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Treatment of bromoamine acid wastewater using combined process of micro-electrolysis and biological aerobic filter

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ABSTRACT

The wastewater originated from the production of bromoamine acid was treated in a sequential system of micro-electrolysis (ME) and biological aerobic filter (BAF). Decolorization and COD_{Cr} removal rate of the proposed system was investigated with full consideration of the influence of two major controlling factors such as organic loading rate (OLR) and hydraulic retention time (HRT). The removal rate of COD_{Cr} was 81.2% and that of chrominance could be up to 96.6% at an OLR of 0.56 $kg\,m^{-3}\,d^{-1}$ when the total HRT was 43.4 h. Most of the chrominance was removed by the ME treatment, however, the BAF process was more effective for COD_{Cr} removal. The GC–MS and HPLC–MS analysis of the contaminants revealed that 1-aminoanthraquinone, bromoamine acid and mono-sulfonated 1,2-dichlorobenzene were the main organic components in the wastewater. The reductive transformation of the anthraquinone derivatives in the ME reactor improved the biodegradability of the wastewater, and rendered the decolorization. After long-term of operation, it was observed that the predominant microorganisms immobilized on the BAF carriers were rod-shaped and globular. Four bacterial strains with apparent 16S rDNA fragments in the Denaturing Gradient Gel Electrophoresis (DGGE) profiles of BAF samples were identified as *Variovorax* sp., *Sphingomonas* sp., *Mycobacterium* sp., and *Microbacterium* sp.

1. Introduction

1-Amino-4-bromoanthraquinone-2-sulfonic acid (bromoamine acid, BAA) is one of the major synthetic intermediates of acid and reactive anthraquinone dyes. It is synthesized through sulfonation and bromination of 1-aminoanthraquinone. Approximately 20 cubic meters of wastewater are discharged per ton of BAA produced. The wastewater contains the raw chemicals, the unseparated final product and the by-products formed during BAA synthesis, most of which are biorecalcitrant. The traditional activated sludge treatment is ineffective to deal with BAA wastewater due to the high level of toxicity, chrominance and salinity. Other potential technologies such as resin adsorption [1], solvent extraction [2], TiO_2-assisted photocatalytic oxidation [3,4] and freeze-dephlegmation [5] can also be applied, while they are not cost-effective. An ideal treatment scheme for BAA wastewater is to pretreat the wastewater by physiochemical processes to remove most of the chrominance and toxicity before biological treatment [6]. The pretreatment processes should be effective, robust and economically feasible.

Recently, ME has been widely used in treatment of wastewater from dye-stuff [7], petrochemical [8], and electroplating industries [9]. It is based on the electrochemical reaction on the surface of electrodes. For example, when a mixture of iron chips and activated carbon (AC) particles is in contact with wastewater (electrolyte solution), numerous microscopic galvanic cells are formed between the particles of iron and carbon, and electrons are supplied from the galvanic corrosion of iron (anode) [10]. The half-cell reactions can be represented as:

Anode (oxidation):

$$2Fe - 4e^- \rightarrow 2Fe^{2+}$$

Cathode (reduction):

$$4H + 4e^- \rightarrow 4[H] \rightarrow 2H_2 \quad \text{(Acidic)}$$

$$O_2 + 4H + 4e^- \rightarrow 2H_2O \quad \text{(Acidic)}$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad \text{(Neutral to alkaline)}$$

It is believed that organic contaminants can be reduced by Fe, Fe^{2+} and [H] during micro-electrolysis. Furthermore, organic contaminants can also be removed through adsorption and co-precipitation by the ferrous and ferric hydroxides formed from

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Table 1
Characteristics of the raw wastewater

Item	Average value	Item	Average value
pH	10.0–11.0	Salinity	4.1%
COD_{Cr}	3707.5 mg L^{-1}	Cl^-	6967.9 mg L^{-1}
BOD_5	150.0 mg L^{-1}	SO_4^{2-}	19975.3 mg L^{-1}
TOC	1102.5 mg L^{-1}	Br^-	510.1 mg L^{-1}
Chrominance	4000 times	BAA	388.3 mg L^{-1}

oxidation and precipitation of Fe^{2+} [10]. Additionally, AC can also adsorb some of the organic contaminants, especially those hydrophobic ones [11]. The ME method has attracted great interests owing to its efficiency, low cost and operational simplicity.

