Safe purification of rural drinking water by biological aerated filter coupled with ultrafiltration

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# Safe Purification of Rural Drinking Water by Biological Aerated Filter Coupled with Ultrafiltration

*Qidong Wu* [a, b], *Chen Chen* [c], *Yongli Zhang* [a], *Peng Tang* [a, b], *Xiaoyu Ren* [a, b], *Jingyu Shu* [a, b], *Xinyu Liu* [a, b], *Xin Cheng* [a, b], *Alberto Tiraferri* [d], *Baicang Liu* [a, b,*]

[a] State Key Laboratory of Hydraulics and Mountain River Engineering, College of Architecture and Environment, Institute of New Energy and Low-Carbon Technology, Sichuan University, Chengdu, Sichuan 610207, PR China

[b] Yibin Institute of Industrial Technology, Sichuan University Yibin Park, Yibin, Sichuan 644000, PR China

[c] Litree Purifying Technology Co., Ltd., Haikou, Hainan 571126, PR China

[d] Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Turin, Italy

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[*] Corresponding author.

Tel.: +86-28-85995998; fax: +86-28-62138325; e-mail: bcliu@scu.edu.cn; baicangliu@gmail.com (B. Liu).

## ABSTRACT

In many rural areas, lakes or reservoirs represent typical water resources. Some of these sources have poor water qualities compared with groundwater or river water, which are commonly used as the main drinking water sources in large modern cities. Drinking water supply in remote rural areas usually suffers from various challenges, such as the high cost of construction and maintenance of centralized drinking water treatment plants and pipe networks due to the dispersed nature of villages, which are often located in varied and complex topographies. In this study, a combined process comprising biological aerated filter (BAF) combined with ultrafiltration was developed to treat polluted reservoir water. Organic matter indexes, turbidity, and chroma were used as indicators for the evaluation of the system performance. In a long-term experiment lasting 260 days, the combined process was tested under different values of critical operational parameters, including filler types and empty bed contact time (EBCT). Furthermore, the microbial communities in different BAF reactors were carefully evaluated at different times, finding that microorganisms with specific functions were enriched in the various BAF reactors. The combined process reached 85.5% removal rate of DOC with an EBCT of 45 min and using granule active carbon (GAC) as filler. Most of the effluents of BAF reactors met the requirements for drinking water in China. The combined system showed practical potential for polluted water treatment in some rural areas.

**KEYWORDS:** Biological aerated filter; Ultrafiltration; Combined system; Rural drinking water treatment; Microbial community.

## 1. Introduction

With the rapid growth of industrialization and population, access to adequate and safe drinking water is an increasing challenge (Li and Wu, 2019; Pedro-Monzonis et al., 2015). Traditional drinking water treatment processes, commonly consisting of coagulation, sedimentation, filtration and disinfection, are capable of meeting the requirements for water quality in modern cities in most cases, and advanced treatment processed are commonly applied in urban water treatment plants to ensure a drinking water of high quality (Shen et al., 2020; Wang, 2016). However, currently around 2.2 billion people still suffer from the lack of safely managed drinking water, especially in remote villages, such as in mountainous, pastoral, and forest areas (Li and Wu, 2019; Song et al., 2020; UNICEF, 2019; WHO, 2018). In fact, rural drinking water access and safety is a global concern, with complex natural and economic conditions, such as related to geography, water source quality, costs and labor issues, limiting the widespread implementation of decentralized drinking water treatment plants (DWTPs). For example, the territory in China counts numerous villages that are dispersedly located. In addition, some reservoirs and lakes in rural areas that serve as the main water source are contaminated due to inadequate environmental protection, thus increasing the challenges of water treatment approaches aimed at ensuring water safety. Science and technology advances should tackle the development of simple and effective water treatment techniques, suitable for local conditions, characterized by low construction and maintenance costs and by the ability to serve areas of flexible sizes (Li and Qian, 2018). Given that a single treatment process can hardly meet the standards for drinking water quality in rural areas of China, combined processes coupling different approaches represent a promising approach to reduce water stress in those areas (Scheili et al., 2015).

Membrane filtration, especially ultrafiltration, is an easily maintainable water treatment technology able to intercept most suspended matter, microorganisms, and large macromolecules in water (Yu et al., 2021; Yu et al., 2020). More importantly, membrane technologies can be widely applied even in rugged terrain. However, dissolved substances and small molecules also exist in polluted water sources, such as odor compounds, ammonia nitrogen, and other organic micropollutants, which cannot be significantly removed using porous membrane processes (Kim et al., 2018; Teodosiu et al., 2018). Moreover, pollutants usually accumulate on the membrane surface and result in membrane fouling, consequently reducing the filtration efficiency, shortening the service life of the membrane, and increasing the operating costs (Shi et al., 2014; Tian et al., 2018). A pretreatment or a synergic process, such as flocculation, advanced oxidation, or biological treatment, is essential to improve the efficiency of overall treatment and the quality of the final effluent. (Gao et al., 2011). In particular, inexpensive biological methods may be suitable for remote areas: they include drip filtration, moving bed biofilm reactors, and membrane bioreactors. Here, it is hypothesized that the biological aerated filter (BAF) is superior to all the competing biological technology due to lower construction costs, easier operability, and the ability to guarantee a continuous and long-term efficiency pollutant removal (Neoh et al., 2016; Udomkittayachai et al., 2021).

BAF is commonly applied in wastewater treatment to remove organic matter, nitrogen, and phosphorus, taking advantage of the activity of microorganisms attached on a filler (Tang et al., 2021). Abu Hasan et.al improved the removal rates of ammonia, Mn(II), and chemical oxygen demand (COD) by investigating the different operation parameters in a BAF system as an additional treatment in DWTP (Abu Hasan et al., 2011). The efficiency of the BAF process is influenced by numerous factors, including the empty bed contact time (EBCT), the properties of the fillers, as well as the activity of various microorganisms, which may form communities on the surface of the fillers and determine the removal efficacy for pollutants in the raw water. Until now, very few studies have investigated the combination of the BAF reactor and ultrafiltration for small-scale drinking water treatment in actual rural areas. For this reason and for the advantages described above, such combined process was applied for rural drinking water treatment in this study, with a schematic diagram of the system shown in Figure 1.

Specifically, in this work we discuss the impact of filler properties, EBCT, and microbial communities on the effluent quality. Also, the treatment efficiency, productivity, and fouling behavior of the ultrafiltration process are evaluated. The stability of the whole system in long-term tests is presented, together with the potential for the application of the process to provide safe drinking water starting from polluted water in rural areas.

