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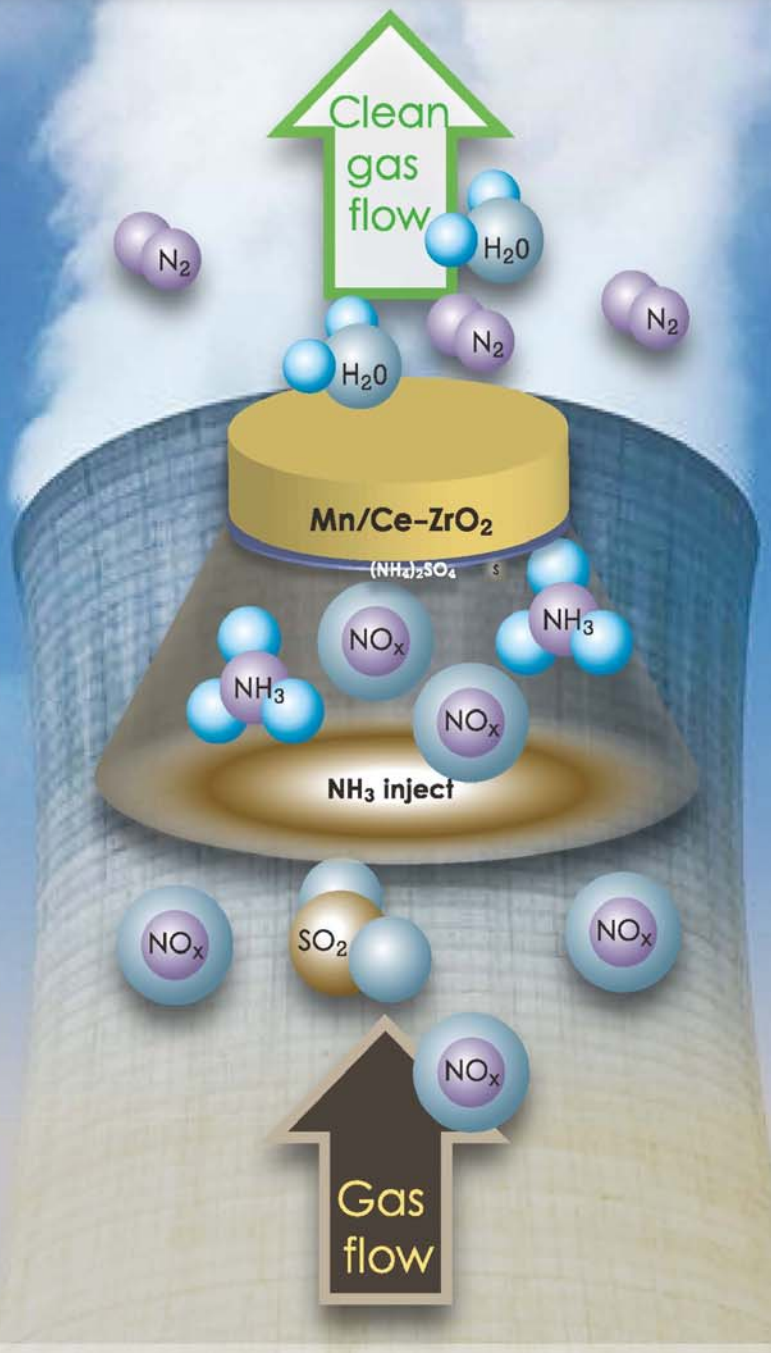


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# Microbial community characterization, activity analysis and purifying efficiency in a biofilter process

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**Abstract**

The growth and metabolism of microbial communities on biologically activated carbon (BAC) play a crucial role in the purification of drinking water. To gain insight into the growth and metabolic characteristics of microbial communities and the efficiency of drinking water treatment in a BAC filter, we analyzed the heterotrophic plate count (HPC), phospholipid, dehydrogenase, metabolic function and water quality parameters during start-up and steady-state periods. In the start-up process of the filter with natural biofilm colonization, the variation in heterotrophic plate count levels was S-curved. The total phospholipid level was very low during the first 5 days and reached a maximum value after 40 days in the filter. The activity of dehydrogenase gradually increased during the first 30 days and then reached a plateau. The functional diversity of the microbial community in the filter increased, and then reached a relatively stable level by day 40. After an initial decrease, which was followed by an increase, the removal rate of $NH_4^+$-N and $COD_{Mn}$ became stable and was 80% and 28%, respectively, by day 40. The consumption rate of dissolved oxygen reached a steady level after 29 days, and remained at 18%. At the steady operation state, the levels of HPC, phospholipid, dehydrogenase activity and carbon source utilization had no significant differences after 6 months compared to levels measured on day 40. The filter was shown to be effective in removing $NH_4^+$-N, $NO_2^-$-N, $COD_{Mn}$, $UV_{254}$, biodegradable dissolved organic carbon and trace organic pollutants from the influent. Our results suggest that understanding changes in the growth and metabolism of microorganisms in BAC filter could help to improve the efficiency of biological treatment of drinking water.

**Key words**: biologically activated carbon; microbial community; microbial biomass and activity; metabolic function; pollutant; removal rate

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## Introduction

In both developing and industrialized nations, a growing number of organic pollutants such as agricultural chemicals are entering water supplies as a result of human activity (Shannon et al., 2008). In China, it has been reported that some parts of the rivers, lakes, reservoirs and ground water supplies are suffering from pollution, and source water quality is facing a severe threat (Sagbo et al., 2008; Wu et al., 2009; Zhang et al., 2010). In recent years, some studies have shown that many organic pollutants have appeared in source water at low concentration (Sun et al., 2002; Wu et al., 2009; Gao et al., 2011; Zhang et al., 2011). For drinking water safety, organic pollutants need to be removed from source water before going into drinking water distribution systems. However, conventional water treatment processes, such as chemical mixing, coagulation, sedimentation, sand filtration, and chlorination, cannot effectively remove some toxic organic contaminants. Therefore, drinking water treatment plants either use advanced technologies or upgrade their existing units. For instance, many advanced water treatment processes, including microfiltration, ultrafiltration, reverse osmosis, advanced oxidation, photocatalytic oxidation, and biofilter processes, have been developed to remove trace organic contaminants from polluted raw water (Huang et al., 2008; Toor and Mohseni, 2007; Seredyńska-Sobecka et al., 2006; Nandy et al., 2007). Of these treatment processes, the biofilter process, which is a cost-effective and environmentally friendly method, has gained popularity in recent years.

