Bio-enhanced activated carbon filter with immobilized microorganisms for removing organic pollutants in the Songhua River
Yu-Nan Gao, Wei-Guang Li, Duo-Ying Zhang and Guang-Zhi Wang

ABSTRACT
Five dominant microorganisms including four kinds of Pseudomonas and one kind of Bacillus were isolated from the Songhua River. The organic pollutants removal potential and community composition of these five dominant microorganisms immobilized on activated carbon filter, which is called the bio-enhanced activated carbon filter (BEAC), were investigated to compare with the naturally formed biological activated carbon (BAC) filter. Songhua River was used as the raw water. The pilot scale test results showed the biomass in the BEAC filter increased initially and then stabilized after 45 d of operation with an average value of 192 nmolPO4/g carbon. The corresponding biological activity reached 1,368 ng ATP/g carbon. The gas chromatography-mass spectrometry (GC-MS) results showed that the BEAC filter degraded the toxic organic substances more effectively than the BAC filter, especially for halogenated hydrocarbons and PAHs (polycyclic aromatic hydrocarbons). Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis revealed the eco-system of five dominant microorganisms did not change in the BEAC filter even on 180 d of operation. Two of the five dominant microorganisms, Bacillus subtilis and Pseudomonas balearica, had high biological activity and were more adaptable to the surface of the carbon media than the other three dominant microorganisms. The scanning electron microscope (SEM) photograph showed a large quantity of microorganisms developed on the BEAC filter. The toxicity test using Deltatox Bioassay Technology Analyzer indicated that the dominant microorganisms were safe to be applied in drinking water treatment process.

Key words | bio-enhanced activated carbon (BEAC), biological activated carbon (BAC), biological activity, biomass, dominant microorganisms

INTRODUCTION
The Songhua River in the Northeast China is the largest tributary of the Heilong River (also known as the Amur River in Russia), flowing 1,927 kilometres from the Changbai Mountains through the Jilin and Heilongjiang provinces. The river flows through 551,000 square kilometres of land with a population of more than 60 million. Since December 2005, the Songhua River has been polluted by nitroaromatic compounds from the explosion at a petrochemical factory located upstream of the Songhua River. The water quality problems had received considerable attention in the recent years (Lang et al. 1993).

Songhua River is the main water source of Harbin City, PRC. Harbin Water Treatment Plant produces 300,000 m³ water per day. The water plant applies conventional treatment processes, including coagulation, sedimentation, anthracite-sand filtration and chlorination disinfection. The existing conventional treatment processes are not suitable for the organic matters and pollutants removal, such as...
synthetic organic chemicals and disinfection by-product precursors (Kim et al. 1997). Recently, the biological activated carbon (BAC) filtration has emerged that overcomes several limitations associated with the conventional water treatment process (Srinivasan & Harrington 2007). In actual water treatment processes, bacteria naturally grow on the surface of granular activated carbon (GAC), which eventually becomes biological activated carbon (BAC) (Servais et al. 1992; David 2008). This naturally occurring active biofilm is capable of processing and biodegrading a significant fraction of entrapped waterborne nutrients in the GAC pores, it also serves to decrease the frequency of filter backwashing and efficiently biologically regenerate exhausted GAC, thereby, extending its service life (Zhang & Huck 1996).

However, this naturally formed BAC filter required a long period of time to achieve biomass on GAC. Biomass grew rapidly after 6 months of filter operation and became steady at a level of 70 nmolPO₄/g carbon after 8 months of filter operation (Bożena et al. 2006). For this reason, water treatment process with immobilized dominant microorganisms has received considerable attention in recent years (Zhao et al. 2006). The dominant microorganisms isolated from the nature with organic matter degradation ability have higher biological activity after acclimatization. In addition, GAC, as a packing material, can support the immobilized microorganisms and act as a buffer for fluctuating loading (Bouwer & McCarthy 1982; Xin et al. 2006). Therefore, GAC with immobilized microorganisms provides both adsorption function of non-biodegradable matters and biodegradation function of contaminants at the start-up period (Yariv 2001; Ye & Ni 2002).