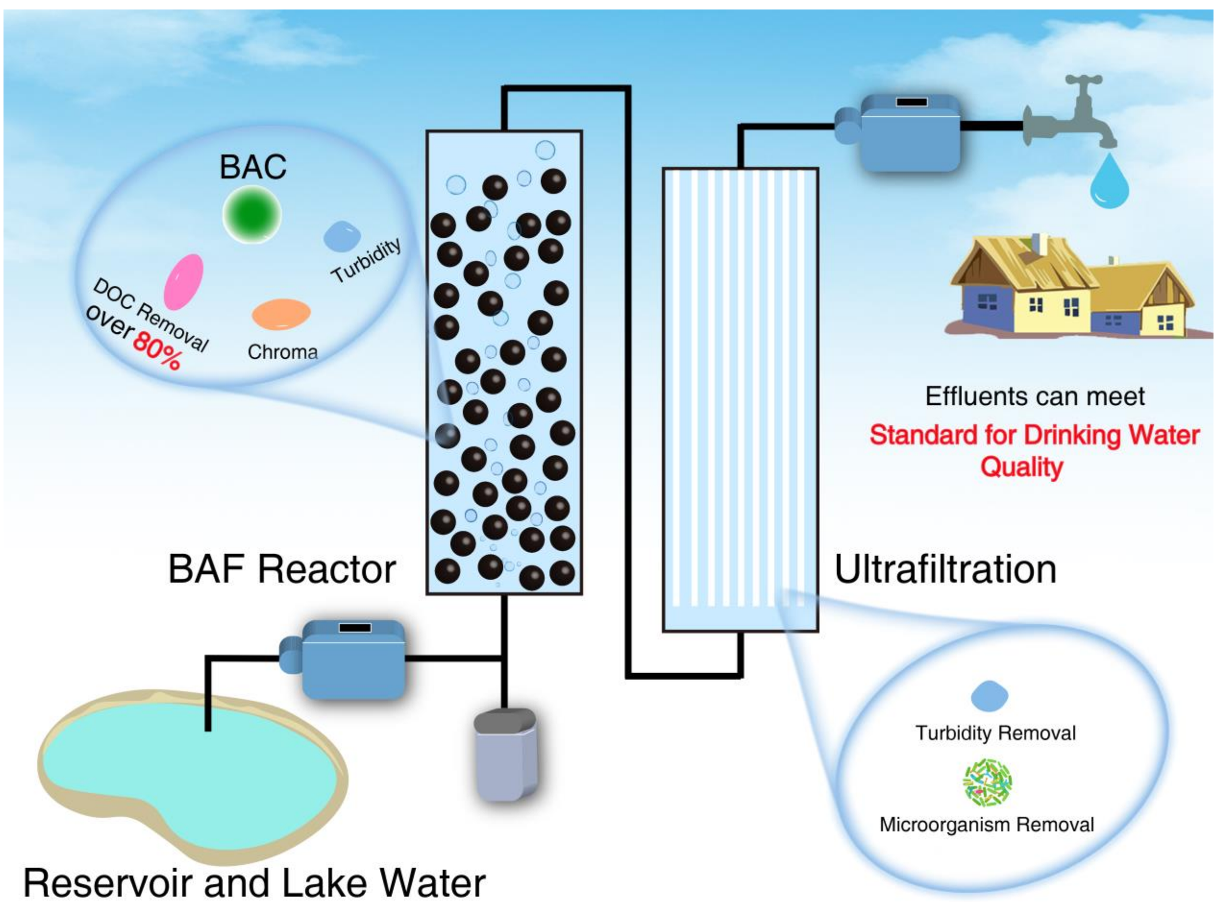

**Figure 1.** The installation diagram of the combined system contains the BAF and ultrafiltration.

## 2. Materials and Methods

*2.1. Materials*

The polluted water was collected from a reservoir in Zhongxian county (Chongqing, China), which serves as the water source for rural villages and towns nearby, and used as raw water. Information regarding the quality of as-collected water is listed in Supporting Information (Table S1). Granular activated carbon (GAC, 12 × 40 mesh, 0.425~1.70 mm) with an iodine number of 950 mg/g was purchased from Calgon Carbon corporation (USA). New and exhausted polyvinylidene difluoride (PVDF) hollow fiber membranes were obtained from Litree Purifying Technology Co., Ltd (Hainan, China), and the exhausted sample was then cut into pieces with 5-10 mm in length and used as filler in one of the BAF reactors. Plexiglass reactors with an inner diameter of 2.0 cm and a height of 30 cm were custom-made and used as the BAF reactors; a schematic diagram of the reactors is shown in Figure S1. Peristaltic pumps (BT-300-2 J, Longer Pump, China) were used to feed the raw water from the tanks to the BAF reactors, and feed the effluents of the reactors to the ultrafiltration hollow fiber membrane modules. Glucose was purchased from a local pharmacy. The LH-D/E reagents used for $COD_{Cr}$ measurement were purchased from Lianhua technology (Beijing, China): LH is the abbreviation for the company name, while D and E are product codes. LH-D is potassium dichromate and LH-E is silver sulfate. Deionized water (DI water) used in this work was produced by an ultrapure water system from Ulupure (Chengdu, China).

*2.2. Methods*

*2.2.1. Operation of BAF Reactors*

Undisturbed sediment samples of about 1 kg from shallow water areas in the reservoir were collected and stored in a 5 L culture tank with glucose (1 g) and raw water (4 L) for microbe enrichment. A volume of roughly 2 L of culture solution was taken from the tank every 3 days, followed by replenishment with glucose (0.5 g) and raw water (2 L). The solution collected from the culture tank was mixed with raw water as the influent during the domestication period of the reactor systems to facilitate rapid growth and adaptation of microorganisms in the BAF reactors. The mixing ratios of collected culture solution and raw water varied from 1:1 to 1:14 over time, until the water quality of the BAF reactor effluents were stable. Owing to the presence of culture solution, the microorganisms accumulated in the BAF reactors and a biofilm formed on the fillers. The domestication period lasted about 45 days. Peristaltic pumps were used to pump the raw water into the BAF reactors and then pump the effluents into the ultrafiltration membrane modules. Meanwhile, the reactors were continuously aerated by aeration pumps with a gas/water ratio equal to 5/1. The BAF reactors were backwashed for 15 minutes with gas and water every 15 days. The influent rate during the domestication and running periods was 0.12 L/h and the aeration rate was 0.6 L/h. For backwashing, the influent rate was 1.5 L/h and the aeration rate was 1.5 L/h. Information of different BAF reactors are listed in Table 1. The water quality of the effluents during domestication is listed in Figure S2 and S3 of the Supporting Information.

**Table 1**. The operation parameters of BAF reactors in this work.

| Operators ID | Fillers | EBCT (min) | Gas-water ratio | Running Time (d) |
|---|---|---|---|---|
| BAF-60-AC | GAC | 60 | 5:1 | 285 |
| BAF-60-M | Shredded hollow fiber membrane | 60 | 5:1 | 285 |
| BAF-45-AC | GAC | 45 | 5:1 | 260 |
| BAF-30-AC | GAC | 30 | 5:1 | 260 |

| | | | | |
|---|---|---|---|---|
| BAF-15-AC | GAC | 15 | 5:1 | 260 |

Note: The running time of the reactors does not include the domestication phase. The reactors were not started simultaneously.

