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Efficiency of a Combined Biological Aerated Filter and Ultrafiltration Process for Removal of Odor Compounds in Rural Drinking Water

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ABSTRACT

Access to safe and clean drinking water is a fundamental need, and the presence of odor compounds can seriously impact consumers' perception, undermining their trust in water quality. The occurrence of *trans-1,10*-dimethyl-*trans*-9-decalol (GSM) and 2-methylisoborneol (2-MIB) is a common issue in surface water, such as lakes and reservoirs, resulting from algae bloom, which exacerbates the odor problems in summer. To address this issue, a combined process of biological aerated filter (BAF) and ultrafiltration was investigated in its efficacy in removing odor compounds under different concentrations and in achieving drinking water standards. Our results indicate that the combined process was effective, achieving abatement of turbidity and organic matter and, specifically, a removal rate of up to 96.5 % for GSM and ~100% for 2-MIB at an influent concentration of 100 ng/L, a concentration commonly associated with odor issues in rural areas. Possible biodegradation pathways of odor compounds are also discussed. Increasing the air-to-water ratio to 5:1 in the combined system greatly improved the removal rate of odor compounds, enabling it to treat raw water with concentrations as high as 300 ng/L for both GSM and 2-MIB. This combined system holds great practical potential in addressing odor issues in rural areas, offering the capability to provide safe, clean, and odor-free drinking water, thereby significantly enhancing the quality of life especially in rural communities.

KEYWORDS: Biological aerated filter (BAF); 2-methylisoborneol (2-MIB); *trans-1,10*-dimethyl-*trans*-9-decalol (Geosmin); Drinking water treatment; Biodegradation products.

1. Introduction

The quality of drinking water and its consumers' acceptance can be substantially influenced by sensory aspects [1]. In extreme cases, consumers may prefer potentially unsafe water with good appearance over aesthetically unacceptable water that is actually safe. Among the factors that affect perception, odor compounds are particularly influential indicators [2]. The odor issues caused by odor compounds can quickly destroy consumers' trust in the safety of their drinking water [3, 4]. *Trans*-1,10-dimethyl-*trans*-9-decalol (Geosmin, GSM, $C_{12}H_{22}O$) and 2-methylisoborneol (2-MIB, $C_{11}H_{20}O$) are commonly detected odor compounds produced by cyanobacteria and actinomycetes that emit earthy-musty odor [5, 6], and have caused increasingly global problems for safe drinking water supply systems [7, 8].

Many countries, including China, have incorporated guidelines for GSM and 2-MIB into their drinking water quality standards. The latest standards in China specified a limit of 10 ng/L for both GSM and 2-MIB. Wang et al. conducted a comprehensive survey of odor compounds in water sources across 31 cities and 98 drinking water treatment plants (DWPTs) in China. The study revealed a high detection frequency of 72% for 2-MIB and 64% for GSM in lakes and reservoirs, respectively [9]. A similar nationwide investigation into odor-related issues reached analogous conclusions [10]. Conventional processes in DWPTs have limited removal effect against odor compounds, and microorganism cell lysis caused by coagulation and flocculation may actually increase the concentrations of the odor compounds in water [11, 12]. Processes such as adsorption on granular activated carbon (GAC) [13-15], advanced oxidation processes (AOPs) based on, e.g., ozone [16, 17], UV and other oxidants were applied to solve the odor problem [18-21]. However, these processes were generally applied as temporary solutions, are expensive, and may create other problems related to water quality or related to the operation of the treatment system. GAC needs to be replaced when its performance deteriorates, and competitive adsorption may take place and reduce the capability of odor compounds removal [4]. AOPs require addition of chemicals and corresponding equipment, and excess oxidants and oxidation products may require substantial post-treatment [22]. These technologies can hardly be applied in DWTPs serving villages in rural areas that are dispersedly located, due to limitations such as cost, availability of labor, and technical expertise [23]. As a result, DWTPs in some rural areas are not well equipped to deal with odor problems. In addition, the water sources in rural areas are commonly nearby rivers, reservoirs and lakes, where algae is prevalent [24]. These water sources can be easily polluted by non-point source pollution caused by agricultural or domestic sewage discharges, accelerating the growth of the microorganisms, which in turn makes the odor problems more common in rural areas than in urban areas [24, 25].

A promising technology for the removal of the odor compounds in water is biological activated carbon (BAC) [26, 27]. The microorganism involved in the BAC process degrade organic micropollutants adsorbed on the material, thus rendering adsorption sites newly available for further adsorption through a material bioregeneration [28]. The combination of biodegradation and adsorption showed excellent performance in the removal of odor compounds from raw water for potabilization purposes [12]. Many studies have investigated the removal efficacy of BAC against GSM and 2-MIB under different conditions like water temperature, empty bed contact time (EBCT), and concentration of natural organic matter [27, 28]. In our previous experiments, a combined system comprising biological aerated filter (BAF) using GAC as filler and ultrafiltration was applied to treat real raw water taken from a reservoir that served as a drinking water source for nearby villages in China. The effluent of the combined system met the standards for drinking water quality in China, but needed further improvement and a more thorough understanding of its functioning and potential [23]. Odor problems also exists in the raw water and the drinking water produced by local DWPT has been reported by the local people. As a result, the potential of the coupled systems for odor removal needs to be further investigated.

In this study, the removal of odor compounds, namely, GSM and 2-MIB, aimed at the purification of water in rural areas is further investigated and improved through BAF systems combined with UF. The combined systems had been employed for the purification of the same actual rural reservoir water for 21 months before this study. The effect of different concentrations of GSM and 2-MIB is investigated, achieved by controlled addition of these compounds in the raw water. The functioning and efficacy of the combined system is thus discussed. Changes in the microbial community within the BAF reactors and possible by-products of GSM and 2-MIB biodegradation are also assessed. In addition, the opportunity of regulating and temporarily augmenting the removal capability of the combined systems for possible odor bursts is also verified, by increasing aeration in the BAF reactor.

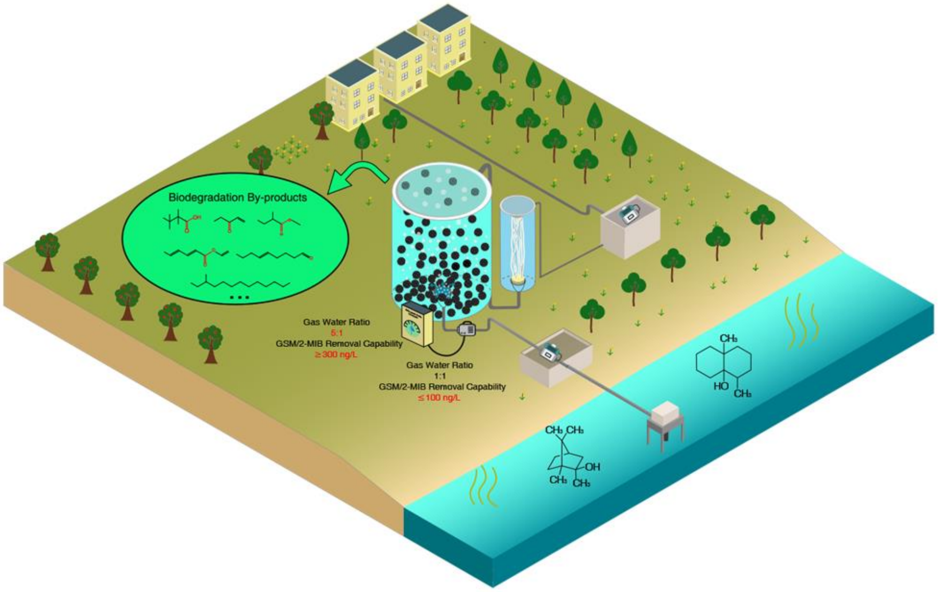

Figure 1. Installation diagram of the combined system comprising BAF and ultrafiltration for odor compounds removal aimed at the potabilization of raw water.

