



## Influence of the type of activated carbon on invertebrate leakage in biological activated carbon filter

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### ABSTRACT

The relationship between the type of activated carbon and invertebrate leakage in biological activated carbon (BAC) filters was investigated in a full-scale test. The results showed that the specific surface area and total pore volume, and the particle size of the activated carbon were the two main factors that affected the invertebrate leakage. The larger the specific surface area and total pore volume were, the larger was the number of invertebrates that grew on the activated carbon and penetrated through the carbon layers. The larger the particle size was, the easier it was for the invertebrates to penetrate through the carbon layers. The BAC filters had similar effects on the removal of organic matter, which was attributed to the similar bacterial community compositions on the activated carbon layers. The dominant bacteria genera on the three BACs were *Bacillus*, *Pseudomonas*, and *Lactococcus*, and their proportions were similar. Hence, on the premise that the carbon particles are not flushed out during backwashing, activated carbon with a smaller particle size should be preferred to that with a larger particle size. Carbon age should not be the only evaluation indicator to determine whether the activated carbon should be regenerated or replaced.

*Keywords:* Type of activated carbon; Invertebrates; Microbial properties; Ozone–BAC

### 1. Introduction

The combination of ozone and biological activated carbon (BAC) process has been widely applied in the production of potable water due to the outstanding removal efficiency of organic contaminants [1,2]. However, biological leakage in the effluent of BAC filters is a problem that has gradually gained increasing attention [3,4]. These leaked microorganisms can include bacteria, protozoa, and metazoa (so-called invertebrates); the invertebrates are mainly discussed here. Studies have shown that the average abundance of invertebrates in the effluent of the BAC filter could be approximately 30 times larger than that in the influent [5]. In addition, the leaked invertebrates from BAC filters may flow into the water supply network directly if no other filtration barrier exists after the BAC filter.

Invertebrates with diameters of 500  $\mu\text{m}$ –3 mm are visible, such as adult copepods and oligochaeta [4]. Once they appear in the water supply network, they may bring about not only aesthetic problems but also could easily cause consumers to lose confidence in the water quality [6–8]. Furthermore, some invertebrates could protect microorganisms from disinfection, which may transport pathogenic microorganisms into the drinking water system and finally reach customers [9–12].

Measures should thus be adopted to control the leakage of invertebrates. Before doing this, the influencing factors of invertebrate leakage from BAC filters should be determined. The influencing factors mainly include water temperature, raw water quality, type of activated carbon, and operating state of the BAC filter, such as filtering velocity and backwashing cycle. Water temperature in particular has an important influence on invertebrate abundance in the filtrate and the activated carbon bed [13,14]. In summer, invertebrate abundance is much higher than in the winter. A previous study suggested that 1) in the warm season, bacteria grow quickly and the con-

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centration of organic matter increases rapidly [13,15], thereby increasing the food source for invertebrate growth; and 2) water temperature influences the invertebrate growth rate, and high water temperature promotes propagation [16]. The backwashing cycle of BAC filters could also affect the invertebrate abundance. In a backwashing cycle, the invertebrate abundance of the filtrate first decreases and then increases [17]. When the backwashing cycle is long, the invertebrate leakage could be severe at the end of the period. Researchers have proposed an optimal operating cycle depending on the changes of the season [18]. However, few studies have been conducted on the effects of the type of activated carbon on invertebrate leakage in BAC filters.

In view of the above, the objective of this study was to investigate the relevance between the type of activated carbon and the leakage of invertebrates. The results will be helpful for the comprehensive understanding of the influencing factors of invertebrate leakage in BAC filters.

2. Methods and materials

2.1. Drinking water treatment plant (DWTP)

A full-scale study was conducted in drinking water treatment plant (DWTP) A located on the lower reaches of the Yangtze River. DWTP A uses reservoir water as raw water and the water qualities are always in good condition. The qualities of the raw water are shown in Table 1.

