicates that most of the COD_{ss} and COD_{col} was removed after the three compartments but that the effluent contained more than 10% of COD_{dis}. R1 removed almost 70% of COD_{dis}, but R2 and R3 removed only

around 20% together.

The TN concentration of the influent was 189.34 ± 13.84 mg/L, and the concentration of effluent from each compartment was 147.91 ± 7.63 mg/L, 138.23 ± 5.98 mg/L, and 134.27 ± 5.59 mg/L, respectively. Most of the TN removal happened in R1, but only $28.78 \pm 5.12\%$ of TN was removed in this ABR in total. Meanwhile, the ammonia concentration of the influent and effluent from each compartment was 139.31 ± 9.29 mg/L, 136.09 ± 7.77 mg/L, 125.67 ± 6.81 mg/L, and 119.26 ± 6.00 mg/L, respectively. Unlike the increased ammonia concentration in the effluent during the experiment for selecting the HRT, the ammonia concentration decreased in the effluent during stable operation. A total of $14.21 \pm 3.93\%$ of the ammonia nitrogen was removed. The ammonia concentration did not always decrease or increase following the flow direction. The TP concentration of the influent and effluent from each compartment was 37.41 ± 5.27 mg/L, 32.75 ± 4.27 mg/L, 29.12 ± 4.20 mg/L, and 25.05 ± 3.81 mg/L, respectively. On average, $32.54 \pm 8.79\%$ of the total TP was removed by the ABR. Besides, $19.11 \pm 4.26\%$ of dissolved TP and $77.64 \pm 7.93\%$ of particulate TP were removed.

3.3. Bacteria community clarification in different compartments under 48 h HRT

250

(a) Bacterial classification histogram at phylum level

251

(b) Bacterial classification histogram at class level

(c) Bacterial classification histogram at genus level

Fig. 4. Bacterial classification histograms at phylum, class, and genus levels.

As shown in Fig. 4(a), at phylum level, *Chloroflexi* (20.15%), *Caldiseirica* (14.22%), *Proteobacteria* (13.92%), and *Firmicutes* (11.33%) were dominant in R1, *Chloroflexi* (24.72%), *Caldiseirica* (13.38%), *Aminicenantes* (12.69%), and *Proteobacteria* (10.27%) were dominant in R2, and *Chloroflexi* (20.63%), *Proteobacteria* (16.01%), and *Aminicenantes* (11.29%) were dominant in R3. Besides, *Firmicutes* in R1, R2, and R3 accounted for 11.33%, 4.85%, and 6.22%

respectively.

Anaerolineae dominated in all three compartments at class level (shown in Fig. 4(b)). The percentage of *Anaerolineae* in R1, R2, and R3 was 19.76%, 24.37%, and 19.58%, respectively. *Caldisericia*, *Delta-proteobacteria*, and *Clostridia* were also abundant in these three compartments.

At genus level (shown in Fig. 4(c)), although *Caldisericum* and the *Aminicenantes* genera *incertae sedis* and *longilinea* were dominant in all three samples, their percentages differed in each compartment. The percentage of *Caldisericum* decreased from R1 to R3, and the percentage of the *Aminicenantes* genera *incertae sedis* and *longilinea* in R2 was the largest followed by R3. Some other genera were also detected, such as *Thermogutta*, *Treponema*, and *Leviline*.

3.4 Archaea community clarification in different compartments under 48 h HRT

(a) Archaeal classification histogram at order level

(b) Archaeal classification histogram at genus level

Fig. 5. Archaeal classification histograms at order and genus levels.

Archaeal classification histograms at order level is shown in Fig. 5 (a). The four main orders in the reactor were *Methanomicrobiales*, *Methanosarcinales*, *Methanobacteriales*, and *Methanomassiliicoccales*. *Methanomicrobiales* was the most abundant order, and its percentage increased from R1 to R3 (R1: 52.12%; R2: 53.36%; R3: 70.28%). *Methanobacteriales* accounted for 21.01% in R1 but few in R3 and R3 (R2: 0.17%; R3: 0.19%). *Methanosarcinales* occupied the second position in the samples (R1: 21.01%; R2: 0.17%; R3: 0.19%). *Methanomassiliicoccales* in R1 accounted for 3.47%, then increased to 9.33% in R2, but decreased to 0.75% in R3.

