Original Article

Bacterial Community and Function of Biological Activated Carbon Filter in Drinking Water Treatment*

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Abstract

Objective It aims to investigate the changes in composition and structure of bacterial communities de-veloping on biological activated carbon (BAC) particles, and the bacterial functions.

Method A pilot plant had been in service for 180 days, aiming to develop bacterial communities on acti-vated carbon naturally. After 180 days of operation, the bacterial communities were determined by dena-turing gradient gel electrophoresis (DGGE) analyses of PCR-amplified 16S rRNA genes. The study on community composition and the phylogenetic relationships of the organisms was complemented by a se-quence analysis of cloned PCR products from 16S rRNA genes. Gas chromatorgaphy-mass (GC-MS) mea-surement was used to determine organic chemical composition of inflow and outflow water on the 300th day. TOC and NH_4^+-N were also tested in this experiment.

Results It showed that the stable bacterial structure did not develop on BAC particles until the 9th month during running time of the BAC filter. The communities were finally dominated by Pseudomonas sp., Ba-cillus sp., Nitrospira sp., and an uncultured bacterium. Stable bacterial communities played an important role in removal of NH_4^+-N and total organic carbon (TOC). Results from gas chromatorgaphy-mass (GC-MS) showed that 36 kinds of chemicals in feed water were eliminated, and concentrations of 5 kinds of chemicals decreased. These chemicals served as nutrients for the dominant bacteria.

Conclusion The findings from the study suggested that the stability of microbial structure was beneficial for improving NH_4^+-N and TOC removal efficiencies. The dominant bacteria had the advantage of biode-grading a wide range of organic chemicals and NH_4^+-N.

Key words: Biological activated carbon; Bacterial community; Denaturing gradient gel electrophoresis

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INTRODUCTION

Songhua River is an important water source for Harbin citizens in China (supplying 300 000 $m^3 \cdot d^{-1}$ drinking water). However, in Dec, 2005, it suffered serious pollution due to the explosion at PetroChina factory located in the upriver and the security of drinking water was thus threatened. Granular activated carbon (GAC) is effective in adsorbing a wide range of organic

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compounds[1-4], unkind taste and odors[5], controlling the formation of chlorinated pollutants[6-7], and reducing the ozonation by-product, bromate[8-9]. So it has been widely used in drinking water treatment. Various studies have shown that GAC used to treat surface water can be heavily colonized by microorganisms to form biological activated carbon (BAC) because of carbon's large surface area, rough surface texture and ability to adsorb organic materials. The attachment of bacteria to activated carbon plays an important role in surface water treatment. For example, the bacteria can utilize the organic materials adsorbed on GAC as growth substances[10-11] and bio-regenerate the activated carbon[12-13]. The classical methods based on cultivation techniques have been used in studying bacterial communities associated with BAC[14]. The predominant microbial genera were *Pseudomonas*, *Chromobacterium*, *Bacillus*, and smaller numbers of nitrogen-fixing bacteria. But they did not cultivate autotrophs or budding, gliding or sheathed bacteria common to aquatic habitats. Moreover, a number of bacteria are not cultivable or identifiable, so it is hard for the cultivation techniques to reflect the bacterial communities authentically.

The denaturing gradient gel electrophoresis (DGGE) method, a new molecular genetic tool used in environmental microbiology, allows the dissection of microbial communities at the level of the phylogeny of their constituents. This method has been widely used to study bacterial community structure of soil[15-16], groundwater[17], lake[18], river[19], and bioreactor[20-21], but rarely used to study or identify the bacteria of the BAC treatment system.

A pilot plant had been in service for 300 days, aiming to develop bacterial communities on activated carbon naturally. The inflow water was taken from contaminated Songhua River and then primary settled and sand filtrated. The goal of this research was to investigate the changes in composition of bacterial communities developing on BAC. The bacterial composition was determined by DGGE analysis of PCR-amplified 16S rRNA genes. The study on community composition and the phylogenetic relationships of the organisms was complemented by a sequence analysis of cloned PCR products from 16S rRNA genes. GC-MS measurement was used to determine organic chemical composition. TOC and NH_4^+-N were also tested in this experiment. By studying the changes in pollutant composition in inflow and outflow water, we analyzed the relationship between bacterial composition and pollutant species in water at the end of BAC operation stage, and inferred the function of the dominant bacteria played on the BAC reactor.