### *2.2.2. Characterizations and Analyses of Influent and Effluent Streams*

The raw water, effluents of BAF reactors, and effluents of membrane modules were collected to monitor turbidity, pH, and total dissolved solid (TDS) directly during the entirety of the experiments. Dissolved organic carbon (DOC), chemical oxygen demands ($COD_{Cr}$), UV absorbance at 254 nm ($UV_{254}$), and fluorescence excitation-emission matrix (EEM) of the samples were determined after filtration through 0.45 μm acetate fiber membrane. A turbidimeter (TL2310, Hach company, USA), a PB-10 pH meter (Sartorious Scientific Instruments Co., Ltd., Germany), an Ultrameter II 6PFC portable multifunctional meter (Myron L Company, USA), a total organic carbon (TOC) analyzer (TOC-L CPH CN200, Shimadzu, Japan), a UV-vis spectrophotometer (Orion AquaMate 8000, Thermo Fisher Scientific INC., MA, USA), and a fluorescence spectrophotometer (F-7000, Hitachi, Japan) were used for analyses. The metal ions were detected using inductively coupled plasma-mass spectrometry (ICP-MS, NexION 1000 G, Perkin Elmer, Waltham, MA) and the anions were detected by Dionex Intergration HPLC ion chromatography (Thermo Fisher Scientific Inc., USA, with AS11-HC chromatographic columns). The ammonia nitrogen was measured using an ammonia nitrogen meter (HI96715, Hanna Instruments Co., Ltd., Italy). Detailed methods can be found in our previous studies and in Text S1 of the Supporting Information (Tang et al., 2020; Tang et al., 2021).

### *2.2.3. Microbial Diversity Sequencing Analysis*

The 16s rRNA gene is the DNA sequence corresponding to the coding rRNA on bacteria. It exists in the genome of all bacteria. The conserved regions and variable regions of 16s-rRNA reflect the relationship and differences among bacteria, respectively. In this study, 2 g filler samples in the BAF reactors were collected at about 5 cm depth from the top, and the same amount of fillers was backfilled immediately after withdrawing. The variation of microbial communities and the dominant functional microorganisms over time were analyzed through the microbial diversity sequencing of the fillers in the continuous-mode BAF reactors at different times using 16s-rRNA. The sequencing of 16s rRNA took place on the $10^{th}$, $60^{th}$ and $110^{th}$ day after the end of the domestication stage. Details for the microbial diversity sequencing analysis are presented in Text S2.

## 3. Results and Discussion

### *3.1. Removal of Organic Matters in Raw Water by BAF Process*

In this work, DOC, $UV_{254}$, and $COD_{Cr}$ values were measured to characterize the organic matter in water samples, and EEM was applied to determine the removal rate of various types of organic pollutants from the raw water. Considering the variation of water quality of influents and effluents of the BAF reactors over time, it can be concluded that the fillers in the BAF reactors reached a stable state. A certain amount of microorganisms were attached on the surface of GAC, transforming this material into biological activated carbon (BAC) during the domestication period (Zhang et al., 2011).

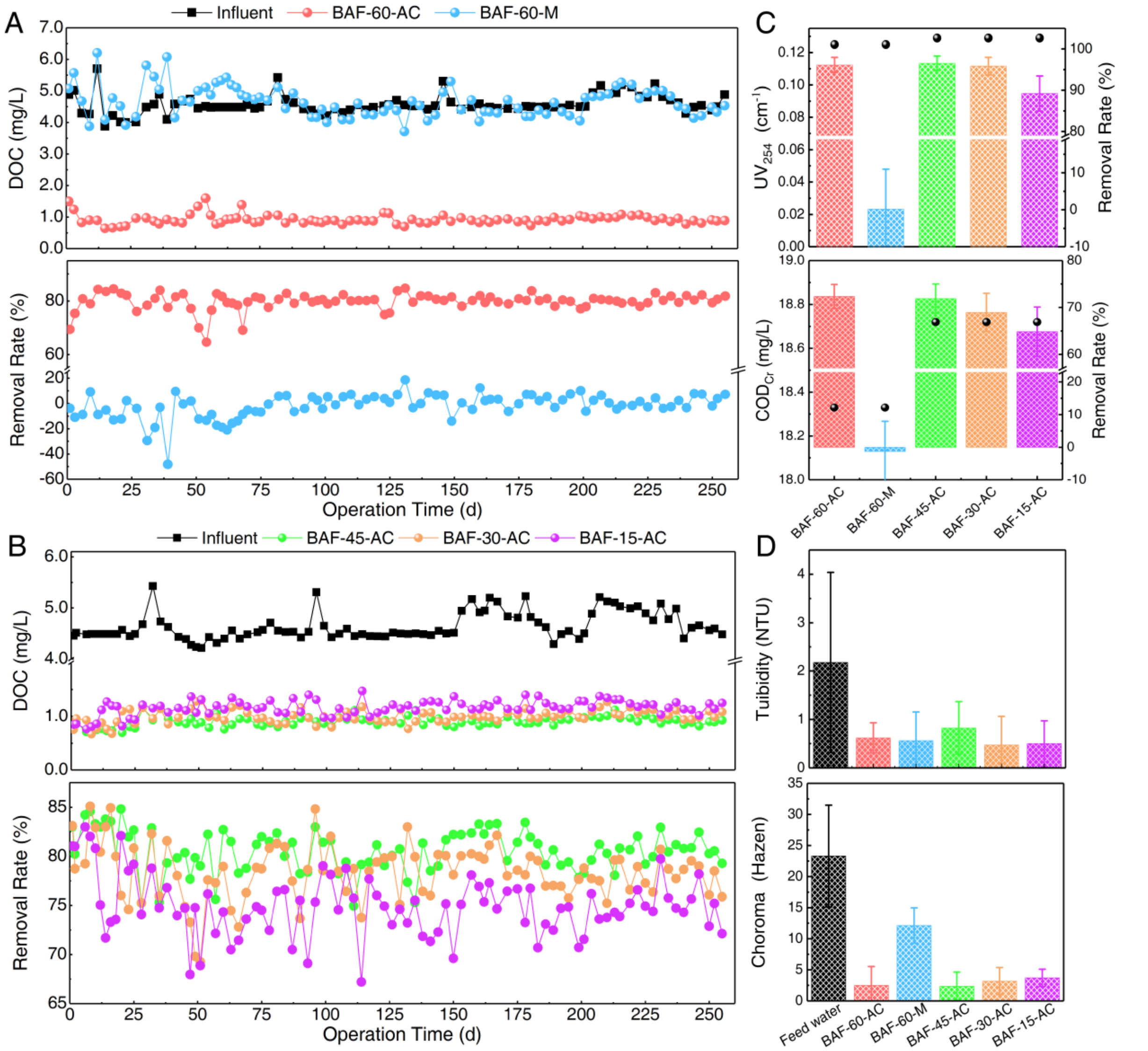


**Figure 2.** Water quality indexes of influent and effluent streams of different BAF reactors during the operation period. A: DOC values of influent and effluent streams of BAF reactors with different fillers and their corresponding removal rates; B: DOC values of influent and effluent streams of BAF reactors with different EBCTs and their corresponding removal rates; C: Average $UV_{254}$ and $COD_{Cr}$ values (black dots referring to the left axis) of influent streams and removal rates (bars referring to the right axis) of different reactors; D: Average turbidity and chroma values of influent streams and removal rates of different reactors.