2. Materials and Methods

2.1. Materials

Water from a reservoir in a rural area of Zhongxian (Chongqing, China), which serves more than 10,000 people in villages and towns nearby, was collected and used as raw water in this study. Information regarding the water and BAF reactors is reported in the Supporting Information (Text S1 and Table S1). Raw water was pumped from influent tanks to the BAF reactors using peristaltic pump (BT-300-2 J, Longer Pump, China) and the effluent streams were pumped to the polyvinylidene difluoride (PVDF) ultrafiltration hollow fiber membrane modules (Litree Purifying Technology Co., Ltd, Hainan, China). Geosmin (GSM, trace CERT®), 2-MIB (trace CERT®), mixed geosmin and 2-methylisoborneol (GSM/2-MIB, trace CERT®) were purchased from Sigma-Aldrich (USA). Influent raw waters with varying odor compound content were simulated by spiking GSM and 2-MIB at three different concentrations, namely, 100 ng/L, 200 ng/L, 300 ng/L. The spiked raw waters served as influent streams immediately after spiking, to minimize volatilization of odor compounds. Wahaha purified water was used in this study for the experiments requiring pure water. Other chemicals and materials used in this study are listed in Text S2. A schematic diagram of the reactors is shown in Figure S1.

2.2. Methods

2.2.1. Operations of BAF

Information related to the functioning of different BAF reactors is listed in Table 1. The influent rate of the BAF reactors was set at 0.12 L/h throughout the experiment. The reactors were aerated with a flow rate of 0.12 L/h (gas/water ratio equal to 1/1) using aeration pumps continuously, and 15-min backwashing was performed every 15 days. The influent rate was 1.5 L/h and the aeration rate was 1.5 L/h for backwashing. The combined systems were operated indoors and the air temperature was controlled by air conditioner at 25±2 ℃. The water temperature remained between 18 and 21 ℃ throughout the experiment.

Table 1. Operational parameters of the AF reactors.

Operators ID	Fillers	EBCT (min)	Gas-water ratio	Running Time (months)
BAF-60	GAC	60	1:1	21
BAF-45	GAC	45	1:1	21
BAF-30	GAC	30	1:1	21
BAF-15	GAC	15	1:1	21

Note: The microbial communities in BAF reactors were mature and already adapted to the raw water.

2.2.2. Characterizations and Analyses of Influent and Effluent Streams

The water samples were collected from influent streams, effluent streams from BAF reactors, and effluent streams from membrane modules. The concentration of GSM/2-MIB and biodegradation by-products were measured immediately after sample collection using gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) system coupled with solid phase micro-extraction (SPME) [29]. Enrichment and extraction of odor components and their biodegradation by-products in water samples were carried out through a SPME fiber, then the SPME fiber was analyzed with GC-MS/MS (Agilent 8890 Series gas chromatograph, HP-5 MS capillary column Agilent, Agilent 7010B mass selective detector, Agilent, USA). The chemical oxygen demand (COD_{Mn}) of the samples were measured based on the acidic potassium permanganate method. The UV absorbance at 254 nm (UV_{254}) and the fluorescence excitation-emission matrix (EEM) of the pre-filtered samples were measured using UV-Vis spectrophotometry (Orion AquaMate 8000, Thermo Fisher Scientific INC., MA, USA) and fluorescence spectrophotometry (F-7000, Hitachi, Japan), respectively. The chroma of the samples was also detected by UV-Vis spectrophotometry. The turbidity of the samples was determined with a turbidimeter (TL2310, Hach company, USA). The detailed methods and parameters can be found in Text S3 and in our previous studies [30, 31].

2.2.3 Microbial Diversity Sequencing Analysis

The 16s rRNA gene exists in the genome of all bacteria and can inform about the prevalence and the relationship between bacteria. During the experiment, 2 g filler were collected at about 3 cm depth from the top of the BAF reactors, followed by filler backfill. The microbial communities of the fillers were analyzed through the 16s rRNA method to gain information about microbial community variation and the dominant functional microorganisms. The sequencing of 16s rRNA took place on the 10th, 60th, and 110th day after the beginning of the test. Details of the microbial diversity sequencing analysis are presented in Text S3.

3. Results and Discussion

3.1. Removal of Odor Compounds in Raw Water by the Combined System

The systems were employed to treat influent streams for 21 months before, during which the microbial communities attached to the BAF fillers adapted to the aqueous matrix [23]. Raw water with different concentrations of GSM/2-MIB (100 ng/L, 200 ng/L, and 300 ng/L) were separately pumped into distinct, parallel systems to assess the odor compounds removal efficacy. Detailed data regarding the concentrations of odor compounds in the influent and effluent streams are presented in Figure 2. As GSM/2-MIB volatilized in the water tank during the experiment, a certain error occurred between the actual and the nominal concentrations. The removal rates of the odor compounds were calculated using the concentrations of GSM/2-MIB in effluent streams and influent streams (actual detected value). The average proportions of the odor compounds escaped from the influent streams can be seen in Table S2 (SI). When treating raw water spiked with 100 ng/L odor compounds, the GSM removal rates were consistent at approximately 100% across different combined systems. For 2-MIB, the removal rates were 98.2% (BAF-60), 97.3% (BAF-45), 96.5% (BAF-30), 94.3% (BAF-15). At higher influent concentration of 200 ng/L, the combined system exhibited slightly lower removal performance. Specifically, the GSM removal rates were 86.9% (BAF-60), 82.5% (BAF-45), 77.2% (BAF-30), and 75.3% (BAF-15), while the removal rates for 2-MIB decreased were 95.4% (BAF-60), 92.7% (BAF-45), 87.6% (BAF-30), and 85.4% (BAF-15), respectively. Similar removal rates were observed with an influent concentration equal to 300 ng/L, namely, GSM removal rates were 88.4 % (BAF-60), 85.0 % (BAF-45), 80.9 % (BAF-30), and 75.3 % (BAF-15), while the removal rates of 2-MIB were 89.8 % (BAF-60), 87.6 % (BAF-45), 86.0 % (BAF-30), and 82.9 % (BAF-15). The various average removal rates are summarized in Figure 2C.