Biofilter technology has been successfully applied to

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remove organic pollutants in source water (Yapsaklia et al., 2010). It relies on indigenous microbial communities to colonize biofilter media and utilize organic compounds as substrates (Velten et al., 2007; Fonseca et al., 2001; Faulwetter et al., 2009). Biodegradation, as part of the biofilter process, has been identified as a major elimination pathway for organic matters in particular in a variety of studies (Molina-Muñoz et al., 2010). Measuring biomass concentration and enzymatic activities is essential to determine the biological characterization of a complex microbial microcosm, such as activated sludge and biofilter processes (Molina-Muñoz et al., 2010; Fonseca et al., 2001). Various methods have been developed to assess the activity or biomass present in a biofilter (Velten et al., 2007), and some studies have assessed microbial biomass, activity and factors affecting these characteristics in drinking water biofilters (Fonseca et al., 2001; Blume et al., 2002; Magic-Knezev and van der Kooij, 2004; Seredyńska-Sobecka et al., 2006; Velten et al., 2007). However, few studies have been conducted to analyze the changes of the microbial biomass and activity in biofilters.

In recent years, use of the Biolog technique to study complex microbial communities has developed rapidly. This method is based on the premise that microorganisms vary in the rate and pattern at which they utilize carbon sources. Therefore, carbon utilization patterns can be used as a measure of microbial community structure and functional potential (Xue et al., 2008). This technique is widely employed for investigation of the microbial community level, physiological profile of the eco-environment and changes in microbial community function, which can be caused by different environmental stresses (Niklińska et al., 2006; Zhou et al., 2008; Leflaive et al., 2008) In a biofilter, a mixed microbial community in the biofilm coating medium degrades the pollutants (Deshusses, 1997; Grove et al., 2004). The metabolic function of microbial communities plays an important role in removing pollutants and operational performance of the biofilter. It is essential to have knowledge of the microbial community function and functional diversity to characterize the role of microbial communities in biofilters. At the same time, the structural and functional analysis of the microbial community by the Biolog technique provides relatively little information regarding the drinking water purification process. Accordingly, it is necessary to analyze the growth and metabolism of microbial communities using the Biolog technique in the water purification process.

The organic contaminants present in raw water are abundant in variety, and their chemical components are very complicated. The levels of most organic contaminants tend to be too low to analyze individually. Therefore, some comprehensive organic indices such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), dissolved oxygen (DO), $UV_{254}$, have been used to describe the total pollution level of organic contaminants. However, a comprehensive organic index does not reflect the content of specific organic contaminants, and thus provides very little information, especially for trace organic contaminants in water. Up to now, the purification efficiency of biofilters for drinking water has usually been evaluated using removal rates, such as for $NH_4^+$-N, $NO_2^-$-N, $COD_{Mn}$, $UV_{254}$, and DO (Tian et al., 2009; Wang et al., 2009). Chen et al. (2007) assayed different water purification processes using the comprehensive indices of $COD_{Mn}$, TOC, $UV_{254}$, AOC, biodegradable dissolved organic carbon (BDOC), and THMs and concluded that a combination of the conventional processes and $O_3$-BAC provides integrated removal of organic matter and meets the required standards (Chen et al., 2007).

In this study, a biofilter has been developed in which microorganisms used granular activated carbon as a base on which to form a biofilm. The first objective was to determine the development of biomass by looking at the changes of microbial activity and metabolic function, as well as water quality transformation, to judge whether the biofilm is mature during the start-up process of a pilot-scale biologically activated carbon (BAC) filter with natural biofilm colonization. The second task was to determine the active microbial biomass by investigating dehydrogenase activity and metabolic function, in order to understand the development of microbial communities in stable operation over a 6-month period. The final objective was to evaluate the removal of organic contaminants by the biofilter.

## 1 Materials and methods

### 1.1 Experimental system

This study was conducted at the Beihekou Tap Water Plant of Nanjing City, China. A pilot-scale drinking water treatment process including a biological treatment unit (biofilter) was established in November 2007. Specifically, the filtration column (biofilter) was 200 cm high with an inner diameter of 25 cm. In the column there was a 100 cm depth of granular activated carbon medium layer with an approximately 40 cm layer of gravel on the bottom. The diameter of the granular activated carbon ranged from 0.5 to 0.7 mm. A number of sampling ports were arranged along the filter column to extract water samples and granular activated carbon media for analysis. In the course of the natural start-up of the biofilter, the biofilter was submerged in the effluent of the sedimentation tank for the first five days. The effluent was then fed to the biofilter at a low-flow rate from the feed water unit, and the media of the biofilter was covered with a stable biofilm. After the completion of start-up, the pilot-scale drinking water treatment process operated at 1.5 $m^3$/hr for 6 months. In the course of drinking water treatment, raw water was characterized by DOC ranging from 1.2 to 3.8 mg/L,

$NH_4^+$-N concentration ranging from 0.02 to 1.18 mg/L, $NO_2^-$-N concentration ranging from 0.024 to 0.200 mg/L, turbidity ranging from 14.0 to 258.0 NTU, pH ranging from 7.74 to 8.06, and temperature ranging from 4.5 to 30°C. The biofilter was backwashed twice a week with air for approximately 5 min (6 L/($m^2$·sec)), followed by water for 12 min (15 L/($m^2$·sec)). The empty bed contact time of the biofilter was 15 min.