MATERIALS AND METHODS

Sampling Device

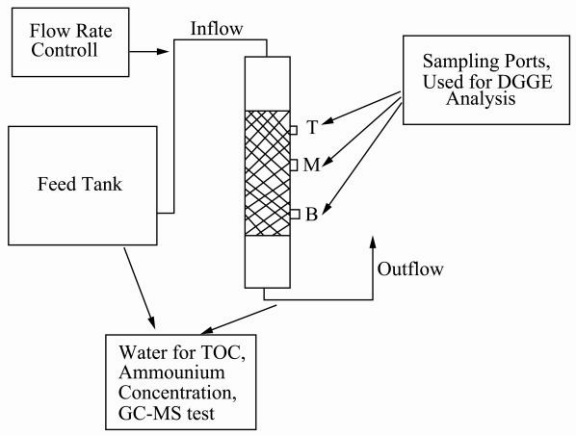
A flow chart of simplified process is given below (Figure 1). A pilot filter was constructed of Lucite tubing to avoid organic contamination. The GAC column of 200 cm high and 6 cm in internal diameter was filled with 3 L dry-heat-sterilized granular activated carbon particles. The column had sampling points at three different positions in the carbon bed. They were named as T (140 cm above the bed bottom), M (100 cm above the bed bottom) and B (60 cm above the bed bottom). Position T was near the inflow and position B near the outflow. Position M was located roughly in the centre of the bed. The pilot filter was fed with primary settled and sand filtrated water continuously at an empty bed contact time (EBCT) of 10 min and was backwashed once a week with air and water. The feed water was taken from Songhua River and its quality parameters are shown in Table 1.

Sample Collection

Water and activated carbon samples were collected from the BAC pilot plant. The pilot plant had been in service for 180 days before water samples were collected, which was aimed at developing bacterial communities naturally. Inflow and outflow water sample collection was conducted every day from the 180th to the 300th day of operation for analyses of concentrations of TOC and NH_4^+. Water samples were collected on the 300th day for the gas chromatorgaphy-mass (GC-MS) analysis. Activated carbon samples were taken from the sampling ports T, M, and B respectively on the 195th day and collected once a month (30 days) until the 285th day. These samples were immediately transported (on ice) to laboratories at Institute of Microbiology of Heilongjiang Academy of Sciences in Harbin for analysis.

Biofilm Developed on BAC

BAC samples were taken from the sampling ports T, M, and B on the 300th day of operation for detecting the biofilm developed on BAC. Biofilm was evaluated by SEM as described previously[22].



[微软用户1]**Figure 1.** BAC process flow Feed tank.

Table 1. Inflow Water Characteristics

Parameter	Min	Median	Max
Temperature (°C)	11	16	23
pH	6.8	7.2	7.4
TOC (mg/L)	3.62	4.63	5.99
$UV_{254\ nm}$ (L/m)	12	14	19
NH_4^+ (mg/L)	0.751	1.033	1.355

Analytical Method for Water Quality

Ammonia plus ammonium were determined colorimetrically[23]. TOC was analyzed by using an Aurora Combustion Total Organic Carbon Analyzer 1030C (OI, America). Composition of the organic chemicals in inflow and outflow water on the 300th day of BAC operation was determined by the GC-MS method (HP MP5890GC/MS) for detecting dominant bacterial biodegradable ability.

DNA Extraction

DNA was extracted from the granular activated carbon with the extraction method described by Zhou et al.[24] The samples of 5 g were mixed with 13.5 mL of DNA extraction buffer (100 mmol/L Tris-HCl [pH 8.0], 100 mmol/L sodium EDTA [pH 8.0], 100 mmol/L sodium phosphate [pH 8.0], 1.5 mol/L NaCl, 1% CTAB) and 100 mL of proteinase K (10 mg/mL) in Oakridge tubes by horizontal shaking at 225 rpm for 30 min at 37 °C. After the shaking treatment, 1.5 mL of 20% SDS was added, and the samples were incubated in a 65 °C water bath for 2 h with gentle end-over-end inversions every 15 to 20 min. The supernatants were collected after centrifugation at 6 000 rpm for 10 min at room temperature and transferred into 50 mL centrifuge tubes. The activated carbon particles were extracted two more times by adding 4.5 mL of the extraction buffer and 0.5 mL of 20% SDS, vortexing for 10 s, incubating at 65 °C for 10 min, and centrifuging as before. Supernatants from the three cycles of extractions were combined and mixed with an equal volume of chloroformisoamyl alcohol (24:1, vol/vol). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids was obtained by centrifugation at 16 000 rpm for 20 min at room temperature, washed with cold 70% ethanol, and re-suspended in sterile deionized water, to give a final volume of 500 mL.