Based on the data reported above, when the initial concentrations of GSM/2-MIB were 100 ng/L, the concentrations of GSM and 2-MIB in the effluent streams of the BAF reactors were reduced to less than 10 ng/L, meeting the latest drinking water standards in China. As the concentration of odor compounds in the influent stream increased, we observed a rise in the volatilization of odor compounds, accompanied by a reduction in the removal rates provided by the combined system. Therefore, the effluents of the combined system may fail to meet the standard for concentrations higher than 100 ng/L. The removal performance of the combined system partially stems from the ability for bioregeneration of the adsorbent fillers in the BAF reactors. Microorganisms played a crucial role in degrading both adsorbed and dissolved odor compounds, as well as other constituents present in the influent water [12].

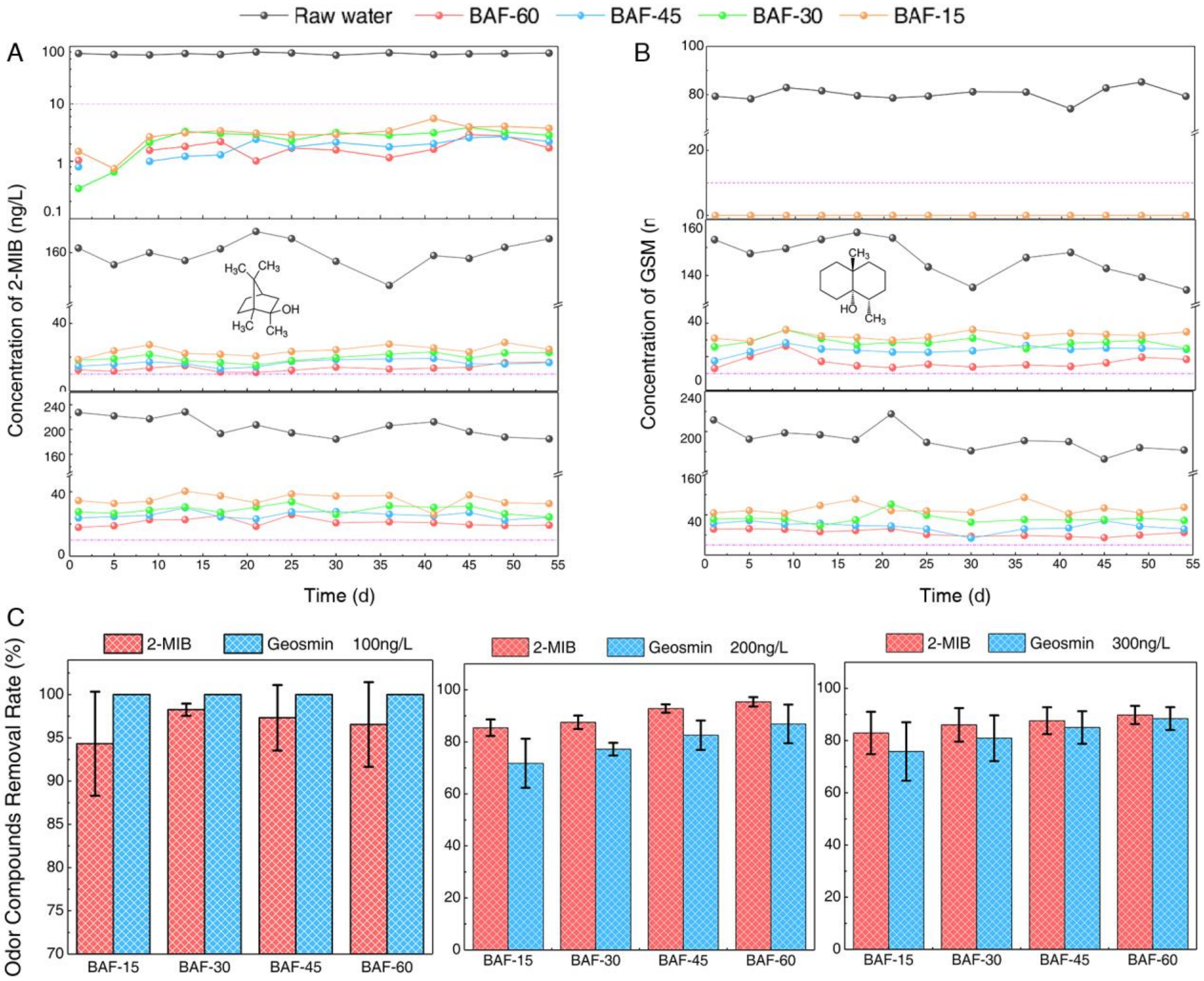

Figure 2. Concentration of odor compounds in influent and effluent streams of different BAF reactors and corresponding average removal rates. A: GSM concentrations in influent and effluent streams of BAF reactors when different initial GSM concentration was spiked into raw water (100 ng/L, 200 ng/L and 300 ng/L from top to bottom); B: 2-MIB concentrations in influent and effluent streams of BAF reactors when different initial GSM concentration was spiked into raw water (100 ng/L, 200 ng/L and 300 ng/L from top to bottom); C: Removal rates observed in different BAF reactors for varying influent concentrations of odor compounds (100 ng/L, 200

ng/L and 300 ng/L from left to right). The dotted lines represent the concentration limits for GSM/2-MIB (10 ng/L) in standards for drinking water quality of China.

3.2. Odorous Compound Biodegradation Pathways and By-products

By comparing the chromatograms and mass spectrometry results of water samples from conical flasks containing BAC and GSM/2-MIB after contact on a vibrating thermostatic water bath, several possible biodegradation by-products of GSM/2-MIB were identified, after excluding the background and possible interfering substances, which are listed in Table S3 (SI). The detailed experimental protocol can be found in Text S3 (SI). Figure 3 illustrates the possible degradation pathways of GSM/2-MIB based on the analytical results.