This study investigated the treatment of BAA wastewater using combined process of iron–carbon ME and BAF. The removal efficiency of COD_{Cr}, color and organic contaminants was investigated, and the bacterial community in the BAF was analyzed. The results provide valuable information for practical BAA wastewater treatment.

2. Materials and methods

2.1. Raw wastewater

The raw wastewater used in this study was obtained from the DanKong Industry and Trade Group Co., Ltd. (Taizhou, Zhejiang, China).

BAA was synthesized in two steps. Firstly, 1-aminoanthraquinone was sulfonated with chlorosulfonic acid in *o*-dichlorobenzene, and 1-amino-anthraquinone-2-sulfonic acid was produced. Secondly, the sulfonated product was brominated by adding bromine solution to give BAA.

The wastewater had a dark red color and a strong pungent odor. The characteristics of the raw wastewater are listed in Table 1. Appropriate amounts of nitrogen ($(NH_4)_2SO_4$) and phosphorus (KH_2PO_4) were added to the wastewater to maintain the COD_{Cr}:N:P ratio of 100:5:1.

2.2. Reactor system

The combined ME–BAF system consisting of two bench-scale reactors was set-up as shown in Fig. 1. Both reactors were made of transparent polyvinyl chloride (PVC) columns. The working volume of the ME and BAF reactors was 1.22 L and 2.74 L, respectively.

Ion chips used as the anode of ME were obtained as scrap metal from a machine shop and were degreased in a 10% hot alkaline solution. They were then soaked in a 2% sulfuric acid solution to remove surface rusts, and finally cleaned by deionized water [10].

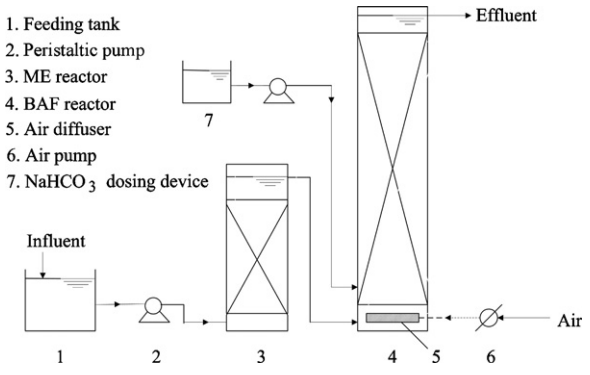

Fig. 1. Schematic diagram of experimental set-up.

The AC particles used were coal based cylinder (Ø 4 mm × 10 mm). Iron chips (300 g) and AC were mixed together with a volume ratio of 1:1 and then added into the ME column. A peristaltic pump (BT01, Xie Da, China) drove the wastewater flow through the ME, with influent entering at the base, and the treated effluent entered the BAF by gravity feed.

In the BAF, both influent and air were introduced at the bottom in a co-current flow mode. A patented functional polyurethane foams (FPUFS) carrier was used as the packing medium to immobilize the microorganisms [12]. The carrier occupies a cube of 512 mm^3, of which the water content and porosity are 2500% and 96%. There are many active chemical groups distributing on the carrier surface, such as –OH, $–NH_2$, –COOH, $–CH_2$, and $–CHOCH_2$, which bond chemically with the microorganisms and immobilize them. The wet media have density of about 1.0 g cm^{-3} and specific surface area of 80–120 m^2 g^{-1}. The dissolved oxygen (DO) level in the BAF reactor was maintained above 2 mg L^{-1}, and pH was adjusted to around 7.0 by adding $NaHCO_3$ solution.

2.3. Experimental procedure

The FPUFS carriers were firstly mixed with the sludge collected from a municipal sewage treatment plant to immobilize microbes, and then were placed in the BAF reactor. The mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), MLVSS/MLSS ratio, and sludge volume index (SVI) of the seed sludge were 12.5 g L^{-1}, 5.8 g L^{-1}, 0.46 mL g^{-1}, and 64 mL g^{-1}, respectively. The ME and BAF reactors were connected in series and started up with diluted BAA wastewater at room temperature (20–30 °C). The pH of influent was adjusted to around 4.0 to facilitate the ME reaction.

During the adaptive phase, the COD_{Cr} of influent was increased from approximately 500 to 1000 mg L^{-1} by reducing the dilution ratio of raw wastewater from 6.7 to 4.0 at a constant HRT of 13.4 h in the ME and 30.0 h in the BAF.