Primers and PCR Amplification

GC-clamp-F357 primer (5′–CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCCCTACGGGAGGCAGCAG-3′) and R518 primer (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the bacterial 16S rRNA gene. PCR mixtures were composed by what follows. 12.5 µL 10×PCR buffer (with $MgCl_2$, TaKaRa), 10 nmol of each deoxyribonucleoside triphosphate, 10 pmol of each primer, 2.5 U of Pfu AmpliTaq DNA polymerase, were combined with DNA-free water to 50 µL in a 0.2 mL Microfuge tube. After the addition of 5 ng of template DNA, the mixtures were incubated in a Ependorf PCR system 2700 (Ependorf) programmed as follows: initial denaturation of double-stranded DNA for 5 min at 94 °C; 20 (touchdown) cycles consisting of 1 min at 94 °C, 1 min at 65 °C, and 1 min at 72 °C with a decrease in the annealing temperature of 0.5 °C per cycle; 10 cycles consisting of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C; and extension for 5 min at 72 °C. All amplification products were analyzed by electrophoresis in 1.0% (w/v) agarose gels, followed by ethidium bromide staining (1.2 mg/liter ethidium bromide in 1×Tris-acetate-EDTA).

Denaturing Gradient Gel Electrophoresis (DGGE)

The PCR products were separated by using DGGE with a D-Code universal mutation detection system (Bio-Rad Laboratories) according to the instruction manual. The PCR products were loaded onto a polyacrylamide gel (8% [w/v] acrylamide in 0.5×TAE buffer [2.42 g Tris base, 0.82 g sodium acetate, 0.185 g EDTA, 1 liter of H_2O; pH adjusted to 7.8 with acetic acid]) with a 30% to 60% denaturant gradient (100% denaturant was 7 mol/L urea and 40% [vol/vol] deionized formamide). The wells were loaded with roughly equal amounts of DNA (about 500 ng), and electrophoresis was carried out in 0.5×TAE buffer at

150 V for 4 h at 60 °C. DGGE gels were stained with $AgNO_3$ as described by Sanguinetti et al.[25]

Cloning and Sequencing

The dominant bands in the DGGE gel were excised. Each excised piece was washed twice with 1 mL of sterilized distilled water. A small chip (less than 1 mm^3) of each piece was used as a direct template for PCR to recover the DNA fragment. The PCR conditions were the same as those for the original PCR. The fragments recovered from the PCR were subjected to DGGE again to confirm the equality of their mobility in comparison with that of the original samples. If a single band appeared in the DGGE gel for one sample, the PCR products were purified with Agarose Gel DNA Purification Kit (TaKaRa, Dalian, China). The re-amplified bands were cloned into *E. coli* JM109 cell (TaKaRa, Dalian, China) by using the TaKaRa pMD18-T Vector (TaKaRa, Dalian, China) according to the manufacturer's instructions. Insert size was confirmed by PCR amplification with the pMD18-T-specific primers M13-47 (5′-GCCAGGG TTTTCCCAGTCACGAC-3′) and RV-M (5′-GAGCGGATAACAATTTCACACAGG-3′). Clones containing a correct insert were re-amplified and screened by DGGE and always compared with their original samples. The sequencing reactions were performed with a DNA sequencing kit, BigDye Terminator v3.0 (Applied Biosystems), and the reaction products were analyzed with an ABI PRISM 3100 genetic analyzer (Applied Biosystems).

Nucleotide Sequence Accession Numbers

The sequences generated in this study have been deposited in the DNA Data Bank of GenBank under accession numbers from FJ978001 to FJ978004.