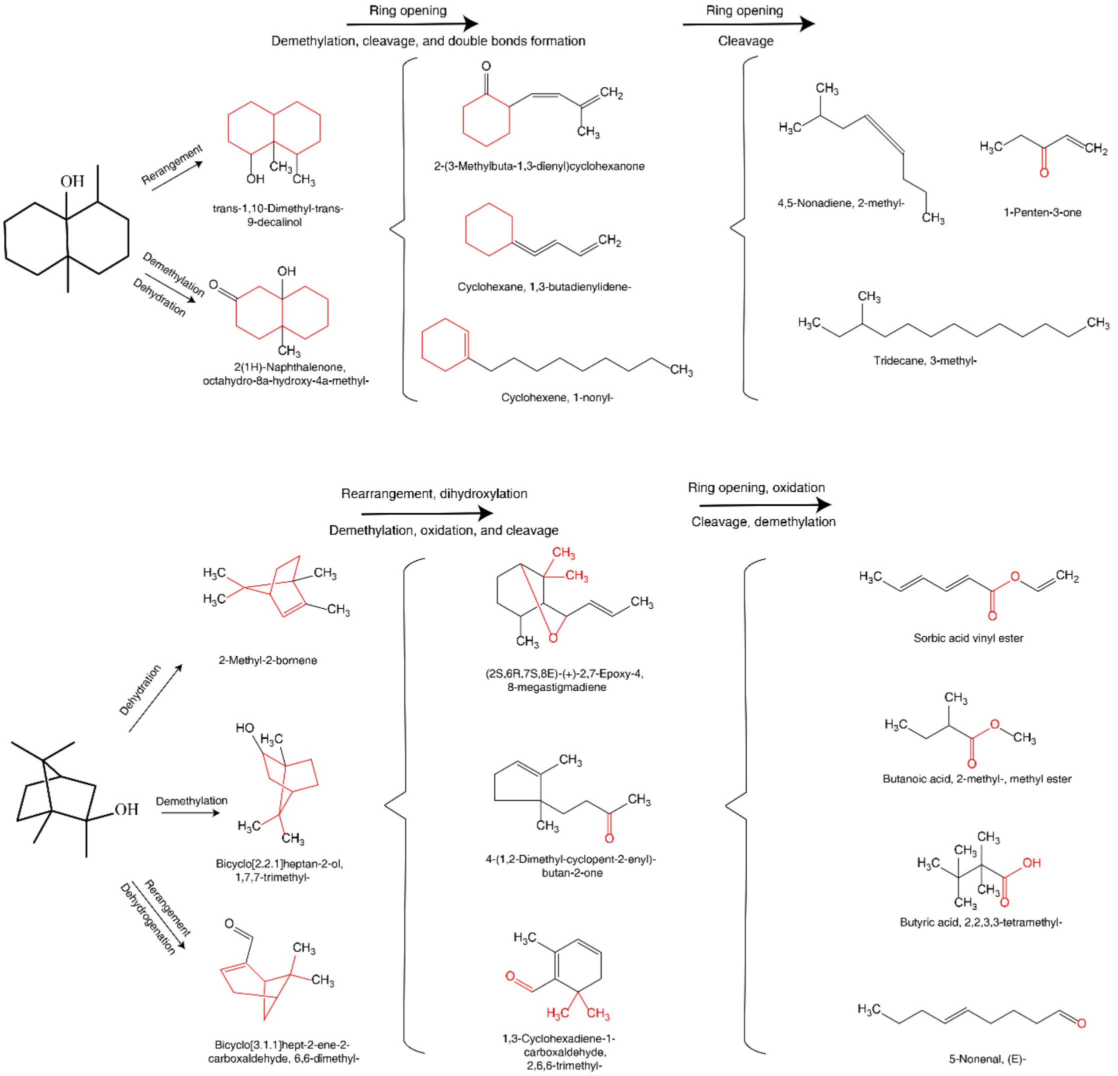

Figure 3. Possible pathway and by-products of the biodegradations of GSM and 2-MIB in the BAF reactors.

As the most likely degradation pathway of GSM/2-MIB involved dehydration and ring opening [32, 33]. Based on literatures concerning the degradation pathways of GSM/2-MIB under background of pure materials [32, 34-36]. The intermediates and biodegradation by-products of GSM/2-MIB were identified through chromatograms and mass spectrometry results. The detailed methods and processes were listed in Text S4 (SI), and the results were presented in Figure 3. This process resulted in the formation of various low molecular weight degradation products, mostly alkanes, are generally considered odorless. In summary, the final possible biodegradation by-products of GSM/2-MIB hypothesized in this study were generally small molecule products, most of which were odorless and harmless.

The degradation of GSM/2-MIB in combined systems mainly depends on the microbial communities in BAF reactors. Variety of relationships, such as synergy, symbiosis, and competition among microorganisms existed in the microbial communities. The locations of the broken bonds of GSM/2-MIB during biodegradation may vary. Besides, even though the BAC was pre-treated with pure water before the degradation experiments, the adsorbed substances of BAC may desorb during the experiment and affect the results. Compounds such as 3-Hexenamide, N,N,2-trimethyl-, (E)-(.+-.)- ($C_9H_{11}NO$) and 2-Butanamine, 3,3-dimethyl-($C_6H_{15}N$), containing nitrogen, were also observed in the chromatograms and mass spectrometry results. Despite several repeated experiments, the chromatograms and mass spectrometry results were slightly different each time. As a result, the complete determination of biodegradation by-products and pathways for GSM and 2-MIB in BAF reactors proved challenging. The possible biodegradation by-products we found in the degradation experiments using pure water with 1 mg/L GSM/2-MIB were odorless and harmless. When treating influent streams with 100 ng/L GSM/2-MIB, the effluent streams of combined systems can be odorless and harmless, indicating effective and safe biodegradation occurring in BAF reactors.

3.3. Removal of Organic Matter

Turbidity and chroma measurements for the raw water, BAF reactor effluent streams, and effluents from the membrane modules are presented in Figure 4A and B. Following BAF reactor processing, effluent streams, except for BAF-15, met the drinking water standard (≤ 1.0 NTU, ≤ 15 Hazen). BAF-15 exhibited an average effluent turbidity exceeding the standard (1.11 ± 0.29

NTU). The subsequent membrane process effectively addressed this issue, resulting in an average decline of 0.43 ± 0.13 NTU between influent and effluent streams. Consequently, the effluent streams of the combined system fully complied with the standard. Furthermore, the ultrafiltration membrane employed in the process can also prevent the leakage of microorganisms from the BAF reactors [23] .

Investigation into the removal of organic matter was conducted by determining the variation in COD_{Mn} values and UV_{254} values, as shown in Figure 4C and D. Organic matter removal predominantly occurred in the BAF step, with average COD_{Mn} values of 0.99±0.40 mg/L (BAF-60), 2.05±0.34 mg/L (BAF-45), 2.04±0.04 mg/L (BAF-30), and 2.12±0.11 mg/L (BAF-15), exiting the BAF reactors. The subsequent membrane processes had minimal impact on organic matter removal, as evidenced by the negligible difference in UV_{254} values between effluent streams from BAF reactors and final effluents. Fluorescence EEM spectroscopy was utilized to identify the removal rates of different dissolved organic components in raw water by the combined system. The spectra were divided into five regions using fluorescence regional integration (FRI) [30, 37]. The spectra of streams related to the BAF-60 combined systems are presented in Figure 4E to G, the spectra of streams collected in other combined systems are reported in Figure S3 (SI). The overall removal rates provided by different combined system is shown in Figure 4H. The dissolved organic matter in raw water mainly comprised of tryptophan protein-like substances (region II), fulvic acid-like matters (region III), and humic acid-like components (region V) (Figure 4E). The various removal rates were estimated as follows: tryptophan protein-like substances (Region II) at 70.3% (BAF-60), 59.3% (BAF-45), 64.5% (BAF-30), and 29.5% (BAF-15); fulvic acid-like matters (region III) at 82.4% (BAF-60), 74.1% (BAF-45), 81.4% (BAF-30), and 61.5% (BAF-15); humic acid-like components (region V) at 83.3% (BAF-60), 70.2% (BAF-45), 76.6% (BAF-30), and 61.3% (BAF-15), respectively. Therefore, the system with a longer EBCT exhibited superior performance in organic matter removal, while a somewhat descending trend was observed as a function of EBCT.