Figure 2A presents the DOC removal efficiency values achieved in the BAF reactors with different fillers: the DOC removal rate of BAF-60-AC was about 80%, higher than that of BAF-

60-M, indicating that the filler type is a critical parameter influencing the efficiency of the biological degradation activity. Figure 2B shows the effect of EBCT on the DOC removal efficiency by the BAF reactors with GAC as the fillers. The average DOC removal rates increased from 75.0 ± 3.7% to 79.5 ± 4.0% with increasing EBCT from 15 to 60 min, indicating that the EBCT also has an important role in ensuring high biological degradation rate of organic matters in the BAF reactors. The results suggest that the BAF reactors with GAC as fillers can adsorb and degrade a significant portion of organic matter in the streams within 15 minutes. In addition, for each 15-minute extension in EBCT, the removal rate of DOC increased by an average of 2%. Higher EBCT values endowed the BAF reactors with higher stability for DOC removal even with varied DOC concentrations in the influent solution.

The $UV_{254}$ parameter can be used to represent the amount of some organic matters that contains C=C double bonds and benzene ring structures, including humic macromolecular substances and aromatic compounds (J.C. Crittenden, 2016). As illustrated in Figure 2C, the removal rates of $UV_{254}$ were 96.1% (BAF-60-AC), 0.17% (BAF-60-M), 96.5% (BAF-45-AC), 95.9% (BAF-30-AC), and 89.2% (BAF-15-AC). The average removal rates of BAF reactors for $COD_{Cr}$ were instead 72.3% (BAF-60-AC), −1.29% (BAF-60-M), 71.9% (BAF-45-AC), 68.9% (BAF-30-AC), and 64.8% (BAF-15-AC). Using GAC as the filler, the BAF reactors showed consistent removal rates for $COD_{Cr}$ and could remove organic matters that contain C=C double bonds and benzene ring structures effectively, while the BAF-60-M reactor (containing membrane pieces as fillers) exhibited a negligible effect on organics. Consistent with results observed for DOC, increasing EBCT also enhanced the performance of BAF reactors in terms of $UV_{254}$ and $COD_{Cr}$ removal.

Turbidity, chroma, pH, TDS, and the concentration of ions that are listed in local drinking water standard were used as indicators related to drinking water quality. As presented in Figure 2D, the average turbidity of the effluents from BAF reactors was always lower than 1.0 NTU, with BAF-60-M resulting in comparable ability of removing turbidity of other reactors, indicating that fillers had negligible impacts on turbidity removal, as opposed to organic matter removal. Note that even when the turbidity of raw water reached values up to 100 NTU, the BAF reactors reduced turbidity to single digit values (1-3 NTU). The chromas of water samples were measured using a Pt/Co-based method (Chang et al., 2020). The BAF process demonstrated a suitable performance in terms of chroma removal rate and showed a significantly better performance when using GAC as the filler. The highest chroma of raw water reached 200 hazen, while all effluent chroma values were less than or equal to 15 hazen.

The influent pH was in the range 7.4-8.0, while the pH of the effluents was always in the range 7.5-8.1 (Figure S4). After treatment in the BAF reactors, the pH of the effluent stream was generally higher than that of influent, with an increase of 0.1-0.4, attributed to the presence of extracellular polymeric substance and small organic matters in the effluent (Ugalde et al., 2013). The TDS of raw water was in the range 130-150 ppm. When treated by BAF reactors, the TDS of effluents was 110-140 ppm, thus decreasing of roughly 10-20 ppm due to adsorption of ions by the microorganisms for growth. Some hazardous ions that were detected during the experiments included arsenic, cadmium, chromium, and lead. The concentrations of these ions and of ammonia in influent and effluent streams of different BAF reactors are reported in Table S2. The removal rates of ammonia in BAF reactors were nearly 100%. The BAF reactors showed a negligible effect on the removal of some ions, such as metal ions, indicating that these ions were not required for microbial growth at significant amounts.