Table 1  
Qualities of raw water

Parameter	Value	GB 3838 – 2002 (Class II)
Chroma, °	6–10	NG
Turbidity, NTU	3–14	NG
pH	7.7–9.0	6–9
COD <sub>Mn</sub> , mg/L	1.5–2.2	≤4
UV <sub>254</sub> , cm <sup>-1</sup>	0.018–0.044	NG
NH <sub>4</sub> <sup>+</sup> -N, mg/L	0.05–0.20	≤0.5

NG: Not given.

Fig. 1 shows that the ozone–BAC process is applied in DWTP A. The BAC system has two parallel groups, and each group has five BAC filters. Group 1 has operated since September 2008, whereas group 2 began operating in April 2013. Until April 2015 (the starting time of the research), the service time of the activated carbon (carbon age) of groups 1 and 2 were 6.5 and 2 years, respectively. Depending on the preparation techniques [19], three types of activated carbon are present in group 1, namely, raw coal crushed carbon, columnar crushed carbon, and briquetted crushed carbon. In group 2, all five BAC filters are used with columnar crushed carbon. BAC filters A and B in group 1 and BAC filter C in group 2 were selected for the full-scale experiments, which were named A (crushed 6.5a), B (columnar 6.5a), and C (columnar 2a). The physicochemical characteristics of the activated carbon are summarized in Table 2. And the basic operating parameters of DWTP A are shown in Table 3.

2.2. Sampling and analysis

2.2.1. Invertebrate and the potassium permanganate index

Samples were collected at DWTP A according to the following distributions: filtrate of BAC filters A, B, and C and filter beds A, B, and C. The carbon samples in the filters were collected from three layers: upper layer 0–50 cm, middle layer 70–120 cm, and lower layer 140–190 cm.

Water samples were taken with a plankton net (10 μm mesh size; Hydro-Bios GmbH, Kiel, Germany), and approximately 300 L of water was filtered to collect invertebrates. Carbon particles in the BAC filter beds were sampled using a stainless-steel sampler. The carbon samples were preserved in sterile plastic pipes. Carbon samples were washed three times by vigorous shaking with sterile distilled water. All washing water was collected and filtered through a 10 μm net to retain the invertebrates.

For invertebrate enumeration, samples were transferred to a counting plate and allowed to settle for 10 min. The entire counting chamber was scanned, and the invertebrates were counted under a microscope (BX-51, Olympus, Japan). Chemical oxygen demand by manganese (COD<sub>Mn</sub>) was analyzed according to the state standard method [20].

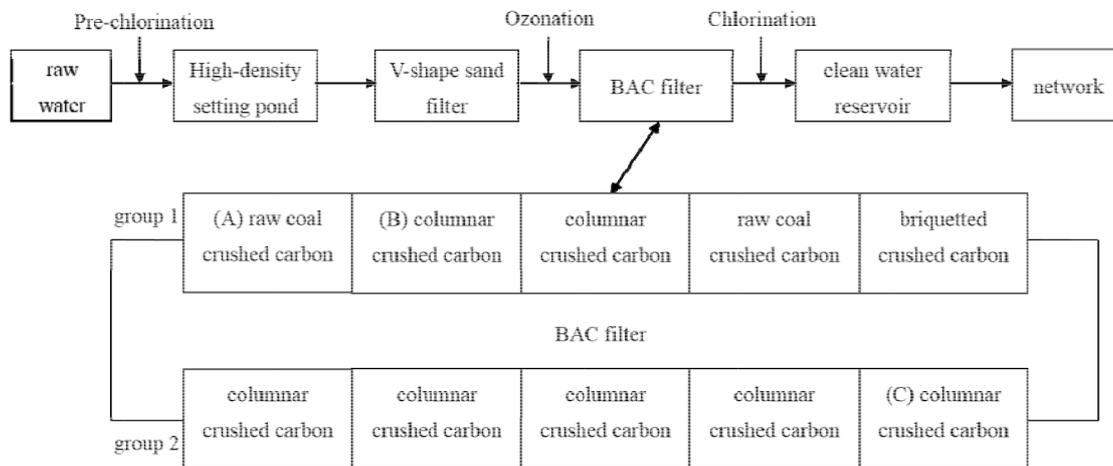


Fig. 1. Ozone–BAC process of DWTPA.