Overall, through joint analysis of COD_{Mn} removal, UV_{254} removal, and EEM results, the BAF reactor characterized by a 60-minute EBCT showed advantages in terms of water product quality over BAF reactors characterized by shorter EBCTs. The combined system achieved effective removal of approximately 80% (BAF-60) of organic matter, even with added odor compounds in the influent streams. Indeed, from an economic perspective, a 30-minute EBCT is preferable, and still ensured meeting conventional water quality indicators and adequate odor substance removal. In this sense, a trade-off does exist between 30 and 60 minutes EBCT between economic and quality considerations

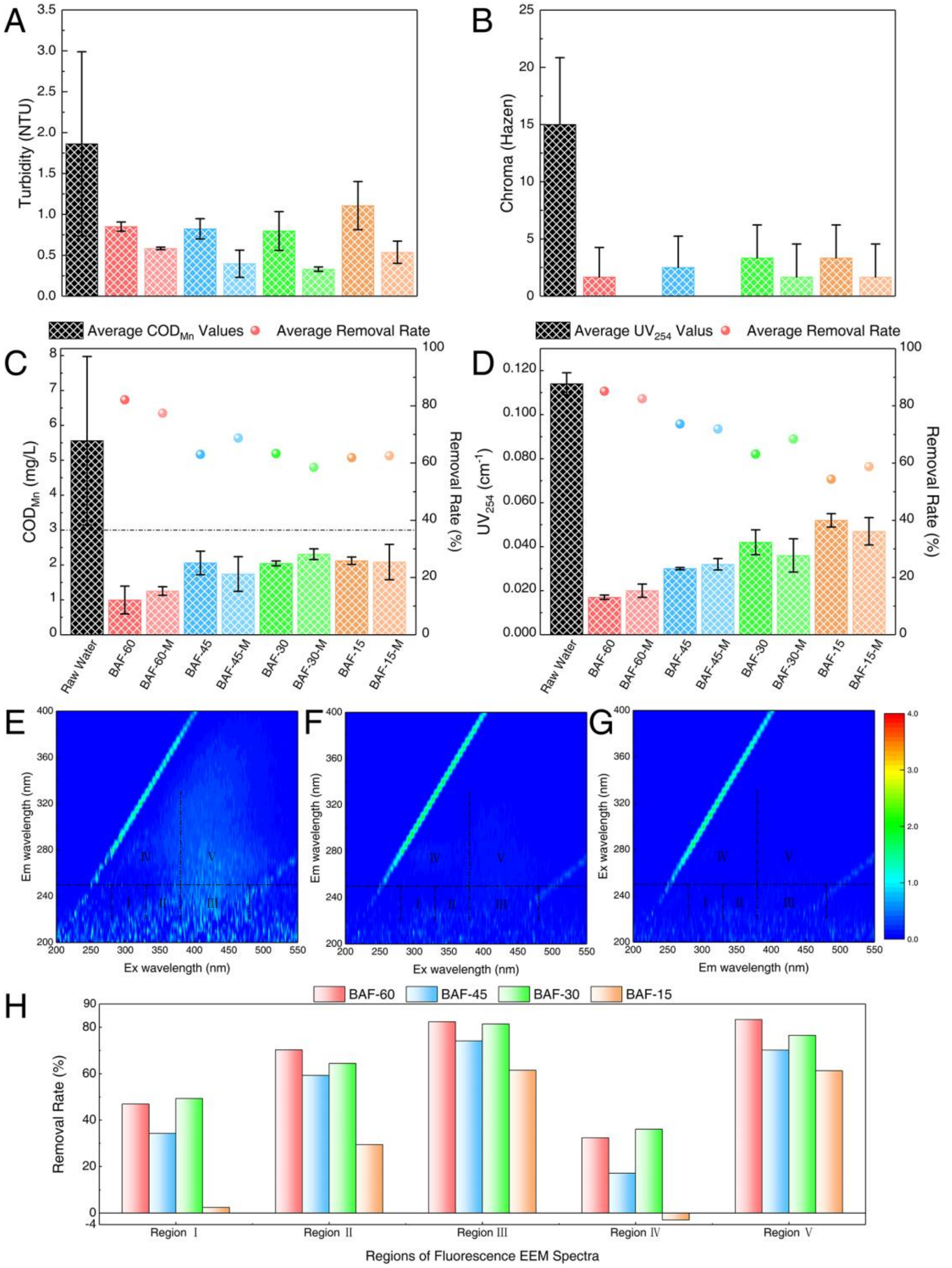

Figure 4. Water quality indexes of water streams in different combined systems. A: Average turbidity values; B: Average chroma values; C: Average COD$_{Mn}$ values (dots referring to the left axis) and their corresponding removal rates (bars referring to the right axis); D: Average UV$_{254}$ values (dots referring to the left axis) and their corresponding removal rates (bars referring to the right axis); E: Fluorescence EEM spectrum of raw water; F:

Fluorescence EEM spectrum of the effluent stream from the BAF-60 reactor; G: Fluorescence EEM spectrum of the effluent stream from the final membrane process; H: Average removal rates of different organic components by the combined system. (Region I: (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 = 250-400/280-380 nm, soluble microbial by-product-like matters), and region V: (Ex/Em = 250-400/380-540 nm, humic acid-like components)). The dotted lines represent the limits for CODMn in standards for drinking water quality of China.

3.4 Microbial Community Structure in the BAF Reactors.

The microbial communities within the BAF reactors played a crucial role in the degradation of odor compounds and organic matter in general, and their composition was analyzed through 16s-rRNA gene sequencing. Table 2 provides an overview of the microbial communities, presenting data on the number of effective sequences, operational taxonomic units (OTUs), and alpha diversity indexes. The reliability of the sequencing results of the biological detection of the microorganism was underscored by the coverage indexes detailed in Table 2. Additionally, the rarefaction curves depicted in Figure 5A, further corroborates the reliability of the obtained microbial community data [30, 38]. The richness index (chao) and the diversity index (shannon) of the microbial community in BAF reactors exhibited an initial increase followed by a subsequent decrease, except for the reactor in BAF-45 combined system. This result may be attributed to the introduction of odor compounds in raw water, leading to structural changes in the microbial communities, followed by subsequent stabilization. Detailed composition of microbial communities in different BAF reactors at the phylum and genus levels are summarized in Figure 5B and C.