Archaeal classification histograms at genus level is shown in Fig. 5 (b). The main genus in R1 were *Methanoregula* (38.66%), *Methanotherix* (22.56%), *Methanobacterium* (20.98%), and *Methanolinea* (7.30%). The percentages of *Methanoregula* and *Methanobacterium* decreased to 21.95% and 0.11%, respectively, in R2. By contrast, the percentages of *Methanotherix* and

Methanolinea increased to 30.48% and 30.21%, respectively. *Methanoregula* decreased to 33.11% in R3, and *Methanothrix* decreased to 26.8%. The percentage of *Methanolinea* increased to 35.98% in R3.

4. Discussion

The ABR performed well at COD removal. However, the removal of TN and TP were not significant. Overall, COD removal was more effective under longer HRT. Considering both of the COD removal efficiency and practical feasibility, 48 h can be used as a reliable HRT.

The COD concentration decreased from one compartment to the next, but the situation was different for the nitrogen concentration. TN decreased from influent to effluent, but the removal efficiency was not very high. However, the ammonia concentration changed differently during different periods. During the HRT selecting period, ammonia increased slightly from influent to effluent. Nevertheless, ammonia decreased in the effluent during the stable operating period. The production of ammonia by the coupling of some mixed bacterial consortia occurs frequently under anaerobic conditions, thereby increasing the ammonia concentration. The increased ammonia concentration was also probably due to the death of bacteria caused by the lack of substrate in the final compartment. Meanwhile, ammonia nitrogen can be used for the growth of bacteria as a necessary source of acid, such as in the assimilation process, which would cause a decrease of ammonia (Hu et al., 2009). Besides, the interception effect due to the fillers also contributed to the decrease of nitrogen. As the running time was increased, so the decreasing ammonia concentration became the main trend. However, no outstanding nitrogen-removal efficiency was observed.

A larger percentage of COD_{ss} was removed by the ABR compared with a previous study of treating BW with a UASB (Luostarinen et al., 2007). The ABR has better interception capability

than a UASB given that the flow pattern in the ABR is more similar to that of plug flow. Besides, the combined packings in the ABR also helped to intercept solids. The high removal efficiency of COD_t, COD_{col}, and COD_{dis} indicated that the ABR can achieve high biological conversion (Luostarinen et al., 2007).

The feed BW used in the present study was not collected from toilets directly but through long-distance sewer pipes, which may have caused decreased particulate phosphorus and increased solute phosphorus. As discussed in some previous studies, the removal of phosphorus in anaerobic systems is due mainly to the interception of particulate pollutants (Hahn and Figueroa, 2015). The high concentration of dissolved phosphorus could be the reason for the low TP removal efficiency. However, phosphorus can also be absorbed by living biomass, a fact that also contributed to the decreased phosphorus concentration. Additionally, a large percentage of TN_{ss} was removed in the ABR. The performance on COD_{ss}, TN_{ss}, and TP_{ss} removal was outstanding and in line with expectation, thereby showing that adding packings is useful.

The concentrations of phosphorus and nitrogen in raw BW are much lower and with smoother fluctuations compared with the COD concentration. Consequently, the aim of pretreating BW is to reduce the COD concentration, and the subsequent processes would be responsible for removing the nitrogen and phosphorus. Previous studies have also shown that most of the decentralized processes for treating domestic sewage show good capacity for nutrient removal (Singh et al., 2015; Adrados et al., 2018; Bunce et al., 2018). Besides, previous researches have shown that calcium phosphate granulation can also help phosphorus recovery, and the level of phosphorus in BW shows the economic value of anaerobic treatment (Cunha et al., 2018a; Cunha et al., 2018b). Nutrient recovery could be another option for further processes.