RESULTS

DGGE Analysis of Bacterial Community Composition on BAC Biofilm

The DGGE profiles of the bacterial communities on the top (T), middle (M) and bottom (B) regions of the BAC column from the 195th day to the 285th day of operation are shown in Figure 2. The DGGE patterns appeared to vary with time. From the 195th day to the 285th day of operation after the BAC pilot in service, the variety of bacterial species decreased. Bands with signal "·" isappeared gradually. From the 255th day, the specific bacterial strains (Band 1-4) began to dominate. On the 195th day, the number of bacterial species in the T region of the BAC column was more than that in the M or B. On the 225th day, their number in the T was equal to that in the M, but more than that in the B. After the 255th day of operation, a stable bacterial structure was developed on the BAC column. The T, M, and B regions of the BAC column were all inhabited by four bacteria, which were Band 1 to 4.

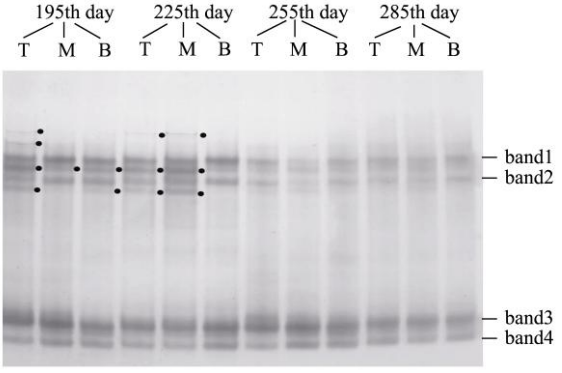


Figure 2. Bacterial DGGE profiles of the BAC treatment system. Above each lane, T, M, and B were sampling ports. The words above T, M, and B (195th day, 225th day, 255th day, 285th day) were sampling time. The word on the right (Band 1, Band 2, Band 3, and Band 4) represented each corresponding band. Bands with signal represent the disappeared bands finally.

Sequencing and Identification of DGGE Fragments

The prominent DGGE bands (Band 1 to 4 in Figure 2) were recovered and sequenced following cloning. Comparison of 16S rDNA sequences with sequences available in GenBank databases revealed high similarity values for these bands. Their closest relatives are shown in Table 2. The organisms represented by the prominent bands (Figure 2 Band 1 to 4) belong to *Pseudomonas* sp. *Bacillus subtilis*, *Nitrospira* sp, and an uncultured bacterium respectively.

Biofilm Developed on BAC

Activated carbon samples of top (T), middle (M), and bottom (B) regions of the BAC filter were collected on the 300th day of operation for observation of the biofilm developed on it. The carbon was coated with sticky and thick biofilm (Figure 3a, 3b, and 3c). A large number of bacteria together with extracellular polymeric substances (EPS) are found to accumulate on the carbon surface and hide in interstices of activated carbon. Since

Table 2. Closest Relative Species of the Selected Clones (Determined by an NCBI BLAST Search)

Band		Identity	Closest Relative	
No.	Accession No.	(%)	Organism	Accession No.
1	FJ978001	96	Uncultured *Pseudomonas* sp.	DQ167038
2	FJ978002	94	*Bacillus subtilis*	AM110930
3	FJ978003	99	*Nitrospira* sp.	AJ224044
4	FJ978004	97	Uncultured bacterium	AY989627

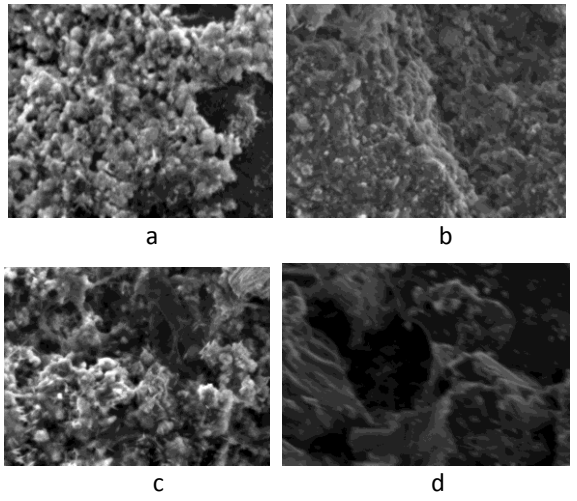


Figure 3. Scanning electron micrographs of representative pellets of carbon. a: BAC in the top region (T) of the filter. b: BAC in the middle region (M) of the filter. c: BAC in the bottom region (B) of the filter. d: Granular activated carbon without bacteria. (BAC samples were taken on the 300th day of operation).

interstices in activated carbon were filled with bacteria, EPS and degradation products, soluble organic chemicals could not pass through. As a result, activated carbon could not play a role in adsorption of organic chemical.