In this study, five dominant microorganisms for organic micropollutants degradation were isolated from the Songhua River. The objective of this study was to evaluate the biodegradation capacity and eco-system of the dominant microorganisms immobilized on the activated carbon filter which was called the bio-enhanced activated carbon (BEAC) filter. The biomass and biological activity of the BEAC filter were analyzed by comparing with the naturally formed BAC filter. The ability of the BEAC filter to biodegrade organic matters was studied by gas chromatography-mass spectrometry (GC-MS) analysis. Observations of the microorganisms on the media surface of the BEAC filter were obtained by using the scanning electron microscope (SEM). Denaturing Gradient Gel Electrophoresis (DGGE) of Polymerase Chain Reaction (PCR) amplified 16S rRNA gene was used to compare the bacterial community structure of samples obtained from the BEAC and BAC filter. The results of this study will provide technical support to the Harbin Water Plant upgrade.

### MATERIALS AND METHODS

**Raw water**

The source water was obtained from the Songhua River. The water characteristics of the Songhua River during this study were as following: TOC (total organic carbon), 5.78～7.49 mg/l; UV₂₅₄, 0.082～0.113 cm⁻¹; CODₘᵦₚ(chemical oxygen demand), 2.35～3.46 mg/l; DO (dissolved oxygen), 8.16～8.95 mg/l; Turbidity, 1.89～2.36 NTU; pH, 6.7～6.9; Temperature, 13～21°C.

**Experimental set-up**

The research was conducted in Harbin Water Treatment Plant. The schematic diagram of the experimental set-up is shown in Figure 1. The filters were fed with the surface water from the Songhua River. Raw water was filtered through a sand filter to remove the suspended solids, and then flowed to a water tank (approximately 300 l) which fed the two filters. The feeding water to the BEAC filter flowed through the UV disinfection device to avoid the autochthonous bacteria invasion to the surface of the BEAC filter, which influenced the investigation of the immobilized dominant microorganisms. The feeding water to the BAC filter was not subjected to the UV disinfection because the

*Figure 1 | Experimental setup.*
presence of live bacteria in source water was necessary for the biofilm formation on carbon media. The flow rate to the UV disinfection device was 1 t/h and the wavelength of the ultraviolet radiation was 253.7 nm (UV–C). This would effectively kill the bacteria. The flow rate to the filters was 10 l/h. The filter cylinder was 2.5 m long with a inside diameter of 0.05 m. The 0.6 m-thick granular activated carbon layer was placed on the 0.3 m-thick sand layer which acted as a final barrier for the microorganism and turbidity. The filters were backwashed for 10 min with water at 25% bed expansion. UV disinfected water was used as backwashing water. The backwashing frequency was once a week.

Granular activated carbon was made by Tangshan Co. China. The characteristics of granular activated carbon were shown in Table 1.

### Dominant bacteria acclimation

Dominant bacteria for organic substances degradation were obtained from the Songhua River. After acclimatization by enriched medium and inorganic salt medium alternately for 5 times (Gaudy & Gaudy 1980), four kinds of Pseudomonas and one kind of Bacillus were identified by SHERLOCK Microbial Identification System and regarded as the dominant microorganisms. The enriched medium 1 g l\(^{-1}\) glucose, 0.5 g l\(^{-1}\) peptone, 1 g l\(^{-1}\) K\(_2\)HPO\(_4\), 1 g l\(^{-1}\) MgSO\(_4\), 11 raw water, and its pH value was 7.0–7.2. The inorganic salt medium contained 3.8 g l\(^{-1}\) Na\(_2\)HPO\(_4\)·12H\(_2\)O, 1 g l\(^{-1}\) K\(_2\)HPO\(_4\), 3.0 g l\(^{-1}\) KCl, 0.1 g l\(^{-1}\) NH\(_4\)Cl, 11 raw water, and its pH value was 7.0–7.2. The TOC removal of 5 mL dominant bacteria solution with a bacteria concentration of 1.19 × 10\(^{9}\)CFU/l was 72.5% after 24 h degradation. The initial TOC concentration was 5.7 mg/l.