### 1.2 Sample collection

Water samples (500 mL) were collected in sterile containers from the entrance and exit of the biofilter. Packing media samples 200 g (wet weight) were taken from the upper (0–10 cm), middle (45–55 cm) and bottom (90–100 cm) parts of the biofilter bed on different sampling dates. Samples were stored in 500 mL autoclaved glass bottles at 4°C in the dark until analysis.

### 1.3 Biofilter microbial biomass and activity analysis

Microbial biomass and activity are two important parameters for the design and operation of biological processing of drinking water. To better understand the characteristics of biofilter microbes, it is necessary to comprehensively assess the microbial biomass and activity on the biofilter bed. A variety of methods of measuring microbial biomass and activity have been employed in drinking water treatment processes, e.g. epifluorescence microscopic total cell counts, heterotrophic plate counts (HPC), reduction of 2-(*p*-iodo-phenyl)-3-(*p*-nitrophenyl)-*s*-phenyltetrazolium chloride (INT), phospholipid analysis, uptake of labelled substances, biomass respiration potential (BRP) and adenosine tri-phosphate (ATP) analyses, respectively (Magic-Knezev and van der Kooij, 2004; Velten et al., 2007). For the biomass measurement, the total biomass associated with the biofilter media was measured using lipid phosphate analysis according to the method described by Fonseca et al. (2001). Total heterotrophic bacterial counts were determined by the pour-plate method. Tenfold serial dilutions were prepared in 0.9% saline and 1 mL of $10^{-5}$ sample solution was pour-plated on to R2A agar. Colony-forming units (CFUs) were determined following 72 hr of incubation at 25°C in the dark (Sen and Chandra, 2009). The biological activity was measured by a dehydrogenase assay using the triphenyl-tetrazolium chloride (TTC) method (Sen and Chandra, 2009). Briefly, 5 g samples were incubated with TTC for 24 hr and the formazan formed was extracted with toluene, after which the optical density (OD) was measured colorimetrically at 492 nm.

### 1.4 Biolog EcoPlates™ analysis

The metabolic function of the microbial community in the biofilter was analyzed using the Biolog EcoPlate™ (Biolog Inc., Hayward, CA). The EcoPlate contained triplicate wells of 31 carbon sources and three negative controls with no substrate. Metabolism of the carbon source results in the respiration-dependent reduction of a tetrazolium dye contained in each well containing formazan, which induces the formation of a purple coloration and increases the OD of the solution (Leflaive et al., 2008).

The samples of packing material (25 g) were shaken at 270 r/min for 2 hr in 225 mL of phosphate buffer (pH = 7.4) and were then allowed to settle for 0.5 hr. The supernatants were then prepared for Biolog analysis. The prepared supernatants containing microbes were diluted with additional phosphate buffer to adjust the OD to approximately 0.06 at 420 nm (Grove et al., 2007). The diluted solutions were inoculated into the Biolog Ecoplates (125 μL per well), after which the plates were incubated at 25°C. The OD at 590 nm was determined at 96 hr using a multi-well plate reader (Spectro Max plus 384, Molecular Devices). Microbial activity in each microplate, expressed as average well-color development (AWCD), was calculated as described by Garland and Mills (1991). The data were standardized by subtracting the mean value of the control wells from all well color values and then dividing by AWCD (Kamitani et al., 2006). OD values smaller than 0.06 were treated as zero for numerical analysis (Classen et al., 2003).

### 1.5 Analysis of water quality

The conventional indices of water quality, including $NH_4^+$-N, $NO_2^-$-N and $COD_{Mn}$, were determined according to standard examination methods for drinking water (GB/5750-2006, China), and $UV_{254}$ was measured with an ultraviolet absorption spectrometer (UV752, Shanghai, China). The BDOC was determined according to the bioassay procedure established by Servais et al. (1994). In addition, the trace organic pollutants in the influent water and effluent water of the biofilter were analyzed by gas chromatography/gas chromatography-mass spectrometry (GC-2010, Shimadzu, Japan; Trace DSQ GC/MS, Finnigan, USA).

### 1.6 Statistical analysis

The statistical analysis software SPSS 13.0 (SPSS Inc., USA) was used to conduct the analysis of all data. One-way ANOVA was performed for microbial biomass, microbial activity, diversity indices and water quality indices. An α level of 0.05 was used for the statistical test. For analysis of the functional diversity of the microbial community, the diversity indices were determined and principle component analysis (PCA) of the Biolog Eco microplate data was conducted based on the absorbance values at 96 hr. Diversity was evaluated by calculating the Richness index and Shannon's diversity index (Jiang et al., 2010). The Richness index, as the number of utilized C substrates, and the Shannon's diversity index ($H'$), were calculated using an OD of 0.06 as the threshold for positive response. Shannon's diversity index was calculated as

follows:

$$H' = -\sum p_i \ln p_i \quad (1)$$

where, $p_i$ is the ratio of the relative absorbance value of the $i$th positive well to the total absorbance values of 31 wells.

## 2 Results

### 2.1 Start-up process of the biofilter

The generation of a steady-state biofilm from the microbiota normally present in a biofilter is a very slow process due to the low microbial population and nutrient concentration in the feed water (Seredyńska-Sobecka et al., 2006; Kazimierska et al., 2002). A pilot-scale study was carried out on the start-up and biofilm cultivation performance of the biofilter with granular-activated carbon, and the biomass, biological activity, metabolic function and water quality parameters were monitored during operation. The results demonstrated that the start-up experiment of the steady-state biofilm formation could be completed within about 40 days when the hydraulic retention time (HRT) was 15 min and the water temperature was maintained at 15–22°C.

#### 2.1.1 Variation of biological biomass and activity

Changes in the biomass and biological activity were particularly descriptive of the start-up stages and were continuously monitored (**Fig. 1**). After 5 days of biofilm culturing, the bacterial number in terms of HPC increased rapidly in the upper media layer, and the number went on rising until day 19 and reached approximately $10^7$ CFU/g. However, bacterial cells in the middle and lower media layers reproduced slowly during the first week, and then the number of bacteria began to increase gradually and reached a peak on day 29 ($1.7 \times 10^7$ CFU/g and $7.8 \times 10^6$ CFU/g, respectively). The total lipid-P level was very low in the filter during the first 5 days, then the values increased and reached their maximum values after 40 days. During the initial development period, initial biofilm development proceeded at the highest rate in the upper layer of the filter, while it was 54% slower in the bottom layer (Velten et al., 2011).