At phylum level, the proportion of *Chloroflexi* was the highest no matter in which compartment, and it was in good accordance with some pervious reports. It has been widely reported that *Chloroflexi* is abundant in anaerobic digestion reactors, and the potential capacity of *Chloroflexi* for carbohydrate degradation was reported in previous studies (Bovio et al., 2019; Rivière et al., 2009). *Firmicutes* was also pointed out as being related to the degradation of carbon compounds. These two bacterial phyla are considered to have good tolerance to high organic loading (Xu et al., 2018). *Firmicutes* is also well known as being related to the degradation of volatile fatty acids at both mesophilic and thermophilic temperatures (Tang et al., 2007). Note that the microbes at phyla level showed good potential capacity for COD removal, which coincides with the monitoring of COD removal performance. It was reported that *Bacteoidetes* (42.8%) and *Proteobacteria* (53.9%) dominated in raw samples of BW (Gao et al., 2019b), accounting for more than 90% in the raw BW. In the present study, after anaerobic digestion, the microbial community in the effluent became diverse and the percentages of *Bacteoidetes* and *Proteobacteria* decreased to 6.41% and 16.01%, respectively. Obviously, the aforementioned transformation through long-distance pipes may have also influenced the microbial structure in the influent for the ABR. At class level, *Anaerolineae* dominated in all three compartments, and its portion increased in R2 then decreased in R3. *Anaerolineae* might play a special role in the degradation of carbohydrates and cellular material in the methanogenic digestion process (Narihiro and Sekiguchi, 2007). Depending on the theory of anaerobic digestion and the COD removal in the ABR, the increase of the *Anaerolineae* percentage in R2 verifies that methane production happened mainly in R2. *Clostridia*, which is related to dechlorinate alkylhalides, was also abundant in these three compartments (Mohn and Tiedje, 1992). Some common genera were also found as normal

anaerobic digestion systems, such as *Thermogutta*, *Treponema*, and *Levilinea* (Kong et al., 2019).

Methanomicrobiales was abundant in all three compartments, and its percentage increased to around 70% in R3 from around 50% in R1 and R2. It was also reported that *Methanomicrobiales* was more abundant in the final compartment than in the former compartments, and that *Methanomicrobiales* was likely to play a key role in ABRs with carriers (Zhang et al., 2011). This may be related to the methane production in the posterior compartments but the acid production in the former compartments. The abundance of *Methanomicrobiales* has also been indicated to be related to temperature, and it might be the key methanogenic group in low-temperature bioreactors (O'Reilly et al., 2009; Tian et al., 2018). This gives us confidence to continue to explore the ABR performance under ambient temperature. *Methanobacterium* and *Methanolinea* are both hydrogenotrophic archaea (Su et al., 2019). *Methanolinea* is a typical anaerobic mesophilic hydrogenotrophic methanogen converting H₂ and formate to methane, and it usually dominates in mesophilic systems (Tian et al., 2015). It was predominant in R2 and R3. *Methanobacterium* was observed in large amounts at the front of ABRs along with *Methanosarcina* (Barber and Stuckey, 1999). *Methanoregula* utilizes formate and hydrogen to produce methane. It is an acid-tolerant hydrogenotrophic methanogen and shows a large percentage in all the compartments, especially in R1 (Ban et al., 2013). *Methanothrix* is also responsible for converting acetate to methane (Peng et al., 2018).

The ABR performance at COD removal gives confidence for using it in practice, and the microbial results further show its potential. In practical applications, the PVC can be replaced by bricks. A configuration involving bricks covered by concrete would be easy to construct and has an insulation effect to a certain extent. Meanwhile, constructing the ABR underground also has

better insulation effect than above ground, thanks to the soil layer. However, further studies to explore the scaled-up application under ambient temperature are still required.

5. Conclusion

The proposed ABR achieved a high COD removal efficiency when treating BW. From the perspective of application in decentralized sewage treatment in rural areas of China, 48 h was chosen as the optimal HRT. Thanks to the good biological activity, good COD removal efficiencies were achieved. Average removal efficiencies of 94.05% of COD, 28.78% of TN, 14.21% of ammonia, and 32.54% of TP were attained during 112 d of continuous operation under 48 h HRT. The bacterial species in the three different compartments were similar but had varying percentages which helps confirm the potential capacity on stable operation. Large amounts of degradation-related microorganisms were discovered. The archaea in R1 to R3 exhibited a trend of going from mainly producing acid to mainly producing methane. The distribution of archaea matched former studies on mesophilic ABRs. In general, the ABR is a good choice as a method for pretreating BW and has the potential to be scaled up in application. Constructing with bricks covered by concrete and placing underground can help to the ABR's application in practice.

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