### Bacteria immobilization

The procedures of bacteria immobilization were as following: virgin activated carbon pellets were autoclaved and stuffed into the columns randomly. A 11 concentrated microbial broth was added into the 991 distilled water. Then, 1001 bacteria solution with a bacteria concentration of 2.34 × 10\(^{9}\)CFU/l was pumped into the column of BEAC filter by submerged pump. The bacteria solution flowed from the top to the bottom of the BEAC filter by gravity then flowed back to the bacteria solution tank. During this cycle process, the dominant microorganisms have finished the transfer and adsorption process on the surface of the activated carbon. At the same time, the air was blown into the bacteria solution tank to provide the dissolved oxygen for the bacteria growing and keeping high biological activity. The immobilization process had lasted for 48 h. The biomass deposited on the top layer media after immobilization reached the value of approximately 15 nmolPO\(_4\)/g carbon.

### Analytical methods

#### Biomass

Biomass was detected by the lipid-P analytical method (Lazarova & Manem 1995; Wang et al. 1995). For phospholipid extraction, 0.8 ml of deionized water, 1 ml of CHCl\(_3\) and 2 ml of CH\(_3\)OH were introduced to a vial containing 0.5 g of carbon sample on the filter. The extraction mixture was shaken for 10 min and was then allowed to stand for 2 h. The vial was charged with 1 ml of deionized water and 1 ml of CHCl\(_3\), and the mixture was shaken for 10 min, and then allowed to stand for 22 h. A 1.6 ml sample was taken with a syringe from the chloroform layer. This sample was placed in a vial, and then chloroform was evaporated under the nitrogen atmosphere. Subsequently, 0.9 ml of potassium persulfate reagent was added. The vial was sealed and annealed at 102°C for 2 h and was allowed to stand open for cooling. Next, 0.2 ml of ammonium molybdate reagent in 5.72 N H\(_2\)SO\(_4\) and 0.9 ml of malachite green reagent was added to the vial and allowed to stand for 30 min. Absorbances of samples were measured at the wavelength of 610 nm using the Spekol 11 spectrophotometer. The phosphate concentration was determined from a standardization curve prepared for phosphate concentrations ranging from 0.0 to 10 μM.

The GAC samples utilized for analyses were dried at...
105°C for 76 h and weighed. The amount of biomass was reported as nmolPO₄/g carbon. 1 nmol P corresponds to 10⁸ bacteria of the size of *E. coli*.

**Biological activity**

The biological activity measured by adenosine tri-phosphate (ATP). ATP is a fundamental component of living matter which is involved in metabolic activities and disappears immediately after the death of the cells (Aleksandra & Dick 2004; Silvana et al. 2007). ATP was a parameter suited for the quantification of the active biomass in biological activated carbon filters. The measurement of ATP was based on the production of light in the luciferine-luciferase assay. ATP was released from suspended cells with nucleotide-releasing buffer. The intensity of the emitted light was measured in a luminometer (Celsis Advance™) calibrated with the solutions of free ATP in autoclaved tap water following the procedures given by the manufacture.

**GC-MS**

The organic substances were detected by the GC-MS (Li et al. 2006). A solution consisting of 1:1(v:v) H₂SO₄ was added to the samples immediately until pH < 2 was achieved. A 2 l sample of water was extracted by 30 ml dichloromethane three times for pH 2, 7 and 12. The three extract layers were combined and dried using nitrogen, and the residue 1 ml sample was analyzed by GC-MS. The methods were detailed in Table 2.

**Scanning electron microscope (SEM)**

The activated carbon samples of the BEAC filter were photographed by a Hitachi S-3400N scanning electron microscope to observe the development of the dominant microorganisms. The samples were first immersed stepwise in 20, 40, 60, 80 and 100% of ethanol. Each step had a minimum duration of 20 min, except the last step, during which the samples were left in the pure ethanol for 1 h. Then, the samples were placed in the critical point dryer where the chamber was slowly flushed 12 times over a period of about 2 h with CO₂ until the specimens were above the critical point (Ranjard et al. 2000).

**PCR-DGGE**

Denaturing Gradient Gel Electrophoresis (DGGE) of Polymerase Chain Reaction (PCR) amplified 16S rRNA gene was used to analyze the bacterial community structure of samples obtained from the different operation period of the BEAC filter. (Muyzer & Smalla 1998; Lapara et al. 2002; Tang et al. 2004).

**DNA extraction** The samples of 5g were mixed with 13.5 ml of DNA extraction buffer 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 × g 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 × g for 20 min at room temperature, washed with cold 70% ethanol, and resuspended in sterile deionized water, to give a final volume of 500 ml.