Table 2  
Properties of the activated carbons used in the study (2015.4)

Activated carbon	A (crushed 6.5a)	B (columnar 6.5a)	C (columnar 2a)
Origin	Coal	Coal	Coal
Physical form	Granular	Granular	Granular
Iodine value (mg/g)	202	271	513
Methylene blue value (mg/g)	<75	<75	<75
Phenol value (mg/g)	30	43	87
Effective particle size (mm)	0.70	0.81	1.01
Specific surface area (m <sup>2</sup> /g)	39	216	577
Total pore volume (cm <sup>3</sup> /g)	0.03	0.128	0.281

Table 3  
Basic operating parameters of DWTPA

Operating parameters	DWTP A
Pre-chlorination dosage, mg/L	0.8–1.0
Retention time of pre-chlorination, min	30
Main ozone, mg/L	0.8
Empty bed contact time of BAC filter, min	10
Residual ozone concentration, mg/L	≤0.1
Filtering velocity of BAC filter, m/h	12
BAC operation cycle, h	120
Chlorination dosage, mg/L	0.5

## 2.2.2. Illumina MiSeq sequencing and data analysis

### 2.2.2.1. DNA extraction and PCR amplification

Microbial DNA was extracted from three activated carbon samples obtained from the upper layer of each BAC filter using the E.Z.N.A.<sup>®</sup> Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The V3-V4 region of the bacteria 16S ribosomal RNA gene was amplified by PCR using primers 338F and 806R, where the barcode is an eight-base sequence unique to each sample.

### 2.2.2.2. Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor<sup>™</sup> -ST (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP\*\*\*\*\*).

### 2.2.2.3. Processing of sequencing data

Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.9.1) with the following criteria: (i)

The 300 bp reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window, with the truncated reads shorter than 50 bp discarded; (ii) exact barcode matching, 2 nucleotide mismatch in primer matching, with reads containing ambiguous characters removed; (iii) only sequences that overlapped longer than 10 bp were assembled according to their overlap sequence, with reads that could not be assembled discarded.

Operational Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (<http://rdp.cme.msu.edu/>) against the silva (SSU123)16S rRNA database using a confidence threshold of 70% [21].

## 3 Results and discussion

### 3.1. Invertebrate leakage tendencies in the filtrate of the three types of BAC filters

#### 3.1.1. Invertebrate leakage characteristics in a backwashing cycle

As shown in Table 3, the backwashing cycles of the three BAC filters were all 120 h. During a backwashing cycle, the invertebrate abundance in the filtrate of each BAC filter was detected every day (Fig. 2a). During detection, the variation range of the water temperature was 19–19.8°C. As shown in Fig. 2a, the leakage characteristics of the total invertebrate abundance of the three BAC filters presented similar tendencies in a backwashing cycle. The total abundance of invertebrates from the effluent of the BAC filters initially decreased and then increased; this finding coincided with previous research results [18]. At the same stage of the backwashing cycle, the order of the invertebrate abundance in the filtrate of the three filters was filter A (crushed 6.5a) < B (columnar 6.5a) < C (columnar 2a).

#### 3.1.2. Comparison of invertebrate abundance and species

From April to August, the invertebrate abundance in the filtrate of each BAC filter was detected every month (Fig. 2b). Depending on the results of Fig. 2a, the sampling time was set on the same day of the backwashing cycle to guarantee the comparability. As shown in Fig. 2b, the inver-