As can be seen from **Fig. 1a** and **b**, the changing profiles of biomass in different media layers showed similar modes, with an S shaped curve during the start-up time. Notably, the development of the microbial community in the biofilter had an obvious lag phase, exponential phase, stationary phase, etc. As shown in **Fig. 1c**, during the start-up progress, activity of dehydrogenase (TTC-DHA) in the upper media layer rose higher than in the middle and lower media layers. TTC-DHA activities at different depths reached their peak within 30 days, then TTC-DHA decreased slightly and leveled off, when the steady-state values were 34.37, 26.75 and 21.37 μg TF/(g·hr), respectively.

The correlation analysis showed that the HPC in the upper, middle and lower media layers was positively correlated with TTC-DHA, and correlation coefficients were 0.974, 0.995 and 0.993, respectively.

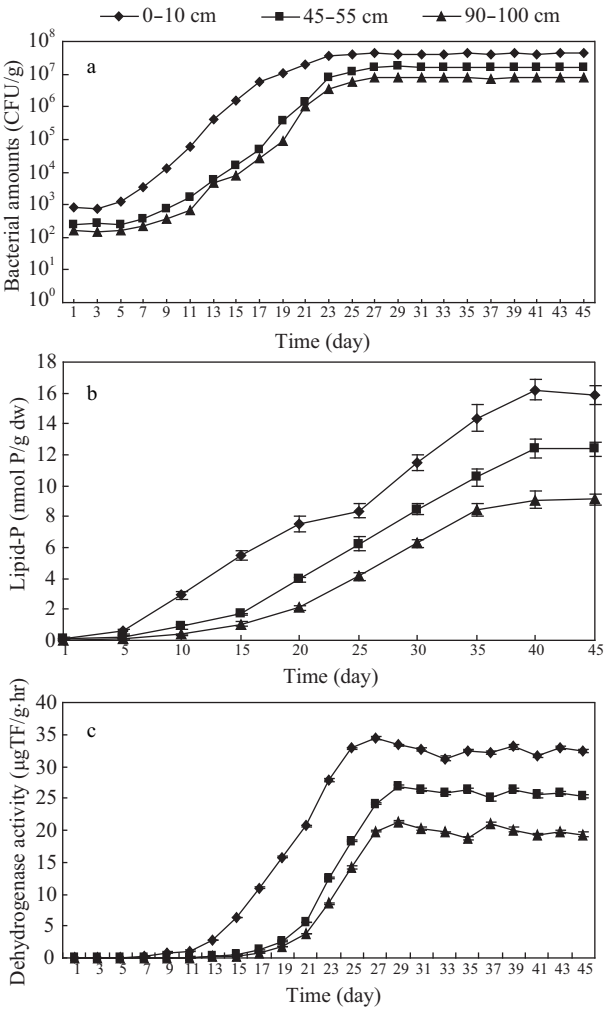


**Fig. 1** Variation of heterotrophic plate count (HPC) (a), lipid-P (b) and dehydrogenase activity (c) of microbial community at different heights of the filter layer. Error bars represent the standard deviation of replicate ($n$ = 3) samples.

#### 2.1.2 Metabolic function of microbial community in biofilter

The Biolog technique based on colorimetric theory was used to analyze the metabolic potential of environmental bacterial communities, and to provide information about the community-level physiological profile. As can be seen from **Fig. 2a**, the substrate utilization rate of the microbial community in the biofilter tended to increase over the start-up process. The carbon source utilization rate of the microbial communities in different media layers of the biofilter ranged from 12.9% to 25.8% on the first day. The substrate utilization rate by the microorganisms in the upper media layer was greater than that in the lower media layer. On day 30, the microbial communities in the upper media layer of the biofilter displayed the maximum carbon source utilization rate of up to 80.0%.

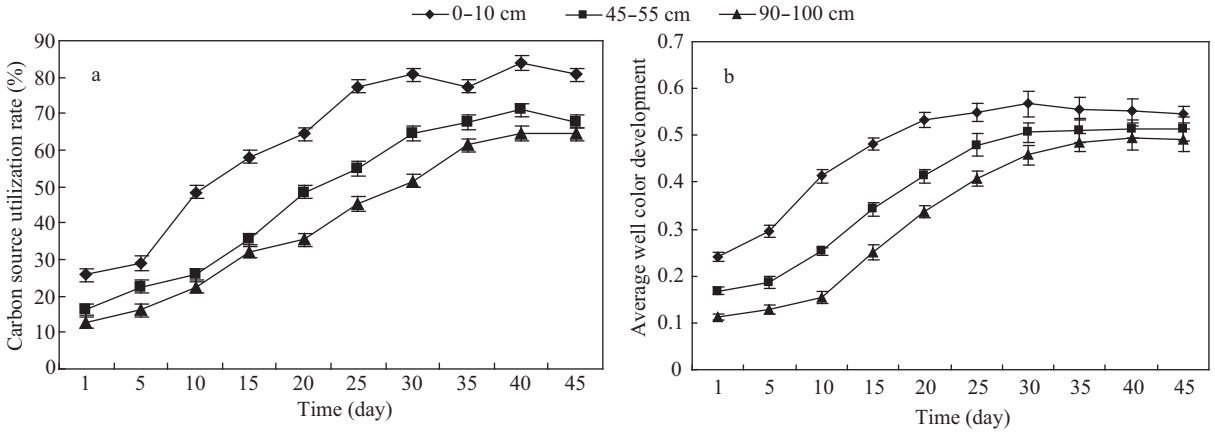


**Fig. 2** Utilized rate of carbon sources (a) and metabolic capability (b) of microbial community at different heights of the filter layer. Error bars represent the standard deviation of replicate ($n$ = 3) samples.

