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Evaluation of long term stability of seeded bacteria in a bio-enhanced activated carbon filter used for treating drinking water

Duo-Ying Zhang [a,b], Wei-Guang Li [a,*], Hai-Nan Gong [c], Lei Zhang [a], Xu-Jin Gong [a], Bai-Yin Liu [d]

[a] School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China
[b] Department of Food Science and Environmental Engineering, East University of Heilongjiang, Harbin 150086, China
[c] Institute of Advanced Technology, Heilongjiang Academy of Sciences, Harbin 150040, China
[d] Chinese Research Academy of Environmental Science, Beijing 100012, China

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ABSTRACT

A bio-enhanced activated carbon (BEAC) filter immobilized with 5 bacteria was operated for 180 days to investigate the seeded bacterial stability. The TOC and ammonium removal efficiency of the filter was analyzed continuously every day. The biofilm, bioactivity and bacterial community structure was evaluated by SEM, ATP test and DGGE respectively. At the initial stage (0 day), low abundance of the bacteria led to low bioactivity (202.97 ng ATP g^{-1} carbon), which resulted in low TOC and ammonium removal efficiency (63.98% and 30.68%). On the 120th day, The TOC and ammonium removal efficiency improved with the increase of bioactivity (increased to 1436.8 ng ATP g^{-1} carbon) and climbed to 81.47% and 51.97%, respectively. DGGE results showed that from day 0–120 of the BEAC operation, 5 seeded bacteria were stable with little change. On the 180th day, there were 4 indigenous bacteria appeared in DGGE profiles, which affected TOC removal efficiency and bioactivity. Fluorescence in situ hybridization results showed that, on the 180th day, the seeded bacteria were 92.98% of total count of bacteria, which were dominant in BEAC filter. In this study, BEAC with relatively stable seeded bacteria is a promising approach to maintain performance of the filter.

1. Introduction

Songhua River is one of the main water supply resources for Harbin citizens in China. With the enlargement of the upstream cities areas, the water quality of Songhua River is getting poor. According to the report of Datacenter of Ministry of Environmental Protection of P. R. China from Jul. 2011 to Jun. 2012 (http://datacenter.mep.gov.cn/), chemical oxygen demand (COD) of the water from Songhua river was 3 mg l^{-1}–8 mg l^{-1}, and ammonium concentration was 0.1 mg l^{-1}–2 mg l^{-1} As a result, the standard of water supply (GB5749-2006) can't be met using such water resource by conventional drinking water treatment process, such as coagulation, sedimentation and filtration.

The bacteria immobilized on the surface of granular activated carbon (GAC), can remove pollutants via the process of bio-adsorption, biodegradation or bioregeneration therewith improving drinking water quality (Vinitnantharat et al., 2001; Wang et al., 2007; Ong et al., 2008). GAC covered with the bacteria is called biological activated carbon (BAC), which has been proved to be an effective drinking water treatment method for removing dissolved organic matter and ammonium (Andersson et al., 2001; Tian et al., 2009). However, BAC requires a long time to achieve sufficient amount of biomass (Gao et al., 2010). Bio-enhanced approach is a kind of technique to be applied to remove pollutants by introducing specific competent strains or consortia of microorganisms (Fantroussi and Agathos, 2005). It can improve the start-up of a reactor (Wilderer et al., 1991), enhance reactor performance (Jianlong et al., 2002), protect the existing microbial community against adverse effects (Mohan et al., 2005), and accelerate the onset of degradation (Hu et al., 2008).

During the previous study, we specifically immobilized functional bacteria on the surface of GAC to form bio-enhanced activated carbon (BEAC) filter, and study its performance. The results

* Corresponding author. Box No. 2602, School of Municipal and Environmental Engineering, 73 Huanghe Road, Harbin 150090, China. Tel./fax: +86 451 86283003.
E-mail addresses: duo0314@163.com, duoying_zhang@126.com (D.-Y. Zhang), hitlwg@126.com, weiguanglihit@hotmail.com (W.-G. Li), ghn717@163.com (H.-N. Gong), lei_zhang25@yahoo.com (L. Zhang), xujin_gong@126.com (X.-J. Gong).

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showed that the BEAC expressed higher operational performance than BAC (Gao et al., 2010). The performance of the BEAC was affected by backwashing because of the variation of bacterial abundance and activity (Gao et al., 2009). Air (8–10 l m^{-2} s^{-1}) plus water flush was found to be the proper backwashing way.

The suitable bacteria for the bio-enhanced approach should be competitive and compatible with indigenous microbial communities, so that they will be persistent (Yu and Mohn, 2001). Gao et al. (2010) found that the ultraviolet disinfection before the BEAC filter effectively controlled the bacteria in the influent. In this way, it can prevent indigenous bacteria from invading in the BEAC filter and improve the stability of seeded bacteria. However, the knowledge about the influence of the indigenous bacteria on the seeded bacteria is still limited. The fate and the activity of the introduced bacteria of BEAC filter running in a long term are unknown.

In this study, five seeded bacteria with high TOC biodegradability and dehydrogenase activity were isolated from BAC filter running for 180 days (Zhang et al., 2011a). These bacteria were immobilized on the activated carbon to form BEAC. The bacterial communities and activity dynamics were analyzed during the BEAC operation for 180 days. The abundance of seeded bacteria was tested by fluorescence in situ hybridization (FISH). The purpose of the study was to evaluate the stability of the seeded bacteria in BEAC filter and investigate the effect of indigenous bacteria in the influent on the seeded bacteria.

