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Low-strength electronic wastewater treatment using immobilized cells of TMAH-degrading bacterium followed by activated carbon adsorption

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ABSTRACT

An immobilized-cell process followed by activated carbon adsorption was developed to treat the tetra-methyl ammonium hydroxide (TMAH) containing low-strength electronic wastewater. The isolated TMAH-degrading bacterium (*Mycobacterium* sp. TMAH-W0418) and activated sludge were entrapped in polyethylene glycol cubes. The packing rate of 60% (v/v), biomass ratio between *Mycobacterium* and activated sludge of 2:3 (w/w), and total biomass of 7,000 mg/L were found to be optimal for removing dissolved organic carbon (DOC) and TMAH with the concentrations around 1 and 0.2 mg/L, respectively. Granular activated carbon was used to further decrease the DOC and TMAH in the effluent, and the concentrations of DOC and TMAH in the effluent of the combined system were below 1 mg/L and almost 0 mg/L, respectively. Scanning electron microscope results confirmed that cells were effectively immobilized in the polymer matrix. Thick biofilm on the surface of both polymer carriers and activated carbon granules was observed. The microorganism concentration estimated by protein assay increased and the immobilized-cell activity maintained constant during the operation period. From these observations, it was concluded that immobilized-cell process combined with activated carbon system could successfully treat low-strength electric wastewater to yield effluent with DOC level below 1 mg/L.

Keywords: Activated carbon; Immobilized-cell process; Low-strength electronic wastewater; TMAH

1. Introduction

Recently, water consumption has increased significantly in the sector of electric industry as it grows fast throughout the world. Wastewater reuse can be an alternative source of water, and thereby reduce the pollution load to the water environment [1,2]. Low-strength electronic wastewater contains isopropyl alcohol (IPA), dimethyl sulfoxide, tetra-methyl ammonium

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hydroxide (TMAH), acetone, acetic acid, etc. [3]. Among these compounds, TMAH, which is utilized in the etching steps of the semiconductor and liquid crystal display manufacturing process, is poisonous and corrosive and also eutrophic to the water environment [4]. TMAH concentration in electric wastewater is relatively high compared to the other contaminants and its removal from wastewater has become a very important issue for the electronic industry [2].

The technologies such as ion exchange, ultra filtration, and reverse osmosis are effective but are significantly expensive and generate concentrated wastes that require subsequent treatment [1]. Anaerobic biological processes involving diverse microbes for industrial wastewater treatment have been successfully applied in full-scale for decades, but their application to TMAH treatment at industrial scale has not been fully established. Furthermore, information on microbial community treating TMAH anaerobically is quite limited [5]. Aerobic biodegradation might be an effective means for treating TMAH-containing wastewater while there is little report regarding TMAH degradation in activated sludge process and this is probably because TMAH is known to be resistant to biodegradation [6]. However, aerobic biodegradation of TMAH can be achieved by a specific strain that can grow on TMAH. For this case, cell-entrapping immobilization is an ideal way of maintaining its biomass within the system, separating the specific microorganism from other microorganisms, and minimizing the inhibitory effects of TMAH and other toxic substances [7–9].

In this study, an immobilized-cell process followed by activated carbon adsorption was developed to treat the THAM-containing low-strength electronic wastewater. For the optimization of the immobilized-cell process, the effects of packing rate of immobilized carriers, biomass ratio of *Mycobacterium* to activated sludge (M:S), and total cell concentration in the carrier were evaluated in terms of the removal rate of dissolved organic carbon (DOC) and TMAH. Granular activated carbon and zeolite were compared as absorbents for removing contaminants from the effluent of the immobilized-cells process for its reuse.

2. Materials and methods

2.1. Cultivation and immobilization

The medium used for the enrichment of the TMAH-degrading bacteria from the activated sludge contained 5 g/L of TMAH, 1 g/L of $(NH_4)_2SO_4$, 0.7 g/L of K_2HPO_4, 0.3 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4$, and mineral salt solution (in mg/L): H_6BO_6 0.6, $CoSO_4{\cdot}7H_2O$ 0.4, $ZnSO_4{\cdot}7H_2O$ 0.32, $MnCl_2{\cdot}4H_2O$ 0.082, $Na_2MoO_4{\cdot}2H_2O$ 0.0648, $NiCl_2{\cdot}6H_2O$ 0.064, $CuSO_4{\cdot}5H_2O$ 0.028, $FeSO_4{\cdot}7H_2O$ 0.5, and $CaCl_2{\cdot}2H_2O$ 200 [2]. Since the medium contains TMAH as a carbon source and no energy source except $(NH_4)_2SO_4$, only TMAH-degrading bacteria can grow in this medium. Activated sludge collected from an electronic wastewater treatment plant of the S electronics (Tangjeong, Korea) was suspended in 300 mL of the mineral medium (2,600 mg/L MLSS), and temperature of the culture was kept at 30˚C with 120 rpm agitation in the dark. When the total organic carbon (TOC) concentration reached about 200 mg/L, the pellet obtained after centrifugation was suspended in the same volume of fresh mineral medium for continuing cultivation. This fed-batch cultivation was repeated until TOC was not reduced further.