### Table 2 | GC-MS analysis method and parameters

<table>
<thead>
<tr>
<th>Item</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparatus</td>
<td>HP MP5890GC/MS</td>
</tr>
<tr>
<td>Column</td>
<td>DB-5 ms (30 m × 0.25 mm × 0.25 μm)</td>
</tr>
<tr>
<td>Internal diameter</td>
<td>250 × 10⁻⁶ m</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Helium</td>
</tr>
<tr>
<td>Mode</td>
<td>Splitless</td>
</tr>
<tr>
<td>Injector temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Max temperature</td>
<td>350°C</td>
</tr>
<tr>
<td>Oven temperature</td>
<td>Initial 60°C&lt;br&gt;60°C → 320°C 15°C/min</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
</tr>
</tbody>
</table>
PCR amplification. 16S rRNA gene were amplified with primers GC-clamp-F357(5'-CGCCCGCCGCCCCCGCCGCCGCCGCCCCCGCCCCCGCCCCCCTACGGGACGCAGC-3') and R518(5'-ATTACCGCGGCTGCTGG-3') which are specific for universally conserved bacterial 16S rRNA gene. PCR mixtures were composed as follows. 12.5 μl 10 x PCR buffer (with MgCl₂, 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 ml 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% (wt/vol) agarose gels, followed by ethidium bromide staining (1.2 mg/litre ethidium bromide).

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% [wt/vol] acrylamide in 0.5 x TAE buffer [2.42 g Tris base, 0.82 g sodium acetate, 0.185 g EDTA, 11 H₂O; pH value 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 x TAE buffer at 150 V for 4 h at 60°C. DGGE gels were stained with AgNO₃.

Samples

The samples for biomass, biological activity measurement and PCR-DGGE, SEM analysis were taken from the carbon media at the 200 mm top layer of the BAC and BEAC filter. The samples for toxicity test were taken from the BEAC filter influent and effluent on 180 d of operation. The analysis results of the biomass, biological activity and toxicity test were the average values of three replicates.

RESULTS AND DISCUSSION

Biomass and biological activity of BEAC and BAC filter

Figure 2 shows the biomass variation of the BEAC and BAC filter during the 180 d of operation. Both of the two filters used the same granular activated carbon as the filter media which can provide the rough porous surface for microbial colonization. The difference was that in one filter the acclimated dominant microorganisms were immobilized artificially on the carbon media and in the other filter bacteria from source water were colonized naturally on the carbon media. Therefore, at start-up there was a little amount of the biomass (15 nmolPO₄/g carbon) in the BEAC filter due to the immobilization. After 15 d of the operation, the amount of biomass started to increase. This indicated that the dominant microorganisms had adapted to the environment of the carbon surface. A rapid growth of biomass was observed from 15 d to 45 d of operation which was associated with a high content of TOC (6.37 mg/L, average value of initial 45 d of operation) in the water feeding to filters. The biomass deposited on the activated carbon reached the maximum value on 60 d of operation. This value was approximately 208 nmol PO₄/g carbon. After 45 d of operation, the stabilization of biomass value was achieved at the average level of 192 nmolPO₄/g carbon (from 45 d to 180 d of operation). After the biomass on the BEAC filter had reached the steady stage, it was less affected by changes in environmental conditions (i.e. temperature,
pH, nutrient concentrations, metabolic products and toxic substances) (Pederson 1990).

Comparing with the BEAC filter, the colonization of autochthonous microbial communities on the BAC filter needed over a relatively long period (30–45 d). The amount of the biomass on BAC filter started to increase after 45 d of operation. Comparing with a period of 4 months reported by Kazimierska et al. (2002) and a period of 6 months reported by Bożena et al. (2006), the colonization of microorganisms in the BAC filter of this study was shorter. Since sand filtration was the only pretreatment process prior to the BAC filter and there was no UV disinfection or ozonation in this study, a large amount of autochthonous microorganisms could easily attached on the surface of the carbon media. Moreover, the high content of the organic substances in influent (the average TOC value was 6.83 mg/l from 45 d to 180 d of operation) was another reason for a shorter period required to form the biomass on the BAC filter. After 160 d of operation, the biomass on the BAC filter exceeded the biomass of the BEAC filter. The average amount of biomass on the BAC filter reached 222.7 nmolPO 4/g carbon (from 160 d to 180 d of operation), which was more than that on the BEAC filter in the same period.