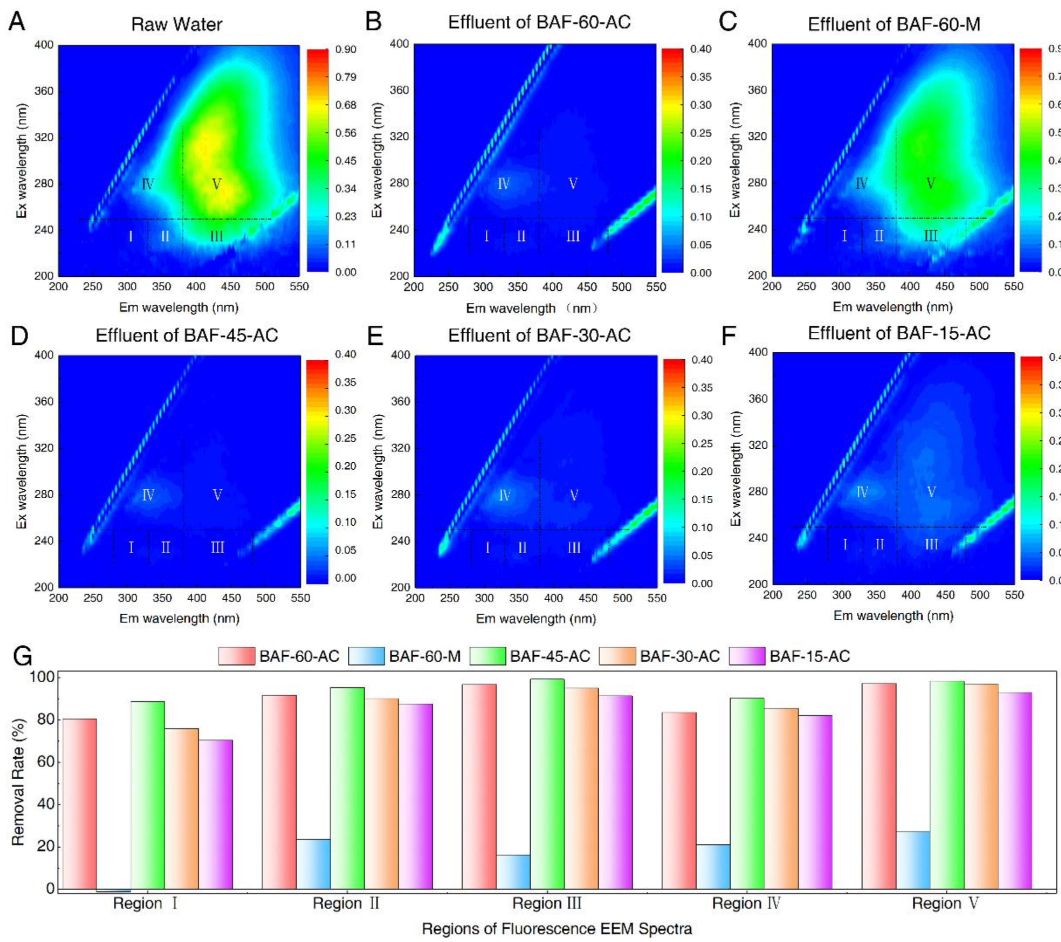


**Figure 3.** Fluorescence EEM spectra of influent and effluent solutions of BAF reactors: A) Influent; B) BAF-60-AC effluent;C) BAF-60-M effluent; D); BAF-45-AC effluent; E) BAF-30-AC effluent; F) BAF-15-AC effluent; G) Removal rates of different BAF reactors. (RegionI: (Ex/Em = 220-250/280-330 nm, tyrosine protein-like substances), region II: (Ex/Em=220-250/330-380 nm, tryptophan protein-like substances), region III: (Ex/Em = 220-250/380-480 nm, fulvic acid-like matters), region IV: (Ex/Em = 250-440/280-380 nm, soluble microbial by-product-like matters), and region V: (Ex/Em = 250-400/380-540 nm, humic acid-like components))

The difference in dissolved organic matter between raw water and effluents of different BAF reactors was also investigated by fluorescence EEM spectroscopy to identify the removal of different components. The EEM spectra can be divided into five regions using fluorescence regional integration (FRI) (Hu et al., 2021; Tang et al., 2020). As shown in Figure 3A, the dissolved organic matter in raw water was mainly composed of three categories, namely, fulvic acid-like matters (region III), soluble microbial by-product-like matters (region IV), and humic acid-like components (region V). Figure 3B-F present EEM spectra of effluents from various BAF reactors, while Figure 3G illustrated the removal rates of each component. When GAC was used as the filler, the removal rates were roughly the same, regardless of the EBCT, while a large difference was observed for BAF-60-M, in which removal rates were not satisfying. The average removal rates of various components when GAC was used were 78.8% (region I), 91.1% (region II), 95.6% (region III), 85.3% (region IV) and 96.4% (region V). For BAF-60-M, the rates were were −1.26% (region I), 23.6% (region II), 16.2% (region III), 21.1% (region IV), and 27.0% (region V). It is well known that the amino acid-like and humic acid-like components are the main components of aquatic humus substances, hence of natural organic matter in surface water (Nerger et al., 2015; Peng et al., 2022). The BAF reactors containing GAC as fillers demonstrated suitable performance in the removal of two such components, thus showing promise in the purification of surface water like reservoirs and rivers in rural areas.

As aforementioned, the BAF-60-M exhibited negligible performance in the removal of organic matter compared with other reactors using GAC as fillers, which is mainly attributed to the different surface roughness of the fillers and the size of the pores on the filler surface. Compared with the shredded hollow fiber membranes, GAC is rougher and rich in micropores, which are more suitable for the attachment and growth of microorganisms (Lu et al., 2022; Lu et al., 2020; Xu et al., 2021). The size of micropores of GAC is larger than that of the ultrafiltration membrane, and the cavities connected to the micropores may have provided a suitable place for the attachment of microorganisms, leading to more biomass growth on GAC. In addition, in the long-term experiments, the microorganisms attached on the GAC would partially degrade the adsorbed organic matters in the micropores, rendering the active sites of GAC that were previously occupied newly available and allowing recovery of the adsorption capacity of GAC fillers (Amini et al., 2018; Smolin et al., 2020). The adsorption capability of the GAC and the biodegradation mediated by the microorganisms attached on the fillers contributed together to the removal of organic pollutants. Moreover, considering the different filling modes, the GAC-filled reactor was tighter and the packed bed more regular than that of the reactor filled with shredded hollow fiber membrane pieces. When the raw water flows in the BAF reactors with aeration, tighter and more regular fillers result in a greater chance of contact between the contaminants in the influent and the fillers, also consistent with the explanation by the van Deemter model used in chromatography (Blanc et al., 2015; Gritti and Guiochon, 2013).

The BAF reactor is the core process of the water treatment in the combined system. The average removal rates for DOC, $COD_{Cr}$ and UV254 of BAF reactors with GAC as filler were 80%, 69% and 94%, respectively. The BAF-45-AC performed best with a DOC removal rate of 85.5%. The BAF-60-M showed a low organic matters removal rate. All reactors presented an adequate performance in terms of turbidity and chroma removal.

### *3.2. Ultrafiltration for BAF Effluents.*

The effluents of BAF reactors were pumped to the ultrafiltration membrane modules with the same flow rates as the raw water. The quality of effluents in different combined systems is presented in Figure 4A and B. The removal rates of ultrafiltration membranes for DOC in the last 50 days can be seen in Figure 4C and D with the detailed results of other reactors in Figure S5. DOC and $UV_{254}$ were used to evaluate the amounts of organic matter in the effluents. Compared with BAF reactors, the ultrafiltration membrane did not change the $UV_{254}$ or DOC significantly. The average removal rates of DOC for the ultrafiltration stage in different systems were 7.2 ± 5.7% (BAF-60-AC), 3.2 ± 3.7% (BAF-60-M), 6.0 ± 4.7% (BAF-45-AC), 6.6 ± 5.3% (BAF-30-AC) and 7.0 ± 5.8% (BAF-15-AC). The organic matters blocked by the membranes were likely untreated macromolecular organic matter and detached biofilms, demonstrating that organic matters in the raw water could be almost completely removed by the BAF reactors, and that backwashing was able to effectively control the amount of biofilm in the systems. Meanwhile, the ultrafiltration membranes can achieve the near-complete removal of microorganisms (including bacteria and viruses), which has been widely observed by common engineering practice. Therefore, the UF process in the combined system can block pathogens, such as *E. coli*. The turbidity and chroma of BAF effluent were instead further reduced during the ultrafiltration stage, by 0.3 ± 0.12 NTU and 1.4 ± 0.41 hazen, respectively. Therefore, the ultrafiltration step further increased the quality of the effluent as a double barrier treatment and allowed meeting of the standard quality values, indicating the excellent performance of the combined process.

The UF process is a second step in the combined dual-barrier system proposed in this study. The average removal rate of the UF process for DOC was 6%. No significant removal was observed for $COD_{Cr}$ or $UV_{254}$. The turbidity and chroma of the effluent were further reduced by 0.3 NTU and 1.4 hazen on average. Therefore, the effluents of the combined system met the local drinking water standard based on the parameters measured in this study.