The enriched and isolated TMAH-degrading bacterium (*Mycobacterium* sp. TMAH-W0418) and activated sludge were suspended in a polyethylene glycol (PEG) aqueous solution containing 22% (w/v) PEG prepolymer (olygomer2910), 1.6% (w/v) crosslinker (bisacrylamide), 0.25% (w/v) initiator (potassium persulphate), and 1.05% (w/v) additive (acetic acid). After mixing for 1 min, 0.5% (w/v) promoter (N,N,N,N′-tetra-methyl ethylene diamine) was added and continued mixing for 5 s. Then the mixture was passed through a polyvinyl chloride tube with inner diameter of 4 mm and left to stand for about 10 min at room temperature for solidification. Thus, an elastic gel containing isolated TMAH-degrading bacteria and activated sludge was obtained. This gel was extruded from the polyvinyl chloride tube and cut at a length of 4 mm. The cubic pellets were washed thoroughly in water before use.

For the optimization of the immobilized-cell process, the effects of packing rate [40, 60, and 80% (v/v)], biomass ratio of M:S [4:1, 3:2, 2:3, and 1:4 (w/w)], and total biomass concentration (1,000, 3,000, 5,000, and 7,000 mg/L) were evaluated based on the removal rates of DOC and TMAH.

2.2. Absorbents

Granular activated carbon (8–12 mesh) and zeolite (12–20 mesh) were used as the adsorbents to further remove the DOC from the effluent of the immobilized-cell process. The adsorbents were washed by deionized water several times until the dust and oil were removed. The washed adsorbents were then dried in a drying oven (C-DO2, Chang Shin Scientific Co., Seoul, Korea) at 105˚C for 24 h. The absorbance was measured by analyzing the DOC concentration after gradually adding 0–2 g activated carbon or zeolite (0.2 g addition per batch of experiment), respectively, into 400 mL wastewater contained in 500 mL beaker. After adding the adsorbents, the mixture was agitated by a jar tester (J-JT6S, JISICO CO., Ltd., Seoul, Korea) (150 rpm, 20˚C) and the DOC was measured after 0.25, 0.5, 1, 2, 3, and 4 h.

2.3. Experimental setup

The combined treatment system consisted of an immobilized-cell process followed by activated carbon adsorption is shown in Fig. 1. Low-strength electronic wastewater was obtained from electronic wastewater treatment plant of the S electronics (Tangjeong, Korea) and its typical composition in mg/L was DOC 4.392, TMAH 1.998, IPA 1.620, ethanol 0.026, methanol 0.590, and acetone 0.022. The low-strength wastewater (2 mL/min) and air (0.5 mL/min) were pumped into the bottom of the reactor (250 mL). The effluent from immobilized-cell reactor was flowed into the adsorption column (250 mL) with granular activated carbon at the packing rate of 10% (v/v).

2.4. Sampling and analysis

The concentrations of DOC, TMAH, alcohols, and acetone were analyzed using a TOC analyzer (SHIMADZU, TOC-5000A), an ion chromatograph (Dionex, ICD-3000), and a gas chromatograph (Young-Lin 600D). The protein concentration was measured using Bradford method [10] twice a week to obtain the indirect concentration of the biomass in the carrier. In order to solubilize and hydrolyze the biomass in the carrier for protein assay, 1 N NaOH was added into the mixture of carrier to pH 12 and mixed for 6 h. Then the mixture was centrifuged at 10,000 rpm for 10 min at 4˚C and the supernatant was subjected to protein assay. The absorbance was measured at 595 nm by a spectrophotometer (Thermo, GENESYS 20). The surfaces of the immobilized-cell carriers and activated carbon before and after eight weeks operation were observed by a scanning electron microscope (SEM) (Hitachi S-4700, Japan).