Figure 3 shows the comparison of biological activity between the BEAC and BAC filters. The biological activity of the BEAC filter could be observed 1 d of operation. After 15 d of operation, the biological activity increased at the same proportion as the growth of the biomass in the BEAC filter. The ATP concentration increased and became stable at an average of 1,368 ng ATP/g carbon after 45 d of operation. However, the biological activity of the BAC filter did not increase at the same proportion as the increase of quantity of the biomass. The ATP concentration of the BAC filter was 23 ng ATP/g carbon after 30 d of operation and started to increase slowly. After 160 d of operation, the amount of biomass on the BAC filter was higher than that on the BEAC filter (Figure 2), but the biological activity of the BAC filter was much less than that of the BEAC filter. The average ATP concentration of the BAC filter after 160 d of operation was only 920 ng ATP/g carbon.

The low ATP concentrations of the BAC filter was attributed to the low biological activity of autochthonous microbial communities colonized on the surface of the BAC filter. Because the dominant microorganisms obtained from the Songhua River were acclimated to increase the biological activity artificially, they were adaptive to the feeding water and the biological activity were higher than autochthonous microorganisms which had not been acclimated. The triphenyl tetrazolium chloride (TTC)-dehydrogenase activity (Klapwijk et al. 1974) of 5 ml pure dominant bacteria solution was 89.56 mgTF/(l h) after acclimatization. However, at the same condition, autochthonous bacteria without acclimatization was only 16.78 mgTF/(l h). Moreover, the feeding water to the BEAC filter was supplied with UV disinfection to avoid the autochthonous microorganisms invasion that competed with the dominant microorganisms for living space and nutrients (Fonseca et al. 2001). For these reasons, although the large amount of biomass was settled on the BAC filter after 160 d of operation, the biological activity of the BEAC filter was still higher than that of the BAC filter.

Biodegradability of organic substances

Table 3 presented the abundance of different organic substances in the influent and effluent streams of the BEAC and BAC filters on 180 d of operation. The influent of two filters contained 69 different organic compounds whose area values were approximate $4.82 \times 10^5$. The organic substances in the influent were mainly alkanes, ketones, alcohols, acids and some toxic chemical substances including halogenated hydrocarbons, chlorobenzenes, aromatic hydrocarbons, and PAHs (polycyclic...
aromatic hydrocarbons). In effluents there were 41 kinds of organic compounds for the BAC filter and 33 kinds of organic compounds for the BEAC filter which can be separated manually using the MSD Chemstation. The area values of organic compounds in the effluent of the BEAC filter (approximate $7.27 \times 10^8$) were less than that of the BAC filter effluent (approximate $1.22 \times 10^9$). As shown in Table 3, the area of each substance in the effluent from the two filters decreased significantly compared to the corresponding value in the influent. The BEAC filter was more effective in total area removal (85.10%) than the BAC filter where the total area was reduced by only 73.95%. The respective removal of halogenated hydrocarbons, chlorobenzenes, aromatic hydrocarbons, PAHs were 63.16%, 100%, 100%, 100% for the BAC filter, and 96.76%, 100%, 100%, 100% for the BEAC filter. For the four toxic chemical substances, the BEAC filter more effectively degraded the halogenated hydrocarbons and PAHs than the BAC filter did. This illustrated that dominant microorganisms were effective in biodegrading the organic substances in Songhua River.