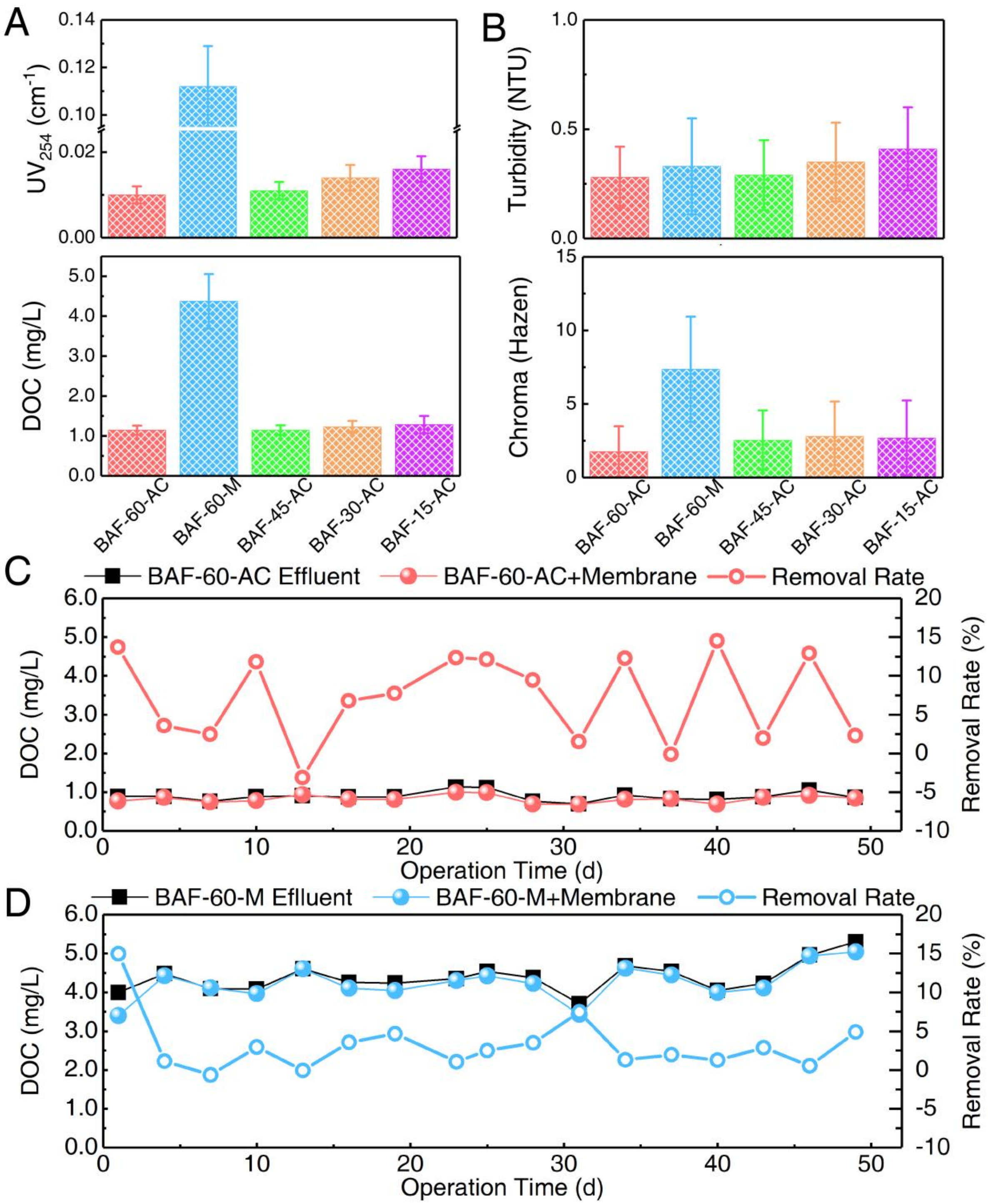


**Figure 4.** Water quality indexes of effluents of the ultrafiltration membrane modules for different BAF reactors during the operation period. A): Average $UV_{254}$ and DOC values of effluents; B): Average turbidity and chroma values of effluents; C): DOC values of influent and effluent solutions of membrane combined

with BAF-60-AC in the last 50 days; D): DOC values of influent and effluent solutions of membrane combined with BAF-60-M in the last 50 days.

*3.3. Microbial Community Structure in the BAF Reactors.*

The microbial community in different BAF reactors was analyzed via the 16s-rRNA gene sequencing. The number of effective sequences, operational taxonomic units (OTUs), alpha diversity indexes for microbial communities in different BAF reactors at different times along the experiments are presented in Table 2. Representative SEM pictures of the treated fillers of each BAF reactors are reported in Figure S6. The coverage indexes (Table 2) and the rarefaction curves of raw water and BAF reactors (Figure 5A) suggest that the amount of biological detection of the microorganism was appropriate and that the sequencing results were reliable (Fang et al., 2018; Tang et al., 2021).

The community richness index (chao) and the diversity index (shannon) in BAF reactors decreased with increasing EBCT, as well as the average OTU number of different BAF reactors, indicative of more bacterial species in the microbial communities in the reactors characterized by shorter EBCT. As shown in Figure 5B, the principal component analysis (PCA) at the OTUs level of the microbial communities of raw water and different BAF reactors indicates that the variations in the proportion of communities were mainly ascribed to the adaptation of the microbial community to the reactor environment under long-term operation conditions. Large differences can be observed between the microbial community of raw water and those of BAF reactors. Figure 5B also suggests that the microbial community structures varied for different running times and BAF reactors, with the BAF-60-M being the reactor with the largest difference compared to the other systems. The membrane-based filler was not suitable for microbial attachment and growth, and it could not absorb substances when compared with GAC.

Hence, the chance for microorganisms to treat organic matter in water was low. On the other hand, the microbial community structure of different BAF reactors with GAC as filler was similar even at different operating times, as can be observed from Figure 5B, demonstrating the importance of the environment to the microbial community in the reactor under long-term operating conditions.