3. Results and discussion

3.1. Optimization of immobilized-cell process

Fig. 2(A)–(F) shows the DOC and TMAH concentrations obtained with different packing rates (A, B), different M:S mixing ratios (C, D), and different total biomass concentrations (E, F) in the immobilized-cell reactor. The concentrations of DOC and TMAH in the effluent from immobilized-cell reactor were reduced to around 1 mg/L and below 0.2 mg/L, respectively, regardless of the packing rate and biomass mixing ratio in the early stages of reactor operation. However, both effluent concentrations of DOC and TMAH began to increase after around 15 days of operation. The packing rate of 60% (v/v) was chosen for subsequent experiments. For the biomass mixing ratio, M:S of 2:3 (w/w) appeared efficient because TMAH concentration increased above 0.5 mg/L after 27 days when M:S mixing ratio was 1:4 (w/w). As shown in Fig. 2(E, F), TMAH and DOC concentrations decreased as total biomass concentration in the immobilized carrier increased and the lowest effluent concentrations of DOC and TMAH were obtained at microorganism concentration of 7,000 mg/L. Therefore, the packing rate of 60% (v/v), M:S mixing ratio of 2:3 (w/w), and total biomass concentration of 7,000 mg/L were chosen for the immobilized-cell process.

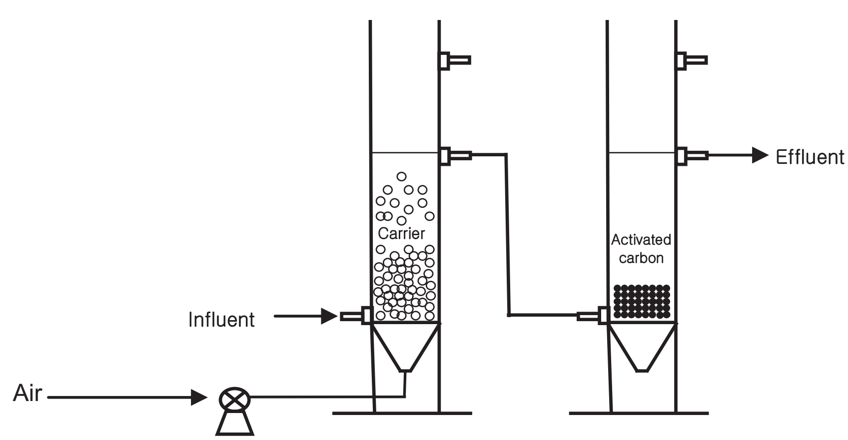

Fig. 1. Schematic diagram of the fluidized bed followed by activated carbon process.