**Table 3** GC-MS analysis of influent and effluent organic components for BEAC and BAC filters

<table>
<thead>
<tr>
<th>Organic components</th>
<th>Influent Class</th>
<th>Area value</th>
<th>BAC filter effluent Class</th>
<th>Area value</th>
<th>Removal (%)</th>
<th>BEAC filter effluent Class</th>
<th>Area value</th>
<th>Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkanes</td>
<td>10</td>
<td>425,385,669</td>
<td>5</td>
<td>45,659,892</td>
<td>89.26</td>
<td>5</td>
<td>55,659,892</td>
<td>86.91</td>
</tr>
<tr>
<td>Halogenated hydrocarbons</td>
<td>4</td>
<td>247,281,169</td>
<td>1</td>
<td>91,094,633</td>
<td>63.16</td>
<td>1</td>
<td>8,009,272</td>
<td>96.76</td>
</tr>
<tr>
<td>Cycloparaffins</td>
<td>5</td>
<td>334,631,933</td>
<td>2</td>
<td>98,621,256</td>
<td>70.52</td>
<td>2</td>
<td>48,276,323</td>
<td>85.57</td>
</tr>
<tr>
<td>Ketones</td>
<td>9</td>
<td>58,585,273</td>
<td>8</td>
<td>$2.18 \times 10^7$</td>
<td>62.85</td>
<td>7</td>
<td>27,037,737</td>
<td>53.84</td>
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<tr>
<td>Alddehydes</td>
<td>2</td>
<td>46,359,148</td>
<td>2</td>
<td>14,631,541</td>
<td>68.43</td>
<td>2</td>
<td>4,424,631</td>
<td>90.45</td>
</tr>
<tr>
<td>Alcohols</td>
<td>14</td>
<td>$2.77 \times 10^9$</td>
<td>11</td>
<td>$2.89 \times 10^8$</td>
<td>89.56</td>
<td>8</td>
<td>$1.08 \times 10^8$</td>
<td>96.10</td>
</tr>
<tr>
<td>Esters</td>
<td>3</td>
<td>14,450,722</td>
<td>1</td>
<td>3,617,550</td>
<td>74.96</td>
<td>1</td>
<td>1,261,507</td>
<td>91.27</td>
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<tr>
<td>Acids</td>
<td>13</td>
<td>731,735,549</td>
<td>10</td>
<td>639,569,582</td>
<td>12.59</td>
<td>7</td>
<td>473,969,582</td>
<td>35.22</td>
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<tr>
<td>Chlorobenzenes</td>
<td>2</td>
<td>112,750,314</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
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<td>Aromatic hydrocarbons</td>
<td>3</td>
<td>14,763,672</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
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<tr>
<td>PAHs</td>
<td>4</td>
<td>62,991,716</td>
<td>1</td>
<td>11,235,642</td>
<td>82.16</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>$4.82 \times 10^9$</td>
<td>41</td>
<td>$1.22 \times 10^9$</td>
<td>73.95</td>
<td>33</td>
<td>$7.27 \times 10^8$</td>
<td>85.10</td>
</tr>
</tbody>
</table>

**PCR-DGGE analysis**

Figure 4 shows the results obtained from DGGE analysis for fragments of 16S rRNA gene of samples taken from BEAC (lane a and b) and BAC (lane c) filters. As shown in Figure 4(a, b), there were five band patterns which represented five dominant microorganisms immobilized on the BEAC filter (Figure 4a) and there was no other band pattern observed even operated 180 d, showing that...
the BEAC filter had no other bacterial invasion (Figure 4b). It was suggested that the eco-system of the population did not change in the BEAC filter. However, the depths of the five band patterns were different and changed with the operation time. After microbial immobilization, the depths of five DGGE band patterns were weak especially for No.4 and No.5 bands, indicating that small amounts of biomass of the five dominant microorganisms were immobilized on carbon surface. After 180 d of operation, the depths of five dominant bacteria became stronger especially for No.2 and No.5 bands, which indicated the biomass of five dominant bacteria had significantly increased and the depth of No.2 and No.5 bacteria were much stronger than the other three dominant microorganisms. These two DGGE bands were excised from the gel, re-amplified, subjected to electrophoresis, and sequenced. The results showed the two sequences had high similarity to Bacillus subtilis (96%) (No.2) and Pseudomonas balearica (99%) (No.5). These two microorganisms, belonged to the five dominant bacteria immobilized on the BEAC filter, were more adaptable to the surface of the filter media.

Figure 4c shows the eco-system of the BAC filter which was different from the BEAC filter. The BAC filter had different groups of microorganisms and there were more bands for the BAC filter than the BEAC filter. This indicated that more autochthonous bacteria existed in influent. However, the depths of band patterns were weaker and the components were more complicated than the BEAC filter. By comparing the PCR-DGGE results of the two filters, it was evident that the five dominant bacteria immobilized on the BEAC filter were not affected by the autochthonous bacteria due to UV disinfection and the BEAC filter operated stably without any change of microbial eco-system.