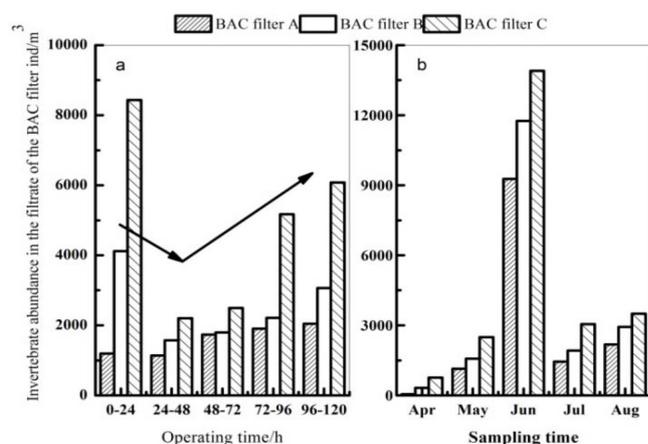


Fig. 2(a). Invertebrate abundance in the filtrate of each BAC filter during a backwashing cycle. (b) Invertebrate abundance in the filtrate of each BAC filter in different months.

tebrate abundance in the filtrate of BAC filter C was the highest, whereas that in the filtrate of BAC filter A was the lowest among the three BAC filters. The monthly average abundance of BAC filters A, B, and C were 2818.74, 3701.52, and 4742.11 ind/m<sup>3</sup>, respectively. This finding indicates that the invertebrate leakage of the BAC filter using the columnar activated carbon was more severe than that of the filter using raw coal crushed activated carbon with the same carbon age. The invertebrate leakage of the BAC filter with a shorter service time was more serious than that of the older filter using the same type of activated carbon. In addition, among the five months, of the most severe invertebrate leakage occurred during June, reaching as high as 10<sup>4</sup> ind/m<sup>3</sup>.

Table 4 shows that a total of eight species of invertebrates were detected in the filtrate of the BAC filters, including rotifers, nematodes, crustacea, and oligochaeta.

Rotifers were the dominant species in the filtrate of BAC filter A, with an average percentage of 90.80%, followed by

Table 4

Abundance and detection rate of the different types of invertebrates in the filtrate of BAC filters ( $n = 5$ )

BAC filter	Species of invertebrate	Range of abundance (ind/m <sup>3</sup> )	Average abundance (ind/m <sup>3</sup> )	Average percentage (%)	Detection rate (%)
A (crushed 6.5a)	Rotifers	22.99–8888	2559.45 ± 3599.87	90.80	100
	Nematodes	22.99–132.78	100.72 ± 67.32	3.57	100
	Crustacea	0–45.64	12.15 ± 18.98	0.43	90
	Oligochaeta	0–176.70	40.07 ± 76.61	1.42	60
	Water mites	0–11.19	4.14 ± 4.63	0.15	60
	Gastrotrichs	0–182.57	36.51 ± 81.65	1.30	20
	Tardigrades	0–271.98	64.87 ± 117.55	2.30	60
	Turbellaria	0–4.15	0.83 ± 1.86	0.03	20
	Total	54.60–9269.74	2818.74 ± 3686.44		
B (columnar 6.5a)	Rotifers	280.14–11488.40	3516.47 ± 4553.71	95.00	100
	Nematodes	4.63–140.70	61.14 ± 51.09	1.65	100
	Crustacea	0–55.81	20.62 ± 23.29	0.56	90
	Oligochaeta	0–98.36	26.92 ± 41.43	0.73	90
	Water mites	0–20.50	5.96 ± 8.45	0.16	60
	Gastrotrichs	0–53.28	15.67 ± 21.96	0.42	60
	Tardigrades	0–131.15	40.54 ± 56.48	1.10	60
	Turbellaria	0–55.81	14.20 ± 23.70	0.38	60
	Total	322.69–11758.16	3701.52 ± 4599.26		
C (columnar 2a)	Rotifers	682.93–12685	4267.20 ± 4808.05	89.99	100
	Nematodes	22.20–118.96	57.22 ± 36.99	1.21	100
	Crustacea	0–146.67	43.06 ± 61.20	0.91	90
	Oligochaeta	3.48–393.30	113.99 ± 167.14	2.40	100
	Water mites	0–70	17.74 ± 29.88	0.37	60
	Gastrotrichs	0–50.76	15.56 ± 21.93	0.33	60
	Tardigrades	0–842.64	207.24 ± 359.82	4.37	90
	Turbellaria	0–65.99	20.09 ± 27.41	0.42	60
	Total	759.57–13903.28	4742.11 ± 5225.77		