When the color and COD_{Cr} removal rate of the combined system was stable, the influence of HRT was investigated. The influent concentration was kept relatively constant (average COD_{Cr} 1000 mg L^{-1}), and the total HRT was incrementally decreased from 43.4 to 29.3 h. The system was operated for at least 10 days at each HRT increment, and measurements were routinely taken of pH, DO, COD_{Cr}, BOD_5, chrominance, and total organic carbon (TOC).

2.4. Analytical methods

2.4.1. Water quality detection

MLSS, MLVSS, SVI, COD_{Cr}, chrominance and salinity were measured using China national standard methods [13]. Decolorization rate was determined spectrophotometrically by monitoring the decrease of the absorbance at the maximum visible absorbance wavelength of the collected wastewater (492 nm) with a UV–vis spectrophotometer (Shimadzu UV1700, Japan), and calculated according to the following formulation:

$$\text{Decolorization rate}\,(\%) = \frac{A_{in} - A_{eff}}{A_{in}} \times 100\%$$

where A_{in} was the absorbance at 492 nm of the influent, and A_{eff} was the absorbance at 492 nm of the effluent.

The UV–vis spectra of the influent and effluent were scanned to investigate the characteristics of the organic contaminants. TOC was measured after filtration by a 0.45 μm fibrin membrane using a TOC analyzer (Jena 3100, Germany). A manometric apparatus (WTW OxiTop IS12, Germany) was used to record BOD_5 concentration. Temperature, pH and DO were measured using selective electrodes. The SO_4^{2-}, Br^-, and Cl^- ions were detected by ion

chromatography (Dionex ICS-2500, USA) using an AS16 analytical column and an AG16 guard column. The separation was performed using 22.0 mmol L^{-1} NaOH eluent.

The concentration of BAA was determined using HPLC (Agillent 1200, USA) equipped with a ZORBAX Eclipse XDB–C18 column (150 mm × 4.6 mm). Gradient elution was used with a mobile phase consisting of acetonitrile (A) and 0.01 mol L^{-1} ammonium acetate aqueous solution (B). The gradient elution started after holding at 5% A for 1 min, and firstly increased linearly to 20% A over 1 min and held 5 min, and secondly increased to 50% A over 4 min and held 3 min, and finally increased to 100% A over 2 min and held 4 min. The flow rate was 1.0 mL min^{-1} and the oven temperature was set at 30 °C. The detection was carried out at the wavelength of 240 and 485 nm.

The organic components of the influent and effluent (HRT = 43.4 h) were analyzed by GC–MS after extraction and HPLC–MS directly. Samples (50 mL) were acidified to pH 2.0 with 2 mol L^{-1} HCl and extracted three times with half volume of methylene chloride. The extracts were combined, dried with anhydrous Na_2SO_4, and concentrated to 0.3 mL. The GC–MS analysis was performed in the EI mode (70 eV) on a 6890N GC with a 5975 MSD (Agilent, USA). An HP-35 type 30 m long capillary column (250 mm × 0.25 mm) was used as the separation column. During analysis, the column temperature was first maintained at 70 °C for 2 min, then raised to 280 °C at a rate of 15 °C min^{-1}, and finally kept at 300 °C for 2 min. The mass scan range was 35–800 *m*/*z*. The substance analysis was undertaken with reference to the NIST05 (National Institute of Standards and Technology) mass spectral library database. HPLC–MS was performed in the ESI mode using a Finnigan LCQ DecaXP ion trap mass spectrometer (Thermo, USA) equipped with a Hypersil C18 column (150 mm × 4.6 mm). The eluent and the gradient elution program was the same to those of the HPLC analysis. Ionization was achieved in the negative mode. In the full scan mode masses were detected from 100 to 700 *m*/*z*.

2.4.2. Microbiology analysis

Morphology of the microorganisms immobilized on the carries in the BAF reactor was observed by a Thermal FE Environment Scanning Electron Microscope (Philip, Holland).