However, the carbon source utilization rate of microbial communities in the middle and lower media layers, after 40 days, only reached 71.0% and 64.5%, respectively. The rate of substrate utilization in the different layers then began to level off. The main carbon substrates, which included L-asparagine, L-serine, α-D-lactose, D-malic acid, D-galacturonic acid, itaconic acid, and pyruvic acid methyl ester, were utilized more by microorganisms in the early stage of biofilm formation, while carbon substrates that were utilized gradually in the mid-term stage of biofilm formation included D-mannitol, 4-hydroxybenzoic acid, N-acetyl-D-glucosamine, D-galactonic acid-γ-lactone, γ-hydroxybutyric acid, Tween 80 and D,L-α-glycerol phosphate. Other substrates that could be not metabolized by microorganisms, even after 45 days, included D-xylose, D-glucosaminic acid, 2-hydroxybenzoic acid, glycogen, α-cyclodextrin and α-ketobutyric acid.

The average well color development (AWCD) in the Biolog plates indicated the metabolic activities of the microbial community. The microbial communities in different depths had different AWCD values, and the AWCD curves declined with increasing depth (**Fig. 2b**). AWCD of the upper media layer increased rapidly, but in the middle and lower media layers, AWCD values increased only slightly. After 40 days the AWCD values reached a relatively stable level in the different layers. The slow increase in AWCD values for the middle and lower media layers was likely due to the fact that most nutrients in the influent were intercepted by the upper media of the filter in the early stage of the start-up process, and only low levels of nutrients could be utilized for the microbial colonization of the middle and lower media layers as a result.

The Biolog Eco microplate data was evaluated using principle component (PC) analysis. The first two PCs, accounting for 54.8% and 22.6% of the total variance, respectively, were chosen for analysis and are shown in **Fig. 3**. The distribution of each substrate on the two axes was studied and as a result axis 1 (PC1) is mainly correlated with β-methyl-D-glucoside, D-galacturonic acid,

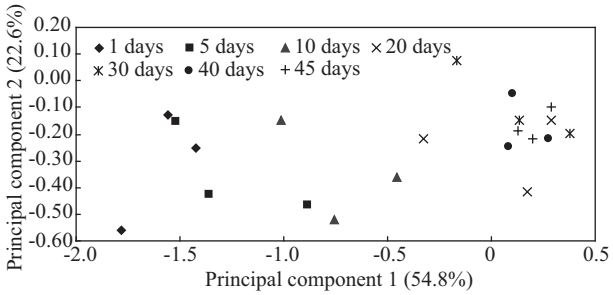


**Fig. 3** Principal component analysis of carbon utilization by microbial community at different heights of the filter layer.

L-asparagine, D-mannitol, Tween 80, 4-hydroxybenzoic acid, glucose 1-phosphate and D-malic acid, while substrates with higher loadings in axis 2 (PC2) mainly included Tween 40, N-acetyl-D-glucosamine, glycyl-L-glutamic acid and α-D-lactose. As seen in **Fig. 3**, the time course profiles for the metabolic function of the microbial communities colonizing media were mainly revealed by PC1. Although the similarities of the metabolic functions were different in the different phases of biofilm formation, the metabolic activities of the microbial communities on day 1 and day 5 of the early stage of biofilm formation shared a high similarity, which was located on the negative side of the PC1. At the same time, the metabolic activities were similar on day 40 and day 45, which is located on the positive side of PC1 axis. In the start-up process, the metabolic functions of the microbial communities moved from the negative side of the PC1 to the positive side at the start-up time. Therefore, the PC analysis illustrated that the metabolic functions of microbial communities in different phases of biofilm formation were distinctive and tended towards a relatively stable level.

**Table 1** shows the diversity of substrate utilization for the biofilm-forming microorganisms, as measured using the Richness index and Shannon diversity index. The functional diversity increased gradually as time went by at the same depth in biofilters, while the indices decreased gradually as the layers became deeper for the same start-up

**Table 1** Functional diversity analysis of microbial communities at different heights of filter layer ($n$ = 3)

| Time (day) | Richness index | | | Shannon index | | |
|---|---|---|---|---|---|---|
| | Upper layer | Middle layer | Lower layer | Upper layer | Middle layer | Lower layer |
| 1 | 8.00 ± 1.00 | 5.33 ± 0.58 | 3.67 ± 0.58 | 0.78 ± 0.07 | 0.54 ± 0.05 | 0.29 ± 0.06 |
| 10 | 15.00 ± 2.64 | 8.33 ± 1.53 | 7.00 ± 2.00 | 1.18 ± 0.12 | 0.62 ± 0.09 | 0.55 ± 0.15 |
| 20 | 19.67 ± 2.08 | 15.33 ± 1.15 | 10.67 ± 3.06 | 1.76 ± 0.14 | 1.07 ± 0.12 | 0.86 ± 0.04 |
| 30 | 24.67 ± 1.53 | 20.67 ± 2.52 | 18.33 ± 2.52 | 2.89 ± 0.09 | 1.91 ± 0.21 | 1.73 ± 0.14 |
| 40 | 25.33 ± 1.53 | 21.33 ± 1.53 | 19.67± 2.52 | 2.92 ± 0.17 | 2.54 ± 0.23 | 2.29 ± 0.06 |
| 45 | 25.33± 2.08 | 21.33 ± 1.53 | 20.33 ± 2.08 | 2.85 ± 0.16 | 2.48 ± 0.15 | 2.34 ± 0.10 |

time. As can be seen on **Table 1**, the Richness index and Shannon diversity index reached a relatively stable level by day 40.

### 2.1.3 Changes of water quality

In the natural start-up of the biofilter procedure, we tested the removal rate of $NH_4^+$-N and $COD_{Mn}$ as well as the changing status of effluent DO. As can be seen in **Fig. 4**, the removal rate of $COD_{Mn}$ dropped from 55% to 16% at the beginning of the start-up process (day 1–day 11), and then fluctuated within a narrow range at 16% after 26 days, and finally stabilized at about 28%. Within the first 5 days of biofilm culturing, the removal rate of $NH_4^+$-N showed a downward trend, then rose slightly and reached 90% on day 21. After 14 days, the rate decreased to 80% and remained at this level thereafter. The consumption rate of DO was slow but rose steadily over 19 days, from the date of biofilm culturing, then fluctuated greatly until day 28, before finally settling at around 18%.