NH_4^+ and TOC Removal by BAC

NH_4^+-N and TOC removal data were estimated as concentrations at the inlet and outlet of the column and removal ratio. They are presented in Figure 5, in the form of running averages (on 30 consecutive values). The results can be divided into two periods of experimentation:

From days 180 to 240, concentrations of NH_4^+-N and TOC in inflow water were at a lower level (with NH_4^+-N concentration below 1 mg/L and TOC below 5 mg/L). The average NH_4^+-N removal rate during this period was 53.8%, and the average TOC removal ratio was 44%. The maximum NH_4^+-N removal ratio was 68.3%, and 47% for TOC removal. Average NH_4^+ concentration in outflow water was 0.41 mg/L and TOC was 2.43 mg/L.

From days 240 to 300, concentrations of NH_4^+-N and TOC in inflow water were increased. The maximum NH_4^+-N concentration in inflow water was 1.41 mg/L and maximum TOC concentration 5.92 mg/L. During this period, the average NH_4^+-N removal rate of the BAC column increased from 53.8% to 67.2%, while the average TOC removal rate increased from 44% to 58.9%. The maximum NH_4^+-N removal rate was 74%. The maximum TOC removal rate was 72.7%. The average NH_4^+ concentration in outflow water was 0.40 mg/L, and that for TOC was 2.22 mg/L.

Biodegradation of Organic Chemicals

The organic chemicals in inflow and outflow water on the 300th day of operation were examined by the GC-MS method in this study (Figure 5). The water samples were collected on the 300th day when the bacterial structure was stable. There were 41 organic chemicals in feed water (with the total area value being 2.32E+09) and 10 in outflow (with the total area value being 1.28E+09). After biodegradation by the microorganisms on the BAC particles, most of the pollutants in water were eliminated except for 5 chemicals (showed in Figure 5), namely 2-Pentanone, 4-hydroxy-4-methyl- (1 in Figure 5a and A in Figure 5b), 2,3,5,6-Detetrahydro-cyclohexanone, 2,6-di-t-butyl-4-hydroxy-methylene- (15 in Figure 5a and E in Figure 5b), 2,6-Bis(1,1-dimethylethyl)-4-nitro-phenol (24 in Figure 5a and G in Figure 5b), Dibutyl phthalate (26 in Figure 5a and H in Figure 5b) and 1-Octadecanamine, N-methyl- (35 in Figure 5a and I in Figure 5b). But the quantities of these five chemicals were decreased. High Creatinine content (C in Figure 5b) detected in outflow water was not

present in feed water. In addition, other four new chemicals appeared in outflow water and they were Ethanamine, N-methyl-2-[(2- methylphenyl) phenylmethoxy]- (B in Figure 5b), 1(2H)-Naphthalenone, octa-hydro-5-hydroxy-4a-me thyl- (D in Figure 5b), 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (F in Figure 5b) and O-Veratramide (J in Figure 5b).

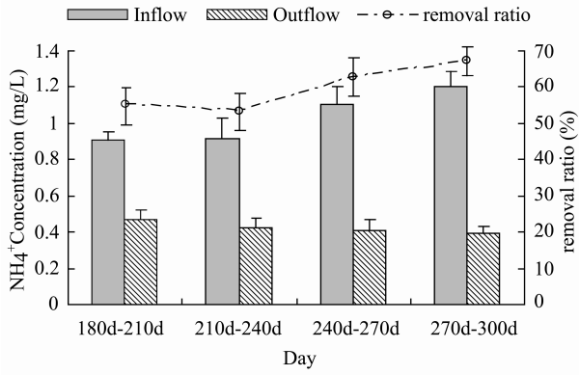
The identification of organic chemicals in water was shown in Table 3 and Table 4.