The bacterial community of samples obtained from the seed sludge and the BAF reactor was analyzed using DGGE of Polymerase Chain Reaction (PCR) amplified 16S rDNA. After operation for 60 days, 20 g carrier samples were taken from the BAF reactor, rinsed in 1.0 mol L^{-1} NaOH at 78 °C for 30 min, and ultrasonicated (20 W, 40 s). Microorganism cells were collected by centrifugation at 10,000 rpm for 10 min and washed twice with phosphate-buffered saline (PBS) solution (pH 8.0). As to the sludge samples, microorganisms were directly concentrated by centrifugation at 10,000 rpm for 10 min and washed twice with PBS solution. The cell pellets were resuspended in 10 mL DNA lysis buffer (100 mmol L^{-1} EDTA–Na_2, 100 mmol L^{-1} Tris–HCl, 200 mmol L^{-1} NaCl, 1% PVP, 2% CTAB, pH 8.0) and 1 mL 20% SDS, incubated at 65 °C for 4 h, and then centrifuged at 12,000 rpm for 10 min. An equal volume of phenol/chloroform/isoamylol (25:24:1) was added in the supernatants to remove protein, and then a mixture of chloroform and isoamylol (24:1) was used to eliminate the phenol. The DNA was deposited by adding an equal volume of isopropanol, washed with 70% ethanol, and dried at room temperature. The DNA pellets were dissolved in 50 μL Tris–EDTA buffer (pH 8.0), and conserved at −20 °C.

The hypervariable V3 region in the 16S rDNA was PCR-amplified using the forward primer GC-357F (5′-CGCCC GCCGC GCGCG GCGGG CGGGG CGGGG GCACG GGGGG CCTAC GGGAG GCAGC AG-3′) and the reverse primer, 518R (5′-ATTAC CGCGG CTGCT GG-3′). The 50 μL reaction mixture contained 5 μL 10 × PCR buffer, 0.2 μM each primer, 200 μM each dNTP, 0.5 μL extracted DNA template, and 0.025 U Taq Polymerase. The PCR protocol included an initial denaturation period of 4 min at 94 °C; 30 cycles of 94 °C for 30 s, 52 °C for 1 min, and 72 °C for 30 s; 72 °C for 7 min. The PCR products were verified by electrophoresis on a 1.2% agarose gel.

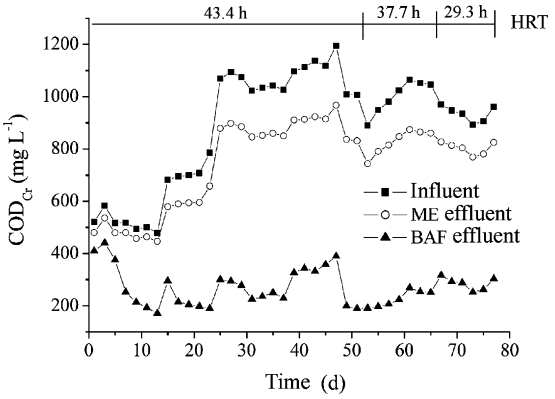

Fig. 2. Chronological curve of COD_{Cr} in influent, ME effluent and BAF effluent.

DGGE was carried out using the Dcode™ Universal Mutation Detection System (BioRad, USA). Approximately 400 ng amplified DNA products were loaded onto 8% (w/v) polyacrylamide gels (consisting of a 37.5:1 of acrylamide to biacrylamide) in 1 × TAE (40 mmol L^{-1} Tris–HCl, 40 mmol L^{-1} acetic acid, 1 mmol L^{-1} EDTA, pH 8.0) using a denaturing gradient ranging from 30% to 60% (100% denaturant contains 7 mol L^{-1} urea and 40% formamide). Electrophoresis was performed at 60 °C, 60 V for 20 min, followed by 180 V for 4 h. The product was then stained with Goldview solution, and photographed by Gel Documentation Systems (GDS-8000, Gene Co., USA).

Selected bands were excised from DGGE polyacrylamide gel, and DNA was retrieved by the crush and soak method [14]. The DNA fragments were reamplified using the forward primer 338F (5′-ACTCC TACGG GAGGC AGCAG-3′) and the reverse primer, 518R (5′-ATTAC CGCGG CTGCT GG-3′), and the nucleotide sequence of the 16S rDNA gene clone was determined by Invitrogen Biotechnology Co., Ltd. (Shanghai, China). The nucleotide sequence was compared with the sequences in the GenBank databases by the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences were aligned by the Clustal X 1.8 program, and the phylogenetic analysis was performed with the neighbor-joining method (MEGA 3.1).