## 2.2 Steady state of biofilter

### 2.2.1 Microbial biomass and activity

HPC, Lipid-P and TTC-DHA in the biofilter were assessed in order to explore the biomass and biological activity levels after about 6 months of operation. As shown in **Table 2**, the biomass and biological activity of the upper layer were higher than those of the lower layer in the biofilter ($P < 0.05$), but the level of HPC, Lipid-P and TTC-DHA at the same depth did not show any significant differences when compared to that measured on day 40 ($P > 0.05$).

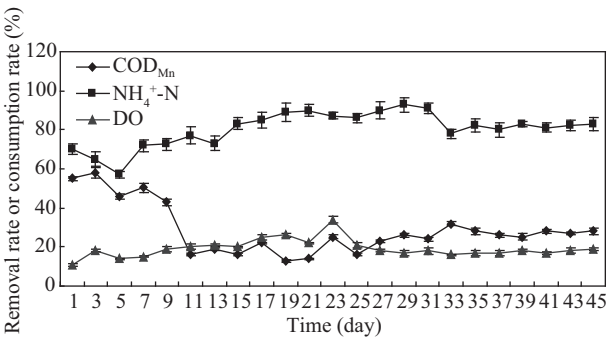


**Fig. 4** Variations of $NH_4^+$-N, $COD_{Mn}$ and DO during the biofilm formation process. Error bars represent the standard deviation of replicate ($n$ = 3) samples.

**Table 2** Biomass and biological activity of different layers

| Parameter | Upper layer | Middle layer | Lower layer |
|---|---|---|---|
| HPC ($\times 10^7$ CFU/g dw) | 4.35 ± 0.04 | 1.65 ± 0.07 | 0.78 ± 0.10 |
| Lipid-P (nmol P/g dw) | 15.67 ± 0.32 | 12.28 ± 0.27 | 9.12 ± 0.19 |
| TTC-DHA (μg TF/(g dw·hr)) | 31.07 ± 0.35 | 26.07± 0.23 | 20.12 ± 0.27 |

HPC: heterotrophic plate count; TTC-DHA: triphenyl-tetrazolium chloride-dehydrogenase.

### 2.2.2 Metabolic activity of microbial communities

After 6 months of operating the biofilter, the values of the average well color development (AWCD) at 96 hr, based on the value of AWCD, were high in the biofilter. The carbon source utilization rates of the microbial community in the upper, middle and lower layer of the biofilter were 80.6%, 67.5% and 61.3%, respectively. There was no significant difference in the utilization rates between those measured on day 40 and those measured after 6 months. For the relative utilization efficiency of six different substrate classes, substrates such as carboxylic acids and polymers were utilized less, while other classes of substrates were utilized more by the microbial communities in the biofilter. The utilizations were further analyzed with principal component analysis (PCA), and the first two components (PCA axes 1 and 2) accounted for 52.5% and 27.3% of the overall variance, respectively. The contribution of each substrate to the two principal components was evaluated based on the factor loading value and the results revealed that component 1 is mainly correlated with D-cellobiose, D-mannitol, putrescine, β-methyl-D-glucoside, *N*-acetyl-D-glucosamine, L-arginine, Tween 80 and *D*-malic acid. In addition, component 2 was primarily correlated with γ-hydroxy butyric acid, glycyl-L-glutamic acid and phenylethylamine. The results showed that the substrates utilized more by microorganisms were the same as in the start-up stage.

### 2.2.3 Operating performance

To evaluate the effectiveness of the biofilter, some water quality indices such as $NH_4^+$-N, $NO_2^-$-N, $COD_{Mn}$, $UV_{254}$ and BDOC were determined. As shown in **Table 3**, the removal rates of $NH_4^+$-N, $NO_2^-$-N and BDOC reached up to 80.0%, 95.8% and 72.0%, respectively.

To investigate the impact of the biofilter on the efficiency of organic micro-pollutant removal, organic micro-pollutants in the water sample were identified by

**Table 3** Purification effect of biofilter ($n$ = 5)

| Parameter | Influent | Effluent | Average removal rate |
|---|---|---|---|
| $NH_4^+$-N (mg/L) | 0.25 ± 0.02 | 0.05 ± 0.01 | 80.0% |
| $NO_2^-$-N (mg/L) | 0.024 ± 0.003 | < 0.001 | 95.8% |
| $COD_{Mn}$(mg/L) | 1.60 ± 0.31 | 1.17 ± 0.25 | 26.8% |
| $UV_{254}$ ($cm^{-1}$) | 0.025 ± 0.002 | 0.021 ± 0.001 | 16.0% |
| BDOC (mg/L) | 0.486 ± 0.085 | 0.136 ± 0.056 | 72.0% |

GC-MS. The results showed that there were 94 species of organic micro-pollutants in the influent (**Fig. 5a**). The main components were alkanes, lipids, benzodiazepines, ketones and phenols, which accounted for more than 90% of the total quantity of organic micro-pollutants. By contrast with organic micro-pollutants identified in the influent, 82 species of organic micro-pollutants were detected in the effluent, showing a reduction of 12 species. In the influent, 49 compounds showed peak areas greater than 0.5% of the total peak area, and these compounds accounted for 52.1% of all species of organic micro-pollutants. However, the number of organic micro-pollutants with peak areas greater than 0.5% of the total peak area detected in the effluent decreased to 34 after the biofilter process (**Fig. 5b**). The total peak area of the organic micro-pollutants detected in the influent was $1.31 \times 10^{10}$, while the total peak areas decreased to $3.43 \times 10^9$ after the biofilter. The removal rates of organic micro-pollutants of the biofilter reached 73.8%. Thus, the organic micro-pollutants in the influent water could be effectively removed by the biofilter process. However, for quantitative analysis of the main organic micro-pollutants, we will do more research in the following stage.