DISCUSSION

In this study, we investigated the dynamics of the structure of bacterial communities developing on BAC, and its function for BAC process. During the past several decades, many reports have focused on bacterial growth and activity[26-28], as well as ecological equilibrium in BAC systems[29], but relatively little is known concerning the process of bacterial community formation. Comparison of dynamics of the bacterial community structure from Day 195 to Day 285 of BAC operation provided, therefore, a unique opportunity to specifically investigate such formation.

During BAC running time, varieties of bacterial species reduced gradually. Bands with signal "." present on the 195th day and the 225th day disappeared finally (Figure 2). Communities were finally dominated by *Pseudomonas* sp. *Bacillus subtillis*, *Nitrospira* sp, and an uncultured bacterium after 255 days of BAC operation. The change of bacterial communities developed on BAC can be explained by two reasons: decrease of water temperature or/and bacterial competition.

Low temperatures have an effect on biological treatment process because of its effects on biochemical systems. From Day 180 to Day 300 of BAC operation, water temperature decreased from



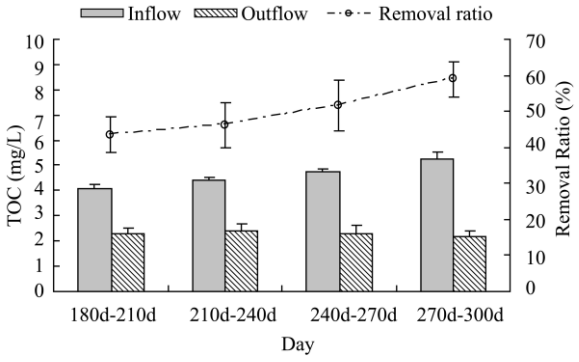


Figure 4. NH_4^+ (left part) and TOC (right part) removal evolution as a function of time in the BAC pilot plant.

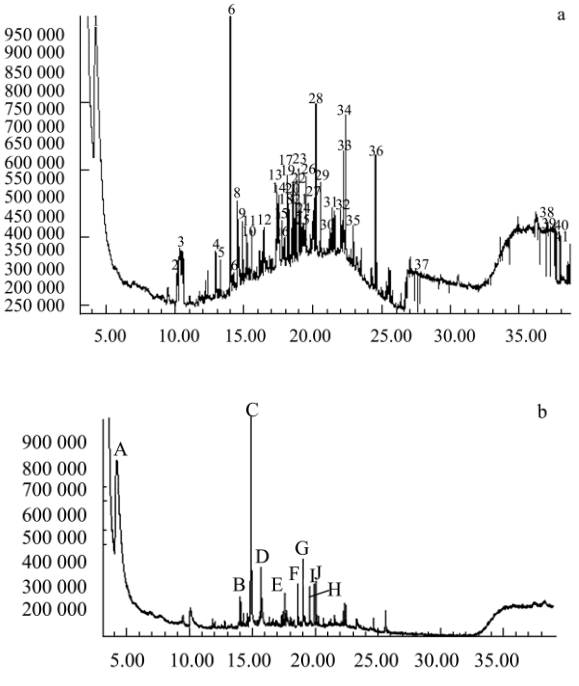


Figure 5. GC-MS profiles showed organic chemicals detected in samples of inflow water (a) and outflow water (b) on the 300th day of BAC operation.

23 °C to 11 °C. Some bacteria could not survive in low temperature conditions, such as below 15 °C. Temperature was a factor limiting bacterial growth and multiplying, which might be the reason that the amount of 4 dominant bacteria was lower on the 255th and 285th days than that on the 195th and 225th days (Figure 2).