3. Results and discussion

3.1. Treatment efficiency of the ME–BAF system

Fig. 2 shows the time history of COD_{Cr} concentration of the wastewater after treatment by the ME and BAF reactor. Most COD_{Cr} removal occurred in the BAF. During the first 13 days, COD_{Cr} of the final effluent decreased gradually to below 200 mg L^{-1}, indicating the biological adaptive process. When the OLR of influent was increased from approximately 0.27 to 0.66 kg m^{-3} d^{-1}, the average removal rate of COD_{Cr} in ME increased from 7.3% to 17.7%. The maximum total COD_{Cr} removal rate of 81.2% was obtained when the OLR was 0.56 kg m^{-3} d^{-1}, and higher OLR inhibited biological activity in the BAF. When HRT was decreased from 43.4 to 37.7 h, COD_{Cr} removal efficiency was hardly affected. However, to obtain high quality of effluent (COD_{Cr} < 200 mg L^{-1}), the influent COD_{Cr} concentration should be no more than 1000 mg L^{-1}. The residual COD_{Cr} which is difficult to be further removed even at long HRT needs tertiary treatment such as activated carbon adsorption. When HRT was further reduced to 29.3 h, the average COD_{Cr} removal rate of ME and the combined system decreased to 14.1% and 69.5%, respectively.

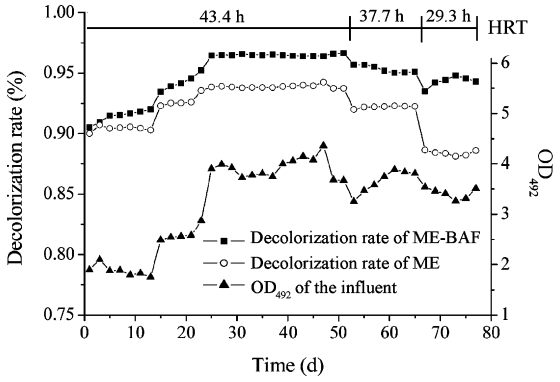

Fig. 3. Chronological curve of decolorization rate after ME and BAF treatment.

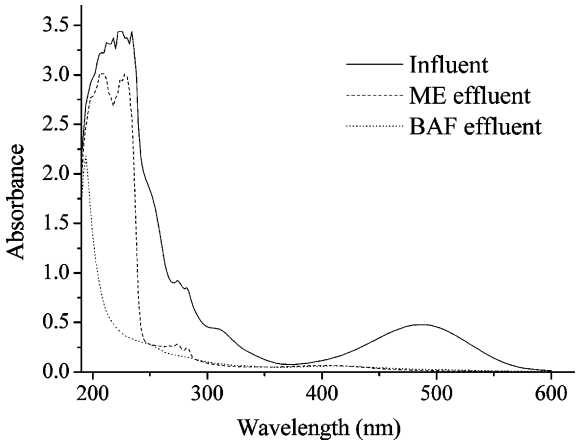

Fig. 4. UV–vis spectra of the influent, ME effluent and BAF effluent (the dilution times of the influent, ME effluent and BAF effluent were, respectively, 10, 10, and 5).

Therefore, a relatively long HRT ensured the efficiency of COD_{Cr} removal in both ME and BAF reactor.

The profile of color removal was illustrated in Fig. 3. During the whole test period, the total color removal rates were high (>91%) under all conditions. Most chrominance was removed by the ME process, and the removal efficiency was robust against variations of influent OLR. The chrominance of the BAF effluent was about 80 times when the influent chrominance was approximately 1000 times comparing with that of distilled water. When HRT was reduced from 43.4 to 29.3 h, the removal rate of color in ME decreased from 94% to 88%, while the followed BAF process ensured low level of chrominance of the final effluent. The HPLC analysis showed that BAA in the wastewater contributed 57% of the chrominance, despite that BAA only occupied 14.6% TOC, however, after ME treatment, 99.6% of BAA was removed (HRT = 43.4 h). Previous studies have demonstrated that Fe–C ME could decolorize dye-stuff wastewater [7,11]. The removal rate of BAA in ME was also influenced by the reduction of HRT and decreased to 96.7% when HRT was 29.3 h. Nevertheless, the total removal rate of BAA reached 99.6% after the BAF treatment.

The UV–vis spectra of the influent, ME effluent and BAF effluent are shown in Fig. 4. The absorbance peak in the visible region at 492 nm disappeared after the ME treatment, and the absorbance between 190 nm and 240 nm decreased significantly after the BAF treatment. It was indicated that the chromophores of the contaminants were destroyed during the electrochemical reaction in ME and caused decolorization, however, the decomposition of aromatic compounds was primarily achieved through the biological metabolism.