## 3 Discussion

The biomass and microbial activity in a biological aerated filter are important parameters, which directly affect the degradation rate of pollutants. The biomass was estimated by HPC, one of the best known and oldest microbiological techniques. Maoyu and Zhang (1989) reported that the number of bacteria estimated by this method correlates well with the values calculated using an indirect microelectrode method. Dehydrogenase activity is recommended as a very sensitive and very simple method to determine bacterial activity (Lazarova and Manem, 1995). The HPC and TTC-DHA were continually monitored in the start-up stage, and the values were low during the early stage of colonization because most microorganisms present on the granular active carbon (GAC) at that time were not adapted to permanent attachment (Servais et al., 1994). About 3 months were required before colonization occurred in the GAC in the filters and a steady state bacterial biomass was reached (Servais et al., 1994). Velten et al. (2011) reported that the biofilm biomass accumulated rapidly on GAC particles in their filter during the first three months of operation. Due to a low content of biogenic substances in the feed water, the settlement of microorganisms in the carbon filter proceeded over a relatively long period (6 months) as reported by Seredyńska-Sobecka et al. (2006). The previous study showed that the biomass accumulated rapidly in the upper layer of the filter, whereas the lower growth rate at the bottom was ascribed to a decreasing availability of organic nutrients downward through the filter (Velten et al., 2011). After the first week of the start-up period in this study, the microorganisms began to reproduce rapidly and become active in the carbon filter, and the HPC count and activity of dehydrogenase gradually increased within 30 days of the start-up process, then reached a plateau. Subsequently, HPC count and dehydrogenase activity reduced slightly as a result of the limited nutrients available for growth and reproduction of the microorganisms along with the competition for substrate between different species of microorganisms, and the toxic metabolic products released by the microorganisms. The characteristics of the microbial community that developed in the BAC filter corresponded well with results previously observed (Seredy ska-Sobecka et al., 2006; Lazarova and Manem, 1995). In particular, the development of the microbial community in the filter had an obvious lag phase, exponential phase, stationary phase, etc. In contrast to the previous study, where the biomass (phospholipids) deposited on the activated carbon reached

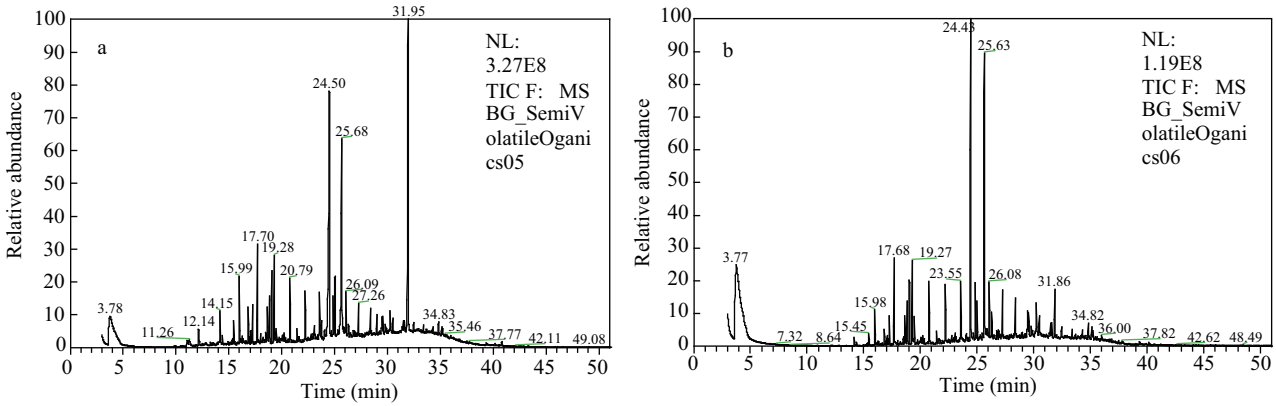


**Fig. 5** GC-MS spectrum of water samples: influent (a), effluent (b).

a maximum value after 6 weeks culture as reported by Seredyńska-Sobecka et al. (2006), our study indicated that phospholipids reached their maximum value 40 days after the filter had been initiated. Delahaye et al. (2003) observed that there was a linear relationship between ATP and HPC in the drinking water distribution network (Delahaye et al., 2003). ATP contents also correlated with dehydrogenase activity values presented by Lazarova and Manem (1995). The change in regularity of dehydrogenase activity is in accord with the change of HPC, and dehydrogenase activity correlated well with HPC ($P< 0.05$) in the study.

The metabolic function of the microbial community in the biofilter increased during the start-up process, and the richness index and function diversity index reached a relatively stable level by day 40. At this time, the biological interactions among different microbial populations in the biofilter were in equilibrium. Therefore, the course of start-up can be completed within about 40 days based on the analysis of the metabolic function of the microbial community. The metabolic functions of the microbial community were different at different depths, and the differences in metabolic functions among various layers were reduced in the start-up process. Carbon sources such as D-xylose, D-glucosaminic acid, 2-hydroxy benzoic acid, glycogen, α-cyclodextrin, and α-ketobutyric acid were hardly utilized by the microbial community during the start-up process. The present results were similar to previous observations by Moll et al. (1998) that some carbon sources, for example, α-cyclodextrin, α-ketobutyric acid and D-glucosaminic acid, were not utilized by drinking water biofilter communities. The metabolic potential of a microorganism toward substrates lies in the biological characteristics of the microorganism, as well as the structural characteristics of substrates. In this study, for these carbon sources hardly used by microorganisms, one possible explanation was that the microbial community developed on the filter bed did not evolve to form a metabolic mechanism available for these substrates. Another possible explanation was that macromolecular compounds (e.g., glycogen and α-cyclodextrin) were poorly utilized by the microorganisms.