Table 2. Number of operational taxonomic units (OTUs), alpha diversity indexes for microbial communities in different BAB-UF reactors at different periods

Sample ID	Number of effective Sequences	OTU at 97% identity	shannon	chao	coverage
Raw Water	38989	718	4.42	850.78	0.995
BAF-60 (10d)	51025	862	3.74	952.32	0.997
BAF-60 (40d)	72597	1198	5.37	1382.80	0.995
BAF-60 (70d)	64598	806	3.44	899.33	0.997
BAF-45 (10d)	56835	994	4.80	1079.01	0.997
BAF-45 (40d)	64779	1018	4.53	1222.97	0.995
BAF-45 (70d)	64638	1091	4.99	1253.39	0.996
BAF-30 (10d)	54184	1188	4.73	1387.27	0.995
BAF-30 (40d)	73737	1145	5.03	1367.24	0.994
BAF-30 (70 d)	60269	891	4.49	1035.58	0.996
BAF-15 (10 d)	60329	1154	4.75	1350.78	0.995
BAF-15 (40 d)	65722	1123	5.22	1362.76	0.995
BAF-15 (70 d)	68396	934	4.72	1100.57	0.996

The *Proteobacteria* dominated the phylum level in all BAF reactors throughout the experiment, constituting an average of 70.8% during the initial period. As the experiment progressed, the proportion of Proteobacteria steadily decreased to an average of 45.0%. Other major phyla in the BAF reactors included *Chloroflexi* (8.1% on average), *Firmicutes* (5.4% on average), *Acidobacteriota* (5.3% on average), and *Nitrospirota* (5.2% on average), collectively accounting for at least 67.3% of the entire community in all systems. The microbial community within the same BAF reactor exhibited changes throughout the experiment, likely influenced by variations in the influent water. The raw water was taken from a reservoir and the experiment spanned the entirety of the winter, with the microbial communities likely affected by changes of both temperature and water composition. The phyla experiencing the most substantial relative changes during the experiment were *Proteobacteria* (a decline of 29.7%), *Nitrospirota* (an increase of 12.9%), *Chloroflexi* (an increase of 9.6%), and *Acidobacteriota* (an increase of 6.9%). The latter three phyla demonstrated adaptability to the conditions associated with raw water in winter. Despite microbial community changes, the removal rate of organic matter by the combined systems was not considerably affected, and the effluent streams consistently met drinking water standards. Figure 5C illustrates that, among the 65 genera, 38 belonged to *Proteobacteria*, while 13 were associated with *Nitrospirota*, *Chloroflexi*, and *Acidobacteriota*. The majority of microorganisms belonging to *Proteobacteria* were heterotrophic, perform respiration and fermentative metabolism using organic matter as carbon source. These dominant microorganisms may play a pivotal role in the degradation of organic matter.

The linear discriminant analysis effect size (LEfSe) method was employed to identify microorganisms with significant influence on the differences in microbial communities within the BAF reactors, and the results are presented in Figure 5D. These disparities were primarily attributed to distinct biodegradation states among BAF reactors with varying EBCTs. Given the low concentrations of GSM/2-MIB, the odor compounds may have exerted only marginal influence on the composition of microbial communities. To further investigate the key microorganisms for the removal of odor compounds, the top 50 genera in abundance were selected for the correlation analysis using the removal rates of GSM/2-MIB 10 days before and after 16s-rRNA sampling. Six genera, such as *Sphaerotilus*, *unclassified_f__Comamonadaceae*, *unclassified_o__Rhizobiales*, *Hydrogenophaga*, *unclassified_f__Rhodocyclaceae*, and

Streptococcus were found to be associated with the removal of both GSM and 2-MIB to varying extents. Specifically, *unclassified_o__Rhizobiales* exhibited a stronger association with 2-MIB removal, while *unclassified_f__Rhodocyclaceae* and *Streptococcus* were more correlated with GSM removal. *SWB02* and *unclassified_f__TRA3-20* were exclusively linked to the removal of 2-MIB. Several genera exhibited instead a negative correlation with the removal of odor compounds, with seven genus, such as *unclassified_f__Anaerolineaceae*, *unclassified_f__Chromobacteriaceae*, *unclassified_c__JG30-KF-CM66*, *Enterobacter*, *unclassified_f__Beggiatoaceae*, *unclassified_p__Latescibacterota*, and *unclassified_f__Gemmataceae*, playing negative roles in the removal of GSM/2-MIB according to the correlation analysis. Additionally, seven genera demonstrated a negative impact on the removal of either GSM or 2-MIB exclusively, including *unclassified_f__JG30-KF-CM45* and *unclassified_c__OM190*. The results are summarized in Figure 5E. Among the top 50 genera in abundance, 22 were found to have an influence on the removal of odor compounds. Notably, certain genera, such as *Hydrogenophaga* and *unclassified_f__Vicinamibacteraceae*, exhibited similar correlations with the removal of organic matter in water [23]. Some microorganisms may primarily utilize odor compounds as a carbon source for growth and propagation, others may use organic matter and be indifferent to odor compounds, adding complexity to the relationships between microorganisms and removal as well as among microorganisms, including competition and cooperation. Despite this complexity, the detected microorganisms consistently provided adequate odor compounds removal, suggesting their potential in cases whereby odor issues may occur. We further classified the 50 genera to phylum level and found that the *Proteobacteria* and *Campylobacterota* are more relevant to GSM/2-MIB removal compared to other phyla. The *Alpha-Proteobacteria* and *Gamma-Proteobacteria* were the main *Proteobacteria* in the BAF reactors.

They are facultative anaerobe, taking organic matter in raw water as the carbon source and conducting respiration and fermentation metabolism simultaneously [39]. While *Campylobacterota* can not use sugars, and relies on amino acids as energy sources, it may participate in the biodegradation processes of GSM/2-MIB as indirect participant. Further experimentation utilizing only odor compounds as carbon sources is needed to further investigate the key microorganisms involved in odor compounds removal.