It should be indicated that many organic components in the BAA wastewater were non-biodegradable. However, the pretreatment of BAA wastewater by ME greatly improved the biodegradability. The BOD_5/COD_{Cr} ratio of the wastewater increased from 0.040 to 0.237 after the ME treatment (HRT = 43.4 h), which implied that the contaminants had become more amenable to biodegradation. Similar results have been reported in treating other organic wastewater by ME [15,16]. The enhancement of biodegradability can be explained by the change or removal of toxic and refractory components in ME. In the present study, the reaction rate of ME was rapid under acid condition, which was nearly twice higher than that of BAF, and the electrode materials were cheap, thus rendering low cost of investment and operation.

3.2. Transformation of organic substances in the combined system

The organic contaminants in the wastewater discharged from BAA production include the raw chemicals, the unseparated final product and the by-products formed during BAA synthesis. Table 2 lists the organic compositions of the influent, ME effluent and BAF effluent, which were measured by GC–MS in order to understand the patterns of removal of organic compounds from the BAA wastewater as it passed through the system. The main organic component of the influent detected using GC–MS was 1-aminoanthraquinone (t_R = 17.49 min), one of the raw materials for the synthesis of BAA (Fig. 5). After treatment by ME, The concentration of 1-aminoanthraquinone in the effluent increased, and new substances were produced such as 1-hydroxyanthraquinone, 1-amino-4-hydroxyanthraquinone, 9,10-dihydroxyanthracene, and 4-bromo-9,10-dihydroxyanthracene. The insoluble 1-aminoanthraquinone was partly adsorbed by the electrodes, however, it was not degraded by the electrochemical reaction. The organic compounds in the ME effluent were effectively removed in the BAF reactor. The less substituted ME products resulting from desulfonation or debromination during the reaction are easier to be biodegraded due to the lower steric hindrance effect and toxicity [17].

Table 2
GC–MS analysis of the organic compounds in the influent, ME effluent and BAF effluent (HRT = 43.4 h)

Organic compounds	Influent	ME effluent		BAF effluent
	Area percentage (%)	Area percentage (%)	Removal rate (%)	Removal rate (%)
1-Aminoanthraquinone	93.1	74.2	−349.4	90.7
Anthraquinone	5.5	0.8	11.5	100.0
1,2-Dichlorobenzene	1.4	ND	100.0	100.0
1-Hydroxyanthraquinone	ND	5.6	*	100.0
1-Amino-4-hydroxy-anthraquinone	ND	2.6	*	100.0
9,10-Dihydroxyanthracene	ND	16.3	*	100.0
4-Bromo-9,10-dihydroxy-anthracene	ND	0.5	*	75.4

ND means the compound was not detected; * indicates a new compound.

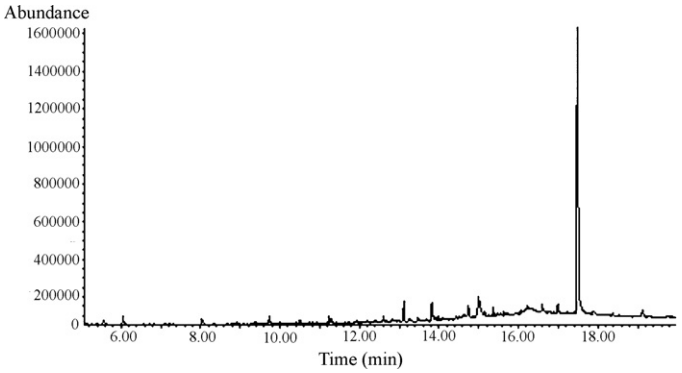

Fig. 5. GC–MS analysis of organic contaminants in the influent (TIC).

Nonvolatile compounds in the influent including BAA (380.4 m/z, 382.2 m/z), 1-amino-anthraquinone-2-sulfonic acid (302.3 m/z) and mono-sulfonated 1,2-dichlorobenzene (225.4 m/z, 227.3 m/z, 229.2 m/z) were detected by HPLC–MS. The mono-sulfonated 1,2-dichlorobenzene which was the by-product during the sulfonation process was the main soluble contaminant. It was hardly removed by the ME process, while efficiently degraded by the biological treatment. The removal rates of BAA, 1-amino-anthraquinone-2-sulfonic acid and mono-sulfonated 1,2-dichlorobenzene by the ME treatment are, respectively, 99.7%, 94.3% and 0.6%, and the final removal rates of them are, respectively, 99.8%, 98.4% and 100.0%.