The removal rate of COD and $NH_4^+$-N as well as the changing status of effluent DO were used to determine the course of biofilm thickening during the process of the natural start-up of biofilm reactors (Phattaranawik and Leiknes, 2011; Guo et al., 2009). Simpson (2008) stated that the model in which BAC filters and the associated biofilms removed or eliminated organic matter from source water was thought to be a three-stage process. The three-stage process was composed of physical adsorption, concurrent adsorption/biological degradation and biological degradation. Initially, BAC biofilters functioned primarily by using a physical adsorption process while the microorganisms in the associated biofilm were in an acclimation period in the study, so COD and $NH_4^+$-N removal efficiencies were high due to carbon adsorption. The adsorption capacity of the BAC declined continually, and the removal rate of COD and $NH_4^+$-N showed a sharp downward trend when the BAC available adsorption sites were gradually occupied either by organic matter and/or microorganisms. At the same time, the biomass and bioactivity of the BAC gradually increased and the biodegradation process enhanced significantly during this time. After the process in which the BAC filters went through physical adsorption, concurrent adsorption/biological degradation and biological degradation, the removal rate of $NH_4^+$-N and $COD_{Mn}$ reached a relatively stable level around day 40. As shown in **Fig. 4**, COD and $NH_4^+$-N removal rates decreased at the beginning of the start-up period (5–11 days), and increased slowly, achieving maximal levels after 21–28 days. The removal efficiencies then decreased slightly and reached stable levels (about 80% and 28%, respectively) on day 40.

Some studies have shown that DO in reactors is a very important parameter because it has a significant influence on the behavior and activity of the heterotrophic and autotrophic microorganisms in various biological systems (Holenda et al., 2008; Tan and Ng, 2008; Guo et al., 2009). DO is also a chief factor in limiting the nitrification process, and its impact becomes even more significant as organic loading increases in the reactor, allowing fast-growing heterotrophic bacteria to compete with nitrifying bacteria for the limited oxygen (Ling and Chen, 2005). According to a previous study, there was an evident relationship between DO concentration and effluent quality (Hashmi and Kim, 2003; Guo et al., 2009). From **Fig. 4**, in the initial stage of the start-up process, various microorganisms, especially heterotrophic bacteria, utilized organic materials for growth and reproduction, and the consumption rate of DO rose steadily as a result. In the middle stage, the consumption rate of DO appeared to fluctuate because of the competition between heterotrophic bacteria and autotrophic nitrobacteria for DO. In the final stage, the consumption rate of DO stayed at about 18% when the competition conditions were in equilibrium.

After 6 months of biofilter operation, there were no significant differences between the microbial biomass and activity, and the metabolic function of the microbial community in the biofilter as compared with that on day 40 (the beginning of the steady state). For the purpose of the biofilter operation we considered that the growth and metabolism of microorganisms in the biofilter were at steady state.

Biofilters have been extensively studied in drinking water treatment, and were proved to be efficient in removing organic matter and ammonia (Andersson et al., 2001; Sang et al., 2003; Li and Chu, 2003). During an 8 month operation, the removal percentage of $COD_{Mn}$ was between 14% and 20% in a bio-ceramic filter (Sang et al., 2003). The ammonia removal efficiency usually ranges from 30% to

93% in biofilters. The submerged membrane bioreactor ran continuously for more than 500 days, in which biological nitrification was even better than organic degradation, and removed more than 95% of $NH_4^+$-N in drinking water treatment (Li and Chu, 2003). In full-scale filters, ammonia removal capacities were more than 90% for two types of GAC when water temperatures were above 10°C, while the ammonia removal in the opened superstructure (> 90%) was higher than in the closed superstructure (45%) at moderate temperatures (4–10°C) (Andersson et al., 2001). As shown in **Table 3**, the removal rates of $NH_4^+$-N and $COD_{Mn}$ were 80.0% and 26.8%, respectively. In fact, the removal efficiencies of COD and $NH_4^+$-N were affected by many factors, such as operational parameters (Hasan et al., 2011), COD/N ratio (Thuan et al., 2003), biofilter media and temperature (Andersson et al., 2001) and microbial community composition. Therefore, the removal efficiencies of COD and $NH_4^+$-N are different compared to other biofilter systems. For nitrite, the concentration was lower than 50 μg/L, which was identical to the previous studies. Li and Chu (2003) determined that the removal rates of $UV_{254}$ and BDOC were 54%–86% in the submerged membrane bioreactor, and Servais et al. (1994) obtained removal rates of $UV_{254}$ and BDOC of 43%–56% in GAC filters. However, in the present study, the removal percentage of $UV_{254}$ was only 16.0%, while the removal percentage of BDOC reached 72.0%.

Some studies have demonstrated that biofilters can effectively remove low concentrations of organic compounds (Qi et al., 2002; García-Peña et al., 2005). The biodegradation of organic compounds depends on biomass accumulation in the biofilters (Song and Kinney, 2000). The pilot plant used in the present study was fed with actual Yangtze River water and there were 94 species of organic micro-pollutants detected in the influent. As shown in **Fig. 5**, the total amount and number of organic micro-pollutants decreased considerably after the biofilter treatment. Based on these findings, the developed biofilter has operated for more than 6 months and reached a steady state in the biological system, and has been shown to perform well when removing organic micro-pollutants. In future studies, we will apply molecular biology techniques and conventional culture methods to analyze the predominant bacteria present and to screen for unique organic micro-pollutant-degrading strains of bacteria in the biofilter.

## 4 Conclusions

Increasing use of biofilm processes in water treatment requires steady development of the microbial communities in biofilters. The experimental results demonstrated that the microbial biomass and activity of the BAC filter increased rapidly after about 1 week and reached steady state on day 29. However, the metabolic capacity of microbial communities as well as the removal rate of $NH_4^+$-N and $COD_{Mn}$ reached a relatively stable level on day 40 during the start-up process. Results from this study suggested that the filter start-up process could be completed within about 40 days. After 6 months of operation, the microbial biomass and activity as well as metabolic function remained steady in the filter. In addition, the results of the water quality analysis showed that the biofilter process was efficient in removing pollutants, especially organic micro-pollutants.

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