Microbiology morphology

In order to observe the morphology of the bacteria on the carbon surface of BEAC filter, three sample pellets of the different operation time were taken from the top media layer for analysis, including carbon sample without use, carbon sample after microbial immobilization, carbon sample on 180 d of operation. The surface characteristics of the samples were analyzed by SEM. From the SEM photograph shown in Figure 5a, the carbon surface was irregular creviced with porous structure which provided the advantage good condition for the bacteria colonization. Figure 5b shows the carbon pellet after immobilization with five kinds of dominant microorganisms. It could be observed that a plenty of bacteria were attached on the micro-pores of carbon and most of the bacteria were bacilliform. The single bacteria existed individually on the surface of carbon. Figure 5c shows the carbon sample on 180 d of operation. The bacteria on the surface of carbon flourished to a great amount and more than the bacteria in the sample (Figure 5b) after immobilization. However, the microorganisms did not cover the whole area of the activated carbon. There was still vacant space on the

<p>| Biological toxicity of BEAC filter samples |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Optical loss %</th>
<th>Toxicity assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>2</td>
<td>Less toxicity, acute toxic risk</td>
</tr>
<tr>
<td>Effluent</td>
<td>0</td>
<td>None acute toxic risk</td>
</tr>
</tbody>
</table>

Table 4
surface of the activated carbon with diameter of 1–10 μm. The uncovered surface could adsorb organic substances which supplied the nutrients for the growth of dominant microorganisms. Such combination effect of microorganisms biodegradation and activated carbon adsorption can improve the organic substances removal ability of the filter and extend the useful life of the carbon (Weber et al. 1978; Scholz & Martin 1997). The discontinuity biofilm adhered to the surface of the activated carbon showed high biodegradation capacity for the organic substances (Xianda et al. 1999; Wang et al. 2008).

Toxicity test of BEAC filter

Deltatox Bioassay Technology by SDI (Strategic Diagnostics Inc.) is an early warning system for monitoring and evaluating drinking water quality. The Deltatox Analyzer use photobiology theory to test toxicity in conjunction with the ATP. It is a rapid analyzer for pathogen and chemical toxicant contained in drinking water. Using the photobacteria optical loss rate as the detecting index, the test assessed the toxicity of the samples from the influent and effluent of the BEAC filter. The test was conducted at the condition of 19.6°C. The results are showed in Table 4. The effluent of BEAC filter had no acute toxic risk with the optical loss of 0%. Carbon media with immobilized dominant bacteria on the surface was safe to be applied in drinking water treatment.

CONCLUSION

Based on the above analysis of the performance of the BEAC filter with dominant microorganisms immobilized on activated carbon media, the conclusions are summarized as follow.

(1) The five dominant microorganisms, including four kinds of Pseudomonas and one kind of Bacillus, were effective on removing organic matters and pollutants from Songhua River. The GC-MS results showed that the BEAC filter had better removal efficiency for toxic chemical substances (especially for halogenated hydrocarbons and PAHs) than the naturally formed BAC filter.

(2) The biomass in the BEAC filter was stabilized after 45 d of operation with the average value of 192 nmolPO₄/ g carbon, and the corresponding average ATP concentration was 1,368 ng ATP/g carbon, more than that of the BAC filter which average ATP concentration was only 920 ng ATP/g carbon.

(3) The PCR-DGGE results showed the five dominant microorganisms immobilized on the BEAC filter were not affected by the autochthonous bacteria even on 180 d of operation. The dominant microorganisms of Bacillus subtilis and Pseudomonas balearica were more adaptable to the living space on the BEAC filter.

(4) The SEM results showed a plenty of bacteria were adsorbed on the micro-pores of activated carbon after immobilization. The dominant bacteria on the BEAC filter flourished to a great amount on 180 d of operation. These microorganisms did not cover the whole area of the activated carbon. There was still vacant space on the surface of the activated carbon with diameter of 1–10 μm.

(5) The toxicity test with Deltatox Bioassay Technology Analyzer indicated that the dominant bacteria were safe to be used in drinking water treatment process.

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