The red color of BAA wastewater was caused by soluble amino-substituted anthraquinone derivatives. Since BAA contributed most of the chrominance, the ME treatment of BAA solution was carried out in a beaker and the reaction products were analyzed to investigate the decolorization mechanism. 1-Aminoanthraquinone, 1-amino-4-bromo-anthraquinone, 4-bromo-9,10-dihydroxyanthracene, 9,10-dihydroxyanthracene, and 1-amino-4-bromo-9,10-dihydroxyanthracene-2-sulfonic acid were detected using GC–MS and HPLC–MS. It was shown that the reductive transformation of the anthraquinone nucleus to dihydroxyanthracene [18] by Fe, Fe^{2+} and [H] during ME reaction destroyed the chromophore, and the desulfonation reduced the solubility, rendering the decolorization.

3.3. Microbiology analysis

The carrier structure consists of some micro-pores, which provide space for gas and liquid to pass through the microorganisms. The microorganisms were immobilized on the carrier by the effects of covalent bonds and ionic bonds between the chemical groups on the carrier surface and the functional groups on the biological membrane (Fig. 6). In practice, the carrier protects the immobilized microorganisms from being shocked by any sudden change of influent OLR or HRT. The predominant organisms were rod-shaped (0.6–1.1 μm long, 0.3–0.5 μm wide) and globular (Ø 0.5 μm). They agglomerated on the carrier under the function of extracellular polymeric substances.

The molecular biological method is an auxiliary to the traditional separation or identification method to analyze accurately the bacterial community of the sludge and biofilm samples [19]. In the current study, 16S rDNA PCR–DGGE technique was used to investigate the change of the microbial community after long-term of wastewater treatment (Fig. 7). There existed many visible bands showing complexity and diversity of microbial ecology in the sludge

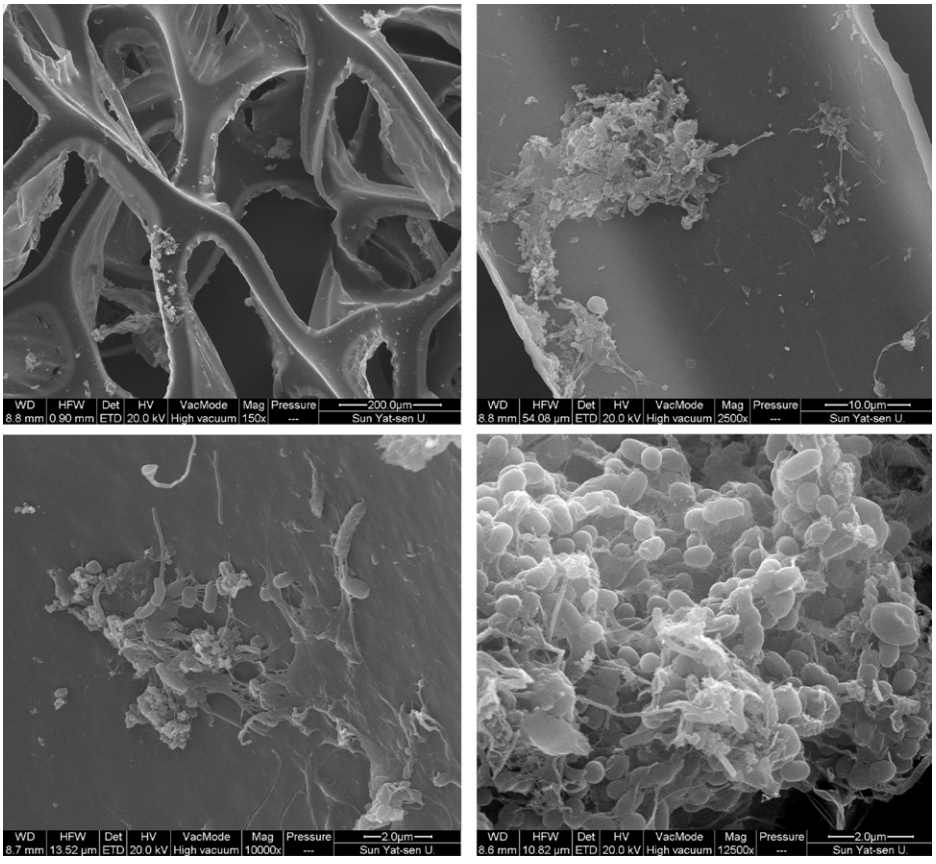

Fig. 6. ESEM photograph of the carrier and the immobilized microorganisms.