$n$ : sampling times; detection rate =  $n(\text{detected})/n(\text{total}) \times 100\%$

nematodes (3.57%), tardigrades (2.30%), and oligochaeta (1.42%). They were also the major species in the filtrate of BAC filter B. However, the average percentages were 95.00%, 1.65%, 1.10%, and 0.73%, respectively. Only rotifers and nematodes were 100% detected in the filtrate of both BAC filters A and B. The detection rates of oligochaeta, gastrotrichs, and turbellaria in the filtrate of BAC filter B were higher than those in the filtrate of BAC filter A. By comparison, the dominant invertebrate species were similar in the BAC filters with the same carbon age. However, the detection rates of the invertebrates in the BAC filter using the columnar crushed activated carbon were higher than those of the filter using raw coal crushed carbon.

In the filtrate of BAC filter C, rotifers were still the dominant species, with an average percentage of 89.99%, followed by tardigrades (4.37%), oligochaeta (2.40%), and nematodes (1.21%). Except for rotifers and nematodes, the detection rate of oligochaeta was also 100%, followed by crustacea (90%), and tardigrades (90%). Compared with BAC filter B, nematodes were the second dominant species in the filtrate of the older BAC filter, whereas tardigrades were the second dominant species in the filtrate of the newer BAC filter. The detection rates of the invertebrates in the filtrate of the newer BAC filter were higher than those in the filtrate of the older one.

### 3.2. Invertebrate distributions on the activated carbon layers

Invertebrates were distributed throughout the depth of the filter bed. The invertebrate abundance in the upper, middle, and lower layers of the BAC filters (ind/kg) are shown in Fig. 3. The invertebrate abundance was very high on the activated carbon C, reaching 24,000 ind/kg on the upper layer, whereas the invertebrate abundance on the upper layer of activated carbon B was 16,015 ind/kg. Invertebrate abundance on the activated carbon A was the lowest, only 4350 ind/kg on the upper layer. These results indicate that the columnar carbon was more beneficial to the growth of the invertebrates than the crushed one with the same carbon age; also, the activated carbon with shorter carbon age

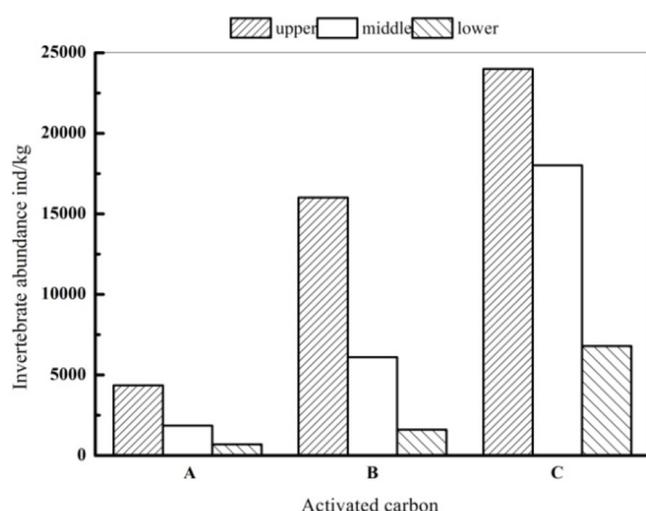


Fig. 3. The vertical distribution of invertebrates from the activated carbon in BAC filters A, B, and C.

was more suitable for the growth of the invertebrates than the older one with the same shape, which showed the same changes in abundance as the filtrate of the BAC filters.