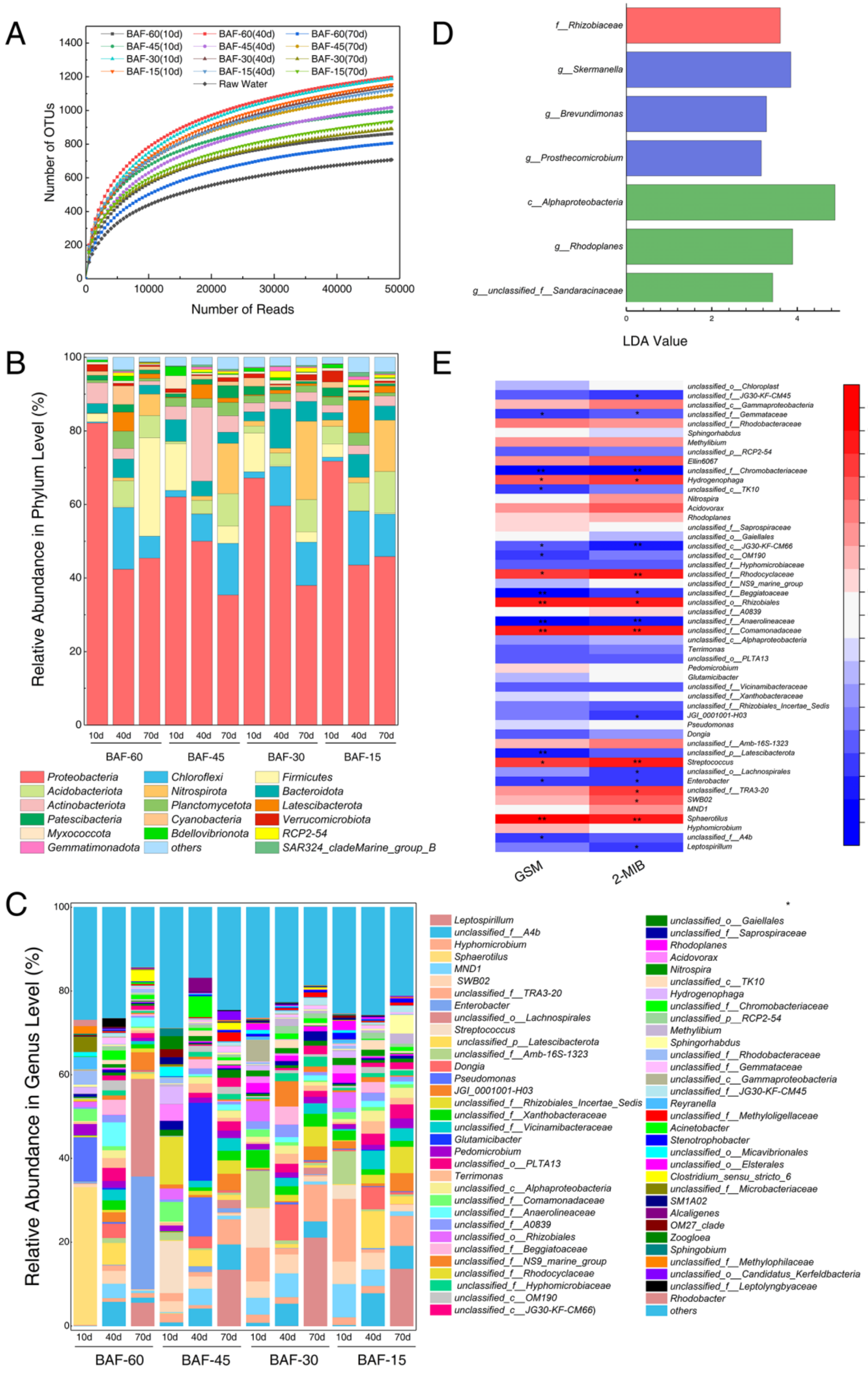
A
BAF-60(10d)
BAF-45(10d)
BAF-30(10d)
BAF-15(10d)
BAF-60(40d)
BAF-45(40d)
BAF-30(40d)
BAF-15(40d)
Raw Water
BAF-60(70d)
BAF-45(70d)
BAF-30(70d)
BAF-15(70d)
Number of OTUs
Number of Reads
0
200
400
600
800
1000
1200
1400
10000
20000
30000
40000
50000
D
f__Rhizobiaceae
g__Skermanella
g__Brevundimonas
g__Prosthecomicrobium
c__Alphaproteobacteria
g__Rhodoplanes
g__unclassified_f__Sandaracinaceae
0
2
4
LDA Value
B
Relative Abundance in Phylum Level (%)
0
20
40
60
80
100
10d
40d
70d
BAF-60
BAF-45
BAF-30
BAF-15
Proteobacteria
Acidobacteriota
Actinobacteriota
Patescibacteria
Myxococcota
Gemmatimonadota
Chloroflexi
Nitrospirota
Planctomycetota
Cyanobacteria
Bdellovibrionota
others
Firmicutes
Bacteroidota
Latescibacterota
Verrucomicrobiota
RCP2-54
SAR324_cladeMarine_group_B
E
unclassified_o__Chloroplast
unclassified_f__JG30-KF-CM45
unclassified_c__Gammaproteobacteria
unclassified_f__Gemmataceae
unclassified_f__Rhodobacteraceae
Sphingorhabdus
Methylibium
unclassified_p__RCP2-54
Ellin6067
unclassified_f__Chromobacteriaceae
Hydrogenophaga
unclassified_c__TK10
Nitrospira
Acidovorax
Rhodoplanes
unclassified_f__Saprospiraceae
unclassified_o__Gaiellales
unclassified_c__JG30-KF-CM66
unclassified_c__OM190
unclassified_f__Hyphomicrobiaceae
unclassified_f__Rhodocyclaceae
unclassified_f__NS9_marine_group
unclassified_f__Beggiatoaceae
unclassified_o__Rhizobiales
unclassified_f__A0839
unclassified_f__Anaerolineaceae
unclassified_f__Comamonadaceae
unclassified_c__Alphaproteobacteria
Terrimonas
unclassified_o__PLTA13
Pedomicrobium
Glutamicibacter
unclassified_f__Vicinamibacteraceae
unclassified_f__Xanthobacteraceae
unclassified_f__Rhizobiales_Incertae_Sedis
JGI_0001001-H03
Pseudomonas
Dongia
unclassified_f__Amb-16S-1323
unclassified_p__Latescibacterota
Streptococcus
unclassified_o__Lachnospirales
Enterobacter
unclassified_f__TRA3-20
SWB02
MND1
Sphaerotilus
Hyphomicrobium
unclassified_f__A4b
Leptospirillum
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
-0.10
-0.20
-0.30
-0.40
-0.50
-0.60
-0.70
-0.80
-0.90
GSM
2-MIB
C
Relative Abundance in Genus Level (%)
0
20
40
60
80
100
10d
40d
70d
BAF-60
BAF-45
BAF-30
BAF-15
Leptospirillum
unclassified_f__A4b
Hyphomicrobium
Sphaerotilus
MND1
SWB02
unclassified_f__TRA3-20
Enterobacter
unclassified_o__Lachnospirales
Streptococcus
unclassified_p__Latescibacterota
unclassified_f__Amb-16S-1323
Dongia
Pseudomonas
JGI_0001001-H03
unclassified_f__Rhizobiales_Incertae_Sedis
unclassified_f__Xanthobacteraceae
unclassified_f__Vicinamibacteraceae
Glutamicibacter
Pedomicrobium
unclassified_o__PLTA13
Terrimonas
unclassified_c__Alphaproteobacteria
unclassified_f__Comamonadaceae
unclassified_f__Anaerolineaceae
unclassified_f__A0839
unclassified_o__Rhizobiales
unclassified_f__Beggiatoaceae
unclassified_f__NS9_marine_group
unclassified_f__Rhodocyclaceae
unclassified_f__Hyphomicrobiaceae
unclassified_c__OM190
unclassified_c__JG30-KF-CM66)
unclassified_o__Gaiellales
unclassified_f__Saprospiraceae
Rhodoplanes
Acidovorax
Nitrospira
unclassified_c__TK10
Hydrogenophaga
unclassified_f__Chromobacteriaceae
unclassified_p__RCP2-54
Methylibium
Sphingorhabdus
unclassified_f__Rhodobacteraceae
unclassified_f__Gemmataceae
unclassified_c__Gammaproteobacteria
unclassified_f__JG30-KF-CM45
Reyranella
unclassified_f__Methyloligellaceae
Acinetobacter
Stenotrophobacter
unclassified_o__Micavibrionales
unclassified_o__Elsterales
Clostridium_sensu_stricto_6
unclassified_f__Microbacteriaceae
SM1A02
Alcaligenes
OM27_clade
Zoogloea
Sphingobium
unclassified_f__Methylophilaceae
unclassified_o__Candidatus_Kerfeldbacteria
unclassified_f__Leptolyngbyaceae
Rhodobacter
others

Figure 5. Results of the microbial community analysis in the raw water and in different BAF reactors: A. Rarefaction curves of OTUs at different times. B. Bacterial community composition at phylum level (> 1%) at different times. C. Bacterial community compositions at genus level (> 1.5%) at different times. D. Results of the Lefse multistage species difference discriminant analysis of microbial communities in different BAF reactors. E. Correlation analysis between microbial community at genus level (top 50) and environmental variables (GSM/2-MIB removal rate). Here, "*" represents a value of $p < 0.05$ and "**" represents a value of $p < 0.01$.