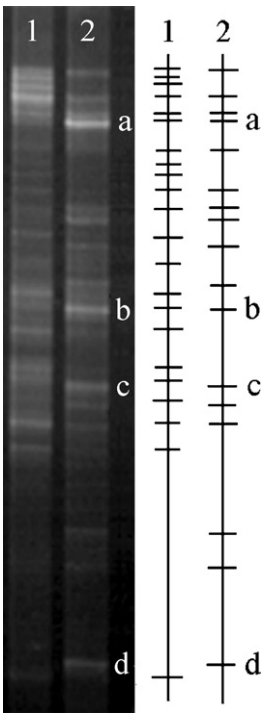

Fig. 7. DGGE of 16S rDNA fragments obtained from raw sludge (lane 1) and BAF reactor (lane 2).

sample (lane 1) and the BAF sample (lane 2). Since the brightness and quantity of the bands reflect the amount and species of the mixture of microorganisms, based on the analysis result, it could be concluded that the long-term contact with the BAA wastewater washed out some species while cultivated several adaptable ones.

Phylogenetic affiliation of the 16S rDNA of bands a–d was summarized in Fig. 8. The bands a–d corresponded to related sequences of *Variovorax* sp., *Sphingomonas* sp., *Mycobacterium* sp., and *Microbacterium* sp., respectively. Since most of the organic contaminants in BAA wastewater were unbiodegradable, those bacteria that were able to grow with the contaminants as carbon sources became the predominant species, for example, the strains of *Sphingomonas* sp. which could degrade BAA as the sole carbon source [20,21]. The presence of BAA-degrading bacteria ensured high

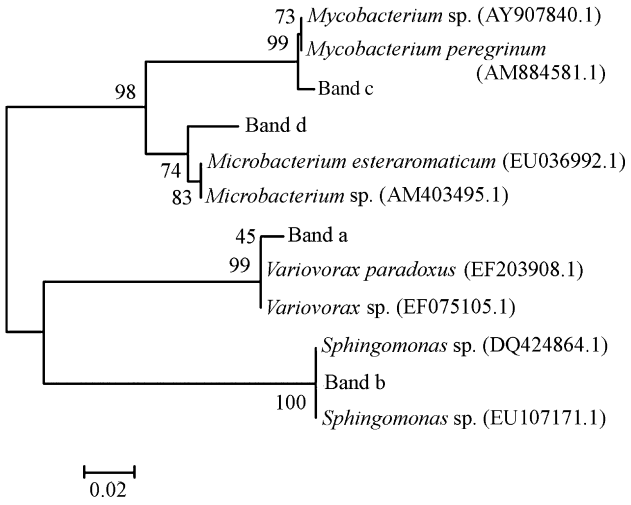

Fig. 8. Unrooted phylogenetic tree of 16S rDNA profiles excised from DGGE.

removal rate of chrominance against the BAA loading shock. The bacterial strains of *Variovorax* sp., *Mycobacterium* sp. and *Microbacterium* sp. have also been reported to degrade various xenobiotic substances [22–28]. These adaptable bacterial species coexisted in the BAF reactor. However, whether they cometabolize the contaminants needs further investigation.

4. Conclusions

The ME–BAF combined system is feasible for treating bromoamine acid wastewater. The HRT was a dominant factor that affected the treatment efficiency. Within the total HRT of 43.4 h, the COD_{Cr} and chrominance removal rate could be up to 81.2% and 96.6%, respectively, at an OLR of 0.56 kg m^{-3} d^{-1}. The chrominance was primarily removed by the ME, accompanied with apparent improvement of biodegradability of the wastewater, whereas COD_{Cr} was mainly removed by the biological process.

The main organic components of the wastewater were 1-aminoanthraquinone, bromoamine acid and mono-sulfonated 1,2-dichlorobenzene. The contaminants were reduced and new compounds were formed in the ME reactor such as 1-hydroxyanthraquinone, 1-amino-4-hydroxyanthraquinone, 9,10-dihydroxyanthracene, and 4-bromo-9,10-dihydroxyanthracene. After the ME–BAF treatment, most of the influent organics were removed.

The long-term operation of the system also resulted in some predominant bacterial species that were adaptive to the specific wastewater in the BAF reactor, such as *Variovorax* sp., *Sphingomonas* sp., *Mycobacterium* sp., and *Microbacterium* sp. Further isolation and identification of these species are of significance to studies of effective biodegradation of BAA wastewater.

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