In line with the results of previous research [22], the biomass on the activated carbon had good correlation with the specific surface area and the total pore volume. A higher specific surface area and total pore volume correspond to higher biomass on the activated carbon. As Table 2 shows, the specific surface areas and total pore volumes of the three types of activated carbon were in the order of  $A < B < C$ . Therefore, the biomass on activated carbon C was the largest, followed by activated carbon B. The biomass on activated carbon A was the smallest. A larger biomass implied that more food could be provided for the growth of invertebrates. Invertebrate abundance on the activated carbon thus increased as the biomass increased. When more invertebrates were present on the BAC bed, the risk of invertebrate leakage increased. In addition, a small particle size corresponds to the formation of a small pore space, which has better interception effects on invertebrates. As Table 2 shows, the particle sizes of the three types of activated carbon were in the order of  $A < B < C$ . Therefore, activated carbon A had the best interception effect on invertebrates. Above all, two factors caused the difference in invertebrate abundance in the different types of BAC filters. First, the larger the specific surface area and total pore volume were, the larger the number of invertebrates that would grow on the activated carbon and penetrate through the carbon layers. Second, the activated carbon with a smaller particle size may reduce the risk of invertebrate leakage.

For each BAC filter, the invertebrate abundance decreased with increasing BAC filter depth because in the down-flow BAC process, the biomass on the activated carbon decreased with increasing BAC filter depth, and the highest biomass was usually found in the upper layer [1]. Therefore, in the different activated carbon layers, the quantity of food for invertebrates was different, which led to differences in the growth of the invertebrates.

### 3.3. Bacterial species diversity analysis

As the output of the invertebrates in the BAC filters was an expression of the accumulation of microorganisms within the filters, the bacteria formed in the BAC filters could be detected.

The bacterial communities of each activated carbon were elucidated by Illumina MiSeq sequencing. Good's coverage estimator on the OTUs from each sample was calculated to assess the diversity captured with the activated carbon samples. The results indicated that this test captured more than 99% of the species of all the samples. The Shannon index and the Simpson index are commonly used to characterize species diversity in microbial communities. A larger Shannon index corresponds to high community diversity; the Simpson index shows the opposite result. Detailed information is shown in Table 5.

The total numbers of estimated OTUs were 218 for activated carbon B and 113 for activated carbon A, thereby indicating that the microorganism community of the columnar activated carbon exhibited higher abundance than that of the crushed carbon with the same carbon age. The columnar carbon also had a higher diversity than the crushed

Table 5  
Coverage and diversity indices from 16s RNA sequencing analysis

Activated carbon	Reads	0.97					
		OTU	Ace	Chao	Coverage	Shannon	Simpson
A (crushed 6.5a)	17988	113	129 (120,150)	125 (117,147)	0.998833	2.64 (2.62,2.66)	0.1101 (0.1079,0.1122)
B (columnar 6.5a)	17988	218	252 (237,279)	260 (239,304)	0.997276	2.9 (2.88,2.92)	0.1012 (0.099,0.1033)
C (columnar 2a)	17988	288	342 (321,375)	353 (324,407)	0.996109	3.12 (3.09,3.14)	0.0906 (0.0885,0.0926)

carbon, as indicated by the higher Shannon index and the lower Simpson index. For activated carbons B and C, the activated carbon with a shorter serving age had higher abundance and more diversity than the older one. A positive correlation was also found between the bacterial abundance, diversity, and specific surface area of the activated carbon. These results were consistent with those reported in section 3.2.

Further analysis was conducted to identify the dominant bacteria species at the genus level. The results are shown in Fig. 4. On activated carbon A, the dominant bacterial communities were *Bacillus* (42.76%), followed by *Pseudomonas* (18.57%) and *Lactococcus* (18.96%). These species were also major bacterial compositions of activated carbon B and C; however, the abundance varied to 40.4%, 18.28%, 18.03% and 38.87%, 16.25%, 18.28%, respectively. By comparison, the three types of activated carbon had high similarities in the dominant bacterial communities because the filters had the same inflow and similar operating parameters. According to the literature [23], *Pseudomonas* and *Bacillus* can effectively remove organic matter. Therefore, in section 3.4, the comparison of organics removal is discussed.