Table 3. Identification of Organic Chemicals in Inflow Water of BAC

NO.	R.T. (min)	Species	Area Value
1	4.196	2-Pentanone, 4-hydroxy-4-methyl-	52463191
2	10.135	n-Hexylmethylamine	983725
3	10.293	1-Propanamine, N,2-dimethyl-	3492061
4	12.890	Urea, N,N-dimethyl-	4125441
5	13.241	N-8-Guanidino-spermidine	2410957
6	13.971	3-tert-Butyl-4-hydroxyanisole	29013793
7	14.450	Phenol, 2,4-bis(1,1-dimethylethyl)-	4909910
8	14.507	Butylated Hydroxytoluene	5146232
9	14.851	3-Ethoxyamphetamine	4846105
10	15.144	Benzeneethanamine, 4-fluoro-.beta,3-dihydroxy-N-methyl	2399348
11	15.502	1-Octanamine, N-methyl-	4415413
12	16.382	Benzenepropanamine, .alpha.-methyl-	3791928
13	17.305	2-Azonia-4-boratapentane, 4-cyano-5,5,5-trifluoro-2-methyl-4-trifluoromethyl-3-trimethylsilyl	5467092
14	17.384	1-Ethynyl-3,5-dimethyladamantane	4825209
15	17.463	2,3,5,6-Detetrahydrocyclohexanone, 2,6-di-t-butyl-4-hydroxymethylene-	5238816
16	17.699	Methylpent-4-enylamine	4470617
17	18.114	Benzenemethanol,3-hydroxy-.alpha.-[(methylamino)methyl]-, (R)-	5110801
18	18.228	Fluoxetine	2752367
19	18.486	1,2-Benzenedicarboxylic acid, dipropyl ester	3957973
20	18.557	Perfluoroglutaramide	3584438
21	18.643	1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)-	1919271
22	18.693	Phenethylamine, p-methoxy-.alpha.-methyl-, (.+/-.)-	3076906
23	18.772	.beta.-Alanine, N-methyl-, ethyl ester	3834210
24	18.915	2,6-Bis(1,1-dimethylethyl)-4-nitrophenol	5135446
25	19.037	dl-Allo-cystathionine	2360246
26	19.430	Dibutyl phthalate	4445584
27	20.031	p-Hydroxynorephedrine	3218009
28	20.167	Hexadecanoic acid, trimethylsilyl ester	9110857
29	20.511	Tetradecanoic acid, dimethyl(isopropyl)silyl ester	3838731
30	21.377	8-Methoxy-6-methyl-2-nitroanthra[2,1-b]furan	2740081
31	21.534	dl-Alanyl-dl-methionine	2305175
32	21.956	2-Methylaminomethyl-1,3-dioxolane	3616078
33	22.157	2H,6H-Pyrano[3,2-b]xanthen-6-one, 5,9,10-trihydroxy-2,2-dimethyl-	5164103
34	22.314	Hexadecanoic acid, tert-butyldimethylsilyl ester	7662928
35	22.886	1-Octadecanamine, N-methyl-	1638836
36	24.489	Octadecanoic acid, tert-butyldimethylsilyl ester	8519084
37	27.609	Benzenemethanol, .alpha.-[(methylamino)methyl]-	1632782
38	36.976	Benzenepropanamine, N-(1,1-dimethylethyl)-.alpha.-methyl-.gamma.-phenyl-	4831739
39	37.656	N-dl-Alanylglycin	883784
40	37.885	2-Pentanamine	2420879
41	37.906	1,2-Propanediamine	676135

Table 4. Identification of Organic Chemicals in Outflow Water

NO.	R.T. (min)	Species	Area Value
A	4.211	2-Pentanone, 4-hydroxy-4-methyl-	66471465
B	13.964	Ethanamine, N-methyl-2-[(2-methylphenyl)phenylmethoxy]-	2690912
C	14.794	Creatinine	32082993
D	15.588	1(2H)-Naphthalenone, octahydro-5-hydroxy-4a-methyl-	4662647
E	17.463	2,3,5,6-Detetrahydrocyclohexanone, 2,6-di-t-butyl-4-hydroxymethylene-	2662582
F	18.486	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	3693115
G	18.915	2,6-Bis(1,1-dimethylethyl)-4-nitrophenol	5122731
H	19.438	Dibutyl phthalate	3400473
I	19.774	1-Octadecanamine, N-methyl-	3318906
J	19.946	o-Veratramide	4262166

WANG[30] has observed that after 38 days of BAC filter operation, the adsorption sites of activated carbon were saturated gradually. In the present study, the surface of BAC was coated with thick biofilm after 300 days of operation. Interstices on activated carbon were filled with bacteria, EPS and degradation products, so activated carbon could not play a role in adsorption of organic chemical. Organic chemicals adsorbed on activated carbon can serve as nutrients for bacterial growth. Many studies showed that bacterial communities of BAC are affected by nutrients adsorbed on activated carbon[28, 31]. In the present study, after 180 days of operation, nutrients adsorbed on activated carbon might become a limiting factor for bacterial community growth. Bacterial community structure in the BAC filter was affected by intra- or interspecific competition for resources, resulting in the change in bacterial structure.