In more detail, the composition of the microbial communities in the raw water and different BAF reactors in terms of phylum level and genus level are shown in Figure 5C and D, respectively. The *Proteobacteria* dominated the phylum during early stages of all BAF reactors and accounted for 72.1% on average; for instance, in the BAF-60-AC, the *Proteobacteria* was 82.4 % of the microbial community. Other major phyla in the early stages of the BAF reactors included *Firmicutes* (6.34% for average), *Bacteroidota* (3.27% for average), *Chloroflexi* (1.56% for average) and *Actinobacteriota* (3.80% for average), constituting higher than 82% of the entire community. A similarly large proportion of *Proteobacteria* in the microbial communities were detected in other drinking water treatment processes (Mahajna et al., 2022; Wang et al., 2017). Differences can be observed compared with the microbial community of the raw water. The five phyla mentioned above also contributed to about 81% of the entire community, while the *Proteobacteria* only accounted for 53.0% and *Chloroflexi* accounted for more than 13.3% in that of raw water. As shown in Figure 5C, The *norank_f__Hydrogenophilaceae* (12.00%), *Methylocystis* (7.37%), *norank_f__norank_o__norank_c__Berkelbacteria* (5.76%), and other 12 kinds of microorganisms accounted for about 60.74% of the microbial community in raw water, while these microorganisms were not a significant fraction of the microbial communities of BAF reactors. The results suggest that adaptation of the microorganisms has taken place in the BAF reactors and the microbial communities on the BAF fillers remained stable over time. In addition to the difference between the microbial community in raw water and those of BAF reactors, the microbial community of the same BAF reactor also changed. The proportion of *Proteobacteria* (from 72.1% to 51.0%) and *Actinobacteriota* (from 3.80% to 1.89%) decreased over time, while that of *Cyanobacteria* (from 1.29% to 8.81%) and *Chloroflexi* (from 1.56% to 4.44%) increased instead. Meanwhile, the proportion of *Firmicutes* slightly decreased at first and then increased in BAF-60-AC and BAF-60-M reactors (from 2.47% to 0.20% and then 37.8%), while the *Firmicutes* decreased in the other BAF reactors (from 8.91% to 0.56%).The results suggest that the *Proteobacteria* were dominant in all systems: as gram-negative bacteria, *Proteobacteria* contain a variety of metabolic species, most of which are heterotrophic and are facultative or obligate anaerobic.(Ahmad et al., 2022; Guarin and Pagilla, 2021). Specifically, *Alpha-Proteobacteria* and *Gamma-Proteobacteria* were the main *Proteobacteria* in the BAF reactors. They are facultative anaerobe, which take organic matter in raw water as the carbon source and carry out respiration and fermentation metabolism at the same time (Tian et al., 2014). Among the 42 genera presented in Figure 5C, 32 belonged to *Proteobacteria*, with *norank_f__norank_o__Rhodospirillales* and *Ellin6067* having significant correlation with the removal of organic matter in the experiments. This result implies that *Proteobacteria* played an important role in organic matter removal. Besides, ammonia oxidizing bacteria also belong to *Proteobacteria* phylum and may play a role in total nitrogen removal based on the ammonia removal shown in Table S5. The second common phylum in the other three reactors was *Cyanobacteria*, the prokaryotic microorganism that can produce oxygen through photosynthesis. Some *Cyanobacteria* are harmful and their metabolites often contaminate water resources (Park et al., 2017). The increase in the proportion of *Firmicutes* and *Cyanobacteria* in some reactors with running time as the temperature and the nutrients in the raw water decreased due to the seasonal change, led to an increase in the proportion of microorganisms with strong viability and autotrophic character.

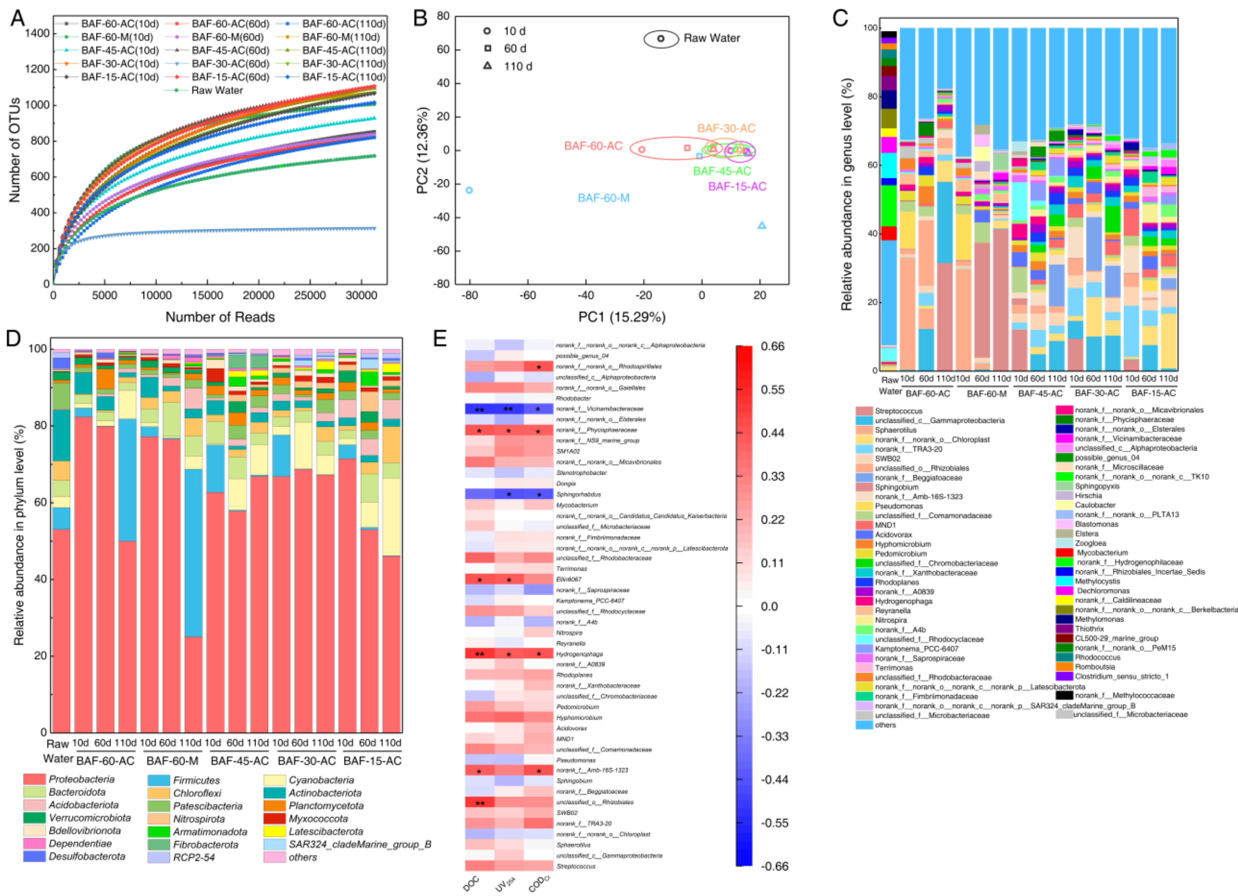


**Figure 5.** Results of the microbial community analysis in different BAF reactors: A) Rarefaction curves of OTUs in raw water and different BAF reactors at different operation times. B) PCA analysis at OTUs level of microbial communities in raw water and different BAF reactors at different operation times. C) Bacterial community compositions at phylum level ( > 1%) in raw water and different BAF reactors at different operation times. D) Correlation analysis between microbial community at genus level (top 50) and environmental variables (organic matter removal rate). Here, "*" represents a value of $p < 0.05$ and "**" represents a value of $p < 0.01$. E) Bacterial community compositions at genus level ( > 1.5%) in raw water and different BAF reactors at different operation times.