3.5 Odor Compounds Removal Efficacy at Different Gas-Water Ratios

The removal of odor compounds in raw water predominantly relies on the biodegradation processes of the microbial communities within the BAF reactors of the combined system. As detailed in section 3.1, the nominal concentration of odor compounds in raw water exceeded the concentrations that was actually detected. This discrepancy may be attributed to volatilization of a certain proportion of odor compounds. The aeration within BAF reactors served to facilitate this process, thereby enhancing the overall efficacy of odor compounds removal, by combining biodegradation to air stripping and by enhancing the activity of microorganisms via oxygen supply. Under normal circumstances, the concentration of odor compounds is low, except for sporadic events, such as sudden surges in the summer, particularly during algae blooms. From an economic standpoint, it would be more judicious to equip DWTPs with a flexible system designed to augment the removal capability of odor compounds only when needed, as opposed to maintaining a dedicated oversized system in continuous operation throughout the year. To address sudden outbreaks of odor compounds without significantly altering the operational state of the systems, an investigation was conducted into the efficacy of temporary changes in the gas-water ratio of the BAF process. Specifically, gas-water ratios of 5:1 and 3:1 were investigated, and the results are summarized in Table 3. The detail procedures were listed in Text S3 and Table S4 (SI). It is difficult for the microorganisms in BAF reactors to adapt to a new environment with changed aeration rate and concentrations of odor compounds in influent streams within a maximum 90 minutes. As a result, we concluded that the microbial communities did not have enough time to adapt to the new environment.

Upon altering the gas-water ratio to 3:1 and maintaining an initial GSM/2-MIB concentration of 200 ng/L, the concentrations of odor compounds in the effluent streams were as follows: 1.42 ng/L (BAF-60), 5.65 ng/L (BAF-45), 8.37 ng/L (BAF-30), and 10.23 ng/L (BAF-15) for GSM; 0.94 ng/L (BAF-60), 1.13 ng/L (BAF-45), 5.63 ng/L (BAF-30), and 12.31 ng/L (BAF-15) for 2-MIB. Subsequently, when the initial concentration was increased to 300 ng/L, the corresponding concentrations in the effluent streams were 9.82 ng/L (BAF-60), 15.27 ng/L (BAF-45), 21.33 ng/L (BAF-30), and 22.92 ng/L (BAF-15) for GSM; 2.11 ng/L (BAF-60), 4.64 ng/L (BAF-45), 7.71 ng/L (BAF-30), and 12.65 ng/L (BAF-15) for 2-MIB. Upon further increasing the gas-water ratio to 5:1, the efficacy of odor compound removal improved. For example, at an initial GSM/2-MIB concentration of 200 ng/L, the GSM concentration in the effluent stream of BAF-15 was 1.91 ng/L, with GSM undetected in the effluent streams of other BAF reactors. Concurrently, 2-MIB concentrations were 1.47 ng/L and 3.26 ng/L for the effluent streams of BAF-30 and BAF-15, respectively, with no 2-MIB detected in BAF-60 and BAF-45. At an initial concentration of 300 ng/L, the GSM concentrations in the effluent streams were 2.32 ng/L (BAF-60), 3.17 ng/L (BAF-45), 6.25 ng/L (BAF-30), and 8.17 ng/L (BAF-15). Furthermore, 1.47 ng/L and 3.26 ng/L of 2-MIB were detected in the effluent streams of BAF-30 and BAF-15, while no 2-MIB was detected in BAF-60 and BAF-45 at this concentration.

Therefore, an increase in the gas-water ratio yielded a substantial reduction in the concentration of odor compounds within the effluent streams of the BAF reactors. In instances where the concentrations of GSM/2-MIB in the influent water increased to 200 ng/L and 300 ng/L without allowing sufficient time for the microbial communities in BAF reactors to acclimate, the combined system demonstrated efficacy in treating raw water with elevated concentrations of GSM/2-MIB, achieving compliance with drinking water standards (GSM/2-MIB concentration ≤ 10 ng/L). Note that, based on existing literature, the concentration of odor compounds in raw waters typically does not exceed 300 ng/L during outbreaks in rural areas in the summer [40, 41]. It is also important to acknowledge that the aeration process constitutes a substantial proportion of the operational costs of the system. Therefore, increasing the gas-water ratio is deemed suitable only when the concentration of odor compounds in raw water surpasses the treatment capability of the biodegradation process.

Table 3. Removal rate provided by BAF reactors under different gas-water ratios for different initial odor compounds concentrations.

Gas-water Ratio	Initial Concentration (ng/L)		BAF-60 (ng/L)	BAF-45 (ng/L)	BAF-30 (ng/L)	BAF-15 (ng/L)
1:3	GSM	300	9.82	15.27	21.33	22.92
	GSM	200	1.42	5.65	8.37	10.23
	2-MIB	300	2.11	4.63	7.71	12.65
	2-MIB	200	0.94	1.13	5.63	12.31
1:5	GSM	300	2.32	3.17	6.25	8.17
	GSM	200	—	—	—	1.91
	2-MIB	300	—	—	3.43	6.31
	2-MIB	200	—	—	1.47	3.26

4. Conclusion

In this study, the efficacy of BAF and ultrafiltration combined systems was explored in the treatment of surface water featuring varying concentrations of typical odor compounds (GSM and 2-MIB). At an influent GSM/2-MIB concentration of 100 ng/L, all effluent streams met the

Standard of Drinking Water Quality of China (GB 5749-2022). Specifically, the BAF-60 system exhibited a remarkable removal efficacy, eliminating 100 % of GSM and nearly 95 % of 2-MIB from the influent stream. Importantly, the introduction of odor compounds into raw water did not compromise the removal capability of the combined systems for other drinking water quality indicators, such as organic matter content, turbidity, and chroma.

Possible biodegradation pathway and biodegradation by-products of GSM/2-MIB were proposed, revealing odorless and non-toxic biodegradation by-products based on the results. Microbial community analysis through 16s rRNA was also conducted and identified likely microorganisms associated with the removal of GSM/2-MIB. The removal efficacy of the aeration of combined system against odor compounds was further assessed by altering the gas-water ratio, indicating that even with an initial GSM/2-MIB concentration of 300 ng/L in raw water, the effluent streams met the corresponding standards at suitable gas flow rates. Considering both efficacy and cost, the combined system with an EBCT of 30 minutes emerged as an optimized choice for rural areas where drinking water sources are impacted by odor compounds.

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