2. Materials and methods

2.1. Preparation of BEAC filter

A bench-scale filter (showed in Fig. 1) was constructed of Lucite tubing to avoid organic contamination. The column was 200 cm high and 6 cm internal diameter filled with 3 liters dry-heat-sterilized granular activated carbon particles (purchased from Tangshan Co. China, http://www.jianxincarbon.com). The column has three sampling points at different positions in the carbon bed. They are named as T (140 cm above the bed bottom), M (100 cm above the bed bottom) and B (60 cm above the bed bottom). Sampling point T is near the inlet and sampling point B near the outlet. Sampling point M is located roughly in the middle of the bed. For sterilizing, the column was filled with 3% hydrogen peroxide solution for 30 min. After the hydrogen peroxide solution was discharged, the sterile water was fed in the column to wash off the hydrogen peroxide solution.

2.2. Seeded bacteria and immobilization

The 5 seeded bacteria were isolated from long term running BAC filter (Zhang et al., 2011a). They were *Pseudomonas stutzeri*, *Pseudomonas putida*, *Pseudomonas pertucinogena*, *Pseudomonas balearica* and *Bacillus subtilis*, respectively. 1 l pure culture of each seeded bacteria (about 10^9 cells) was centrifuged, then the settling bacteria were collected and mixed in 45 l of sterile water. The bacterial mixture was pumped into the sterile BEAC filter from the top of the column and flowed down to the bottom of the column by gravity. During this pumping cycle, the seeded bacteria were immobilized on the surface of the activated carbon. The pumping cycle process had lasted for about 48 h till the settlement of bacteria could be observed by scanning electron microscopy (SEM, with Hitachi S-4500, Hitachi, Ltd. Japan).

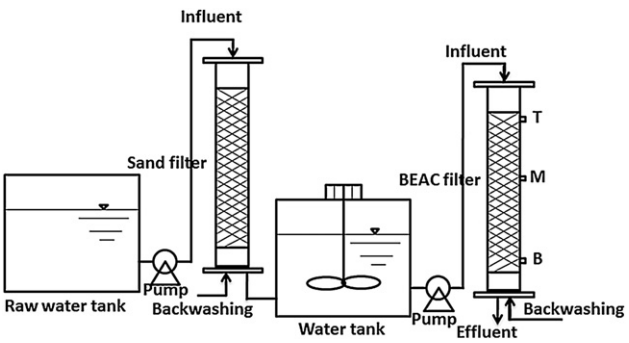

Fig. 1. BEAC process used for continuous experiments.

2.3. Operation of the BEAC filter

Raw water of Songhua River was taken from the water inlet at the Third Water Treatment Plant and transported in drums to the lab for the experiment. After primary settling and sand filtration, the raw water flowed into water tank for adjusting the water quality. Then, the water was continuously pumped into the BEAC filter. For evaluating bacterial community dynamic and its' stability of the BEAC filter, the influent water parameters were controlled as consistent as possible. If necessary, acetate sodium and ammonium sulfate were added into the water tank for adjusting TOC (2.97–3.41 mg l^{-1}) and NH_4^+–N concentration (0.95–1.26 mg l^{-1}). The other parameters were controlled at the values as following: pH 7.0–7.2, temperature 22–24 °C, dissolved oxygen 8.4–8.8 mg l^{-1}. The influent water flow rate was 4–8 l h^{-1}. Empty bed contact time (EBCT) was 10 min with backwashing (8–10 l m^{-2} s^{-1} air scouring plus 12 l m^{-2} s^{-1} water backwashing) once a week. Since then, the BEAC filter was operated for 180 days. During these days, water and activated carbon samples were collected for water quality, biofilm, bioactivity and bacterial community analysis.

2.4. Analytical methods

2.4.1. Water quality analysis

Water sample collection continued every day during 180 days of the BEAC running time for testing NH_4^+ and TOC concentration. Ammonia plus ammonium were determined colorimetrically (Lu, 1987). TOC was analyzed using Aurora Combustion Total Organic Carbon Analyzer 1030C (OI, America). The removal efficiency formula is $(C_0 - C_n)/C_0$. C_0 is initial concentration of TOC or NH_4^+–N (mg l^{-1}). C_n is the final concentration of TOC or NH_4^+–N (mg l^{-1}).

2.4.2. Biofilm and biological activity of the BEAC filter

BEAC samples were taken from the sampling ports T, M and B respectively once a month (30 days) for bioactivity analysis. Bioactivity was detected by ATP based method (Magic-Knezev and Kooij, 2004). BEAC samples were taken from the sampling port T once two months (60 days) for detecting biofilm developed on BAC. Biofilm was evaluated by SEM as described previously (Stewart et al., 1990).

2.4.3. Bacterial dynamics and structure on the BEAC

The BEAC samples were taken from sampling ports T, M and B for testing bacterial dynamics by PCR-DGGE. DNA was extracted from the granular activated carbon by the extraction method as described previously (Zhang et al., 2011b).

GC-clamp-F357 primer (5′-CGCCCGCCGCGCCCCGCGCCCGGCCC GCCGCCCCCGC CCCCCTACGGGAGGCAGCAG-3′) and R518 primer (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the bacterial 16S rRNA gene. PCR mixtures were composed as follows: 12.5 μl 10× PCR buffer (with $MgCl_2$, TaKaRa), 10 nmol of each

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