**Table 2.** Number of effective sequences, operational taxonomic units (OTUs), alpha diversity indexes for microbial communities in raw water and different BAF reactors at different times.

| Sample ID | Number of effective Sequences | OTU at 97% identity | shannon | chao | coverage |
|---|---|---|---|---|---|
| Raw Water | 38989 | 718 | 4.415047 | 850.784 | 0.994849 |
| BAF-60-AC (10d) | 48955 | 854 | 3.73616 | 850.784 | 0.992449 |
| BAF-60-AC (60d) | 41025 | 822 | 4.142537 | 1046.569 | 0.993601 |
| BAF-60-AC (110d) | 44233 | 821 | 3.225351 | 966.203 | 0.991969 |
| BAF-60-M(10d) | 40303 | 1006 | 4.215275 | 1046.719 | 0.996193 |
| BAF-60-M(60d) | 62034 | 840 | 4.18883 | 1076.919 | 0.993697 |
| BAF-60-M(110d) | 46179 | 1071 | 3.96469 | 981.956 | 0.994273 |
| BAF-45-AC (10d) | 54286 | 928 | 4.761545 | 1178.642 | 0.992641 |
| BAF-45-AC (60d) | 40519 | 1096 | 5.145886 | 1135.362 | 0.993057 |
| BAF-45-AC (110d) | 50466 | 1097 | 4.693617 | 1251.205 | 0.990594 |
| BAF-30-AC (10d) | 52352 | 1106 | 4.677481 | 1364.522 | 0.990114 |
| BAF-30-AC (60d) | 34857 | 314 | 4.140391 | 1382.663 | 0.999488 |
| BAF-30-AC (110d) | 53385 | 1072 | 4.768929 | 1326.517 | 0.990018 |
| BAF-15-AC (10d) | 56771 | 1068 | 4.64183 | 1393.298 | 0.990018 |
| BAF-15-AC (60d) | 31255 | 1107 | 5.113004 | 1369.342 | 0.991265 |
| BAF-15-AC (110d) | 50703 | 1016 | 4.878137 | 1361.301 | 0.991585 |

Note: Due to the low biomass, samples of BAF-60-M were tested again for BAF-60-M (10d) and BAF-60-M (60d).

The linear discriminant analysis effect size (LEfSe) method was used to determine the microorganisms with significant abundance differences among the different BAF reactors (Wu et al., 2018; Zhou et al., 2021). As shown in Figure S7, statistically significant differences appeared among the 18 bacterial clades when the LDA (linear regression analysis) threshold was 3.2. Except for BAF-60-AC, each reactor had its own characteristic microorganisms with corresponding abundance was higher than that in other reactors regardless of the number of bacterial clades. The *o__norank_c_WWE3* bacterial clades and *o_0319-6G20* bacterial clades were enriched in BAF-45-AC, *Vicinamibacteria* bacterial clades and *o_Candidatus_Peregrinibacteria* bacterial clades were enriched in BAF-30-AC and BAF-15-AC, respectively. *Brevundimonas* was enriched in BAF-60-M, which might be the bacterial branches existing in raw water of the reservoir. The higher the LDA score obtained by LDA analysis, the greater the impact of corresponding bacterial clades abundance on the difference of the microbial communities of the BAF reactors (Qiu et al., 2020). Based on the experiment, the difference among bacterial branches in the different BAF reactors may be related to the existence of different biodegradation states at the same sampling depth with different EBCT.

In order to further study the activity of microorganisms on the organic matter removal, correlation analysis was conducted between the removal rate of organic matter indexes and microbial community at the genus level (top 50 genus in abundance); see Figure 5E. It was found that *norank_f__Phycisphaeraceae* and *Hydrogenophaga* were correlated with all indicators for organic matter removal. The unclassified_o__*Rhizobiales* and *Hydrogenophaga* ($P < 0.01$), *norank_f__Amb-16S-1323*, *Ellin6067* and *norank_f__Phycisphaeraceae* ($P < 0.05$) were significantly correlated with DOC removal rates. The *norank_f__Amb-16S-1323*, *Ellin6067* genera correlated with $COD_{Cr}$ and organic matters that contain C=C double bonds and benzene ring structure removal, respectively, while *norank_f__norank_o__Rhodospirillales* was only correlated with $COD_{Cr}$ removal. The *norank_f__Vicinamibacteraceae* and *Sphingorhabdus* were negatively correlated with organic removal rate. Among the bacteria with correlation with organic matter removal, only the *norank_f__Phycisphaeraceae* had an average proportion for 0.47% in the whole process, indicating that the quantity of the key bacteria is not a key factor for organic removal. Due to the complex relationships between the different bacterial species in the microbial communities, this analysis only presents a probable assessment, which would need further testng and corroboration.

In summary, after the completion of the domestication stage, the microbial community in the different BAF reactors changed in response to the variation of raw water, which is corroborated by the results shown in Figure 5B, C and D. After the long-term operation of the systems, *Proteobacteria* was the dominant phylum in all communities. Only the fillers with strong biological attachment ability seem to be able to domesticate a stable microbial community, reliably degrade contaminating substances in raw water, and play a role in water treatment. The shorter the EBCT, the more complex the composition of microbial communities in the BAF reactors. Moreover, several microorganisms were found to be strongly associated with the removal of certain pollutants in the raw water, incuding association of *norank_f__Phycisphaeraceae* and *Hydrogenophaga* with organic matters removal, as well as *norank_f__Amb-16S-1323*, *Ellin6067* with the removal of $COD_{Cr}$ and organics containing C=C double bonds and benzene ring structures. These microorganisms may have a great potential for treating the corresponding type of water.

## 4. Conclusion

In this work, a combined system consisting of a BAF reactor and an ultrafiltration membrane was used to treat polluted surface water in a rural area, and the practical potential of the combined technology was evaluated by investigating the variation of organic matter, turbidity, chroma, and other indicators of water qualities in long-term operation. The BAF step removed organic matter, chroma, turbidity and ammonia effectively. In the ultrafiltration membrane stage for treatment of the effluents of the BAF reactors, the ultrafiltration membrane would efficiently block the microorganisms in the effluents. The turbidity and chroma of the water were further reduced by the membrane filtration, but not organic matter at a significant rate.

The combined technology showed suitable performance for some rural water treatment, and possesses potential advantages compared to other techniques, such as convenient operation and maintenance, certain practicality, and economy. However, it is still unclear whether the process can adapt to different water sources in different rural areas. It is worth noting that the experiments were conducted at laboratory scale, and that the full-scale applicability of this approach in rural areas needs to be evaluated in further experiments conducted at a larger scale.

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