### 3.4. Comparison of organic matter removal

COD<sub>Mn</sub> was chosen to indicate the operating performance of the filter because COD<sub>Mn</sub> was the surrogate parameter of organic matter in drinking water in the latest national standard (GB5749-2006) of China [1]. The inflow of the three BAC filters was the same, which meant that the

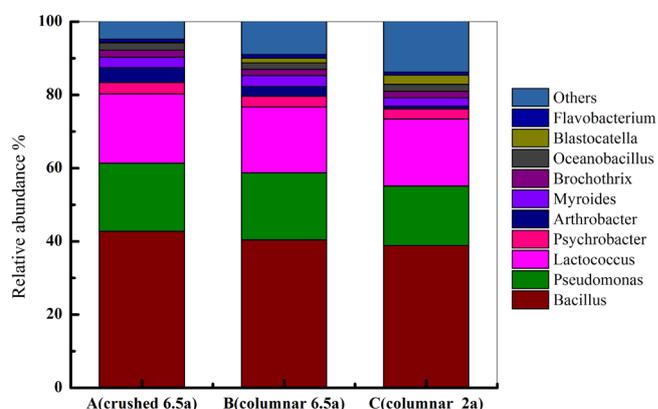


Fig. 4. Microbial population classification on the activated carbon (defined as genus).

concentration of COD<sub>Mn</sub> in the filtrate of the filters could represent the organic matter removal rate.

COD<sub>Mn</sub> concentrations in the filtrate of the three BAC filters were detected every month from September 2014 to September 2015, and the results are shown in Fig. 5. COD<sub>Mn</sub> concentration in the filtrate of the BAC filters remained stable during the investigation period, fluctuating around 1.0 mg/L, which was far below the standard value (3 mg/L). Previous research indicated that biodegradation predominantly accounted for the COD<sub>Mn</sub> removal after running the filter for ~63 days [1]. In this experiment, the runtime of these three BAC filters was more than two years and the biodegradation process played a major role in removing organic matter. The similar effects on the removal of organic matter in the three BAC filters may be explained by the high similarity in the dominant bacterial communities on the activated carbons.

### 4. Conclusions and Suggestions

Invertebrate leakage, microbial properties, and organic removal were compared in the three different types of BAC filters, producing the following main results.

- Specific surface area and total pore volume, and particle size of the activated carbon were the two main factors affecting the invertebrate leakage.

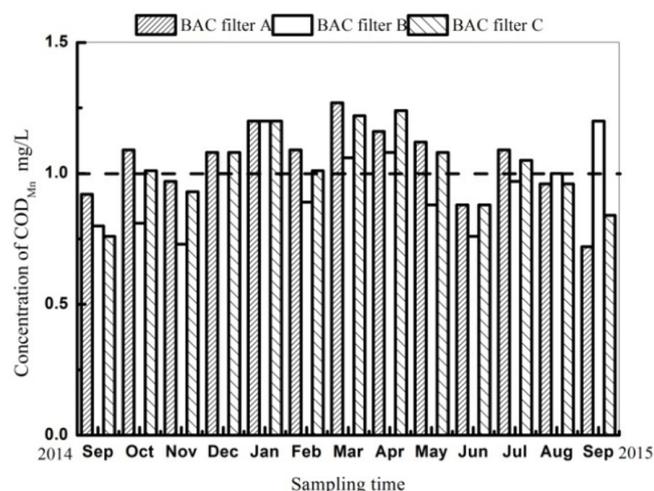


Fig. 5. COD<sub>Mn</sub> concentrations in the filtrate of the three BAC filters from September 2014 to September 2015.

The larger the specific surface area and total pore volume were, the larger was the number of invertebrates that would grow on the activated carbon and penetrate through the carbon layers. The larger the particle size was, the easier it was for the invertebrates to penetrate through the carbon layers.

- The BAC filters had similar bacterial community structures on the activated carbon. The dominant bacteria were *Bacillus*, *Pseudomonas*, and *Lactococcus*, and the proportions were similar.
- The BAC filters had similar effects on the removal of organic matter, which may be explained by the high similarity in the dominant bacterial communities on the activated carbons.

The selection and regeneration of activated carbon were problems in the application of the ozone–BAC process. Hence, if the carbon particles are not flushed out during backwashing, then smaller-sized particles should be preferred. Moreover, carbon age should not be the only evaluation indicator in deciding whether the activated carbon should be regenerated.

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