Because of low temperature, concentrations of NH_4^+-N and TOC in inflow water increased after 240 days of operation and it was observed that the rises in NH_4^+-N and TOC removal efficiencies were achieved during this period (Figure 4). The average NH_4^+-N removal rate of the BAC column increased from 53.8% to 67.2%, and the average TOC removal rate increased from 44% to 58.9%. NH_4^+-N and TOC removal efficiencies were the highest during the 270th to the 300th day of operation. These rises might be attributed to biodegradation because it was highly unlikely that the adsorption of pollutants could be improved during this timeframe (Figure 3). Although the temperature was lower between Day 240 and Day 300 of BAC operation than that between Day 180 and Day 240 of operation. the TOC and NH_4^+-N removal efficiencies were higher. Bacterial community structure was at a stationary phase from Day 240 to Day 300 of operation. These results indicated that the stability of bacterial communities affected pollutant removal efficiency.

The BAC filter was colonized by 4 bacteria. To analyze these 4 bacterial functions, organic chemicals in inflow and outflow water on the 300th day were tested by the GC-MS method.

GC-MS results showed that there were 41 organic chemicals in inflow water (with the total area value being 2.32E+09) and 10 in outflow water (with the total area value being 1.28E+09). 36 kinds of organic chemicals were biodegraded by the bacteria. *Pseudomonas* sp. was reported to bioadsorb and biodegrade a wide range of organic chemicals such as phenol, *p*-nitrophenol, phenanthrene, benzene, toluene, ethylbenzene, xylenes, dibenzothiophene, methyldibenzothiophenes, n-Hexadeca, octadecane, 1,2-dichloroethane, 2,4- and 2,6-diaminotoluene[32-39], and other petroleum hydrocarbons like n-alkanes, aromatic hydrocarbons and polycyclic aromatic hydrocarbons[40]. It was found that *Bacillus* sp. was able to degrade a wide range of aromatic acids such as cinnamic, 4-coumaric, 3-phenylpropionic, 3-(p-hydroxyphenyl) propionic, ferulic, benzoic, and 4-hydroxybenzoic acids[41]. It was also shown that *Bacillus* sp. elaborated extracellular enzymes that degraded some extracellular polysaccharide[42]. In this complex water system, it can be confirmed that the organic chemicals in water serving as nutrient substances were biodegraded.

Nitrospira species play an important role in accumulation of high concentrations of nitrite. However, during BAC operation time, nitrite concentration in influent water was below 0.1 mg/L, which was too low to promote *Nitrospira* species growth. Some studies have observed that

heterotrophic nitrification exists in some *Bacillus* species[43] and *Pseudomonas* species[44]. Nemergut and Schmidt[45] reported that the nitrate reductase inhibitor could inhibit the denitrification function of *Pseudomonas* sp., resulting in nitrite accumulation. The potential explanation for existence of Nitrospira species in BAC was that *Bacillus* sp. or *Pseudomonas* sp. accumulated nitrite during heterotrophic nitrification.

After treated by BAC, both the quantity and variety of chemicals in outflow water were lower than those of inflow water (Figure 5). It showed that 36 chemicals disappeared, the contents of 5 chemicals decreased and 5 new chemicals formed (Table 3 and Table 4). Especially, a high amount of creatinine came into being (C in Figure 5). Creatinine is produced from creatine. Creatine synthesis requires three amino acids, namely methionine, glycine and arginine[46]. In the inflow water, there was N-8-Guanidino-spermidine, dl-Allo-cystathionine, dl-Alanyl-dl-methionine, N-dl-Alanylglycin, which contained amino acid groups. It was inferred that Creatinine was produced from these four chemicals after biodegradation and biosynthesis. In the BAC system, except for bioadsorption and biodegradation, biosynthesis is another important but not limited role of bacteria.

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