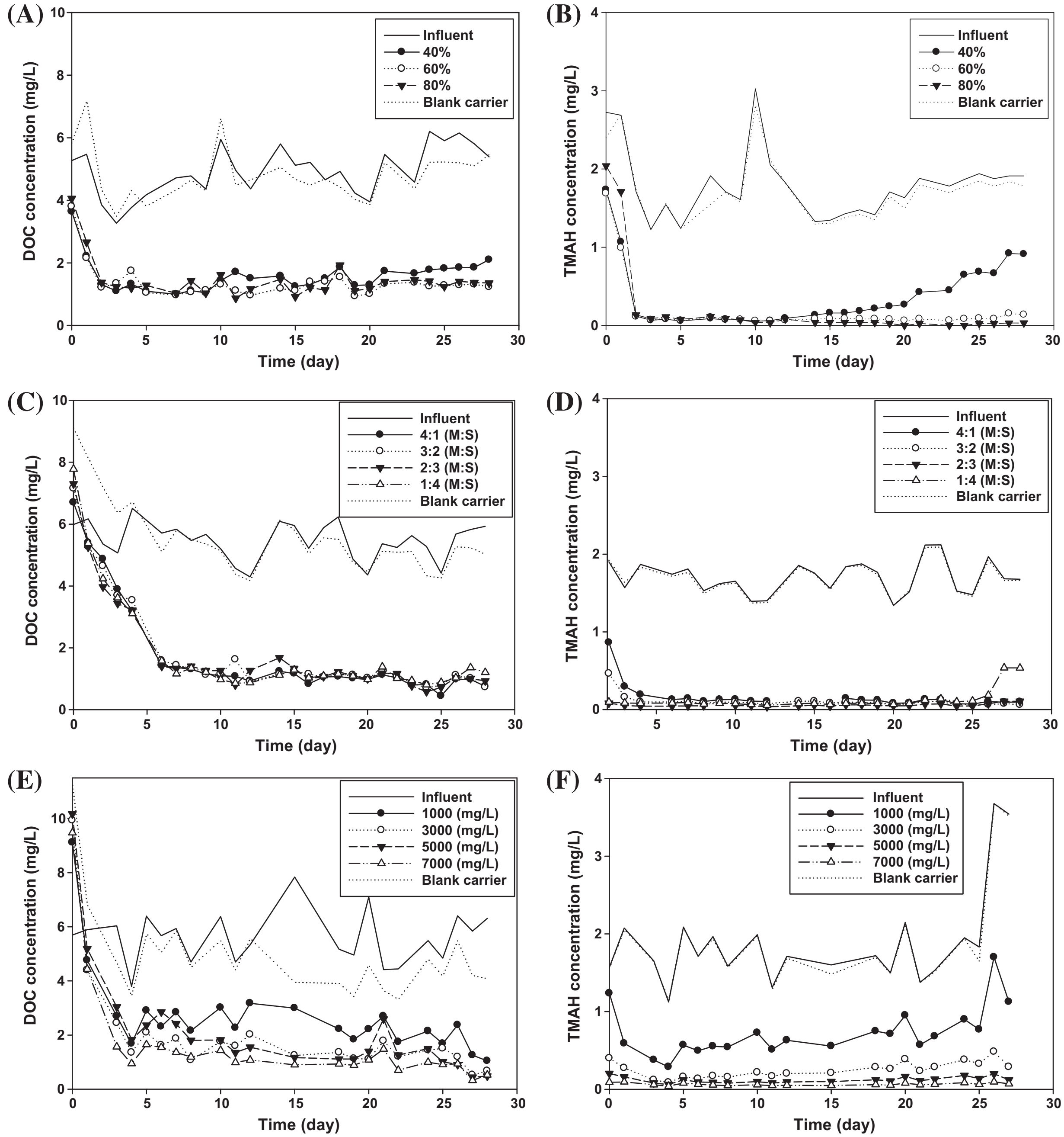

Fig. 2. Influent and effluent concentrations of DOC and TMAH with different packing rates (A, B), different M:S mixing ratios (C, D), and different total biomass concentrations (E, F). For the effect of packing rate, M:S mixing ratio and total biomass concentration were 4:1 (w/w) and 5,000 mg/L, respectively; for the effect of M:S mixing ratio, packing rate and total biomass concentration were 60% (v/v) and 5,000 mg/L, respectively; for the effect of total biomass concentration, packing rate and M:S mixing ratio were 60% (v/v) and 2:3 (w/w), respectively.

3.2. *Activated carbon process following immobilized-cell process*

In order to further remove the DOC and TMAH from the effluent of the immobilized-cell reactor, granular activated carbon and zeolite were used as the adsorbents to adsorb DOC and TMAH. These two adsorbents were packed in the column at the rate of 10% (v/v). Final DOC concentrations at different amount of adsorbent after 2 h of reaction time were shown in Fig. 3 and activated carbon showed better

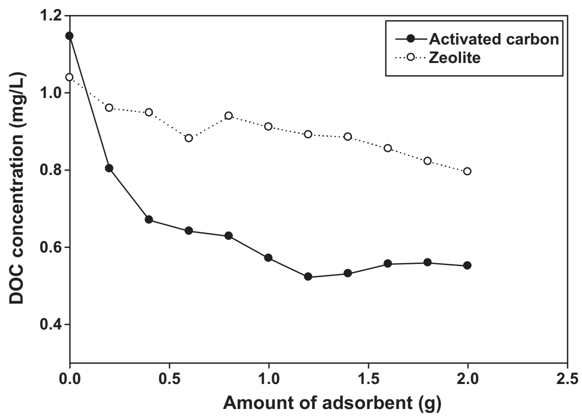

Fig. 3. Final DOC concentrations at different amount of adsorbents after 2 h of operation.

performance. The concentration of DOC decreased from 1.146 to 0.522 mg/L when the activated carbon increased from 0 to 1.2 g and maintained constant when activated carbon was more than 1.2 g. However, the DOC concentration only decreased from 1.039 to 0.795 as the zeolite increased to 2.0 g. Therefore, an activated carbon process was combined with immobilized-cell process to treat the low-strength electronic wastewater.

As shown in Fig. 4(A), the effluent concentrations of DOC and TMAH in the combined system were below 1 mg/L and almost 0 mg/L, respectively, which were lower than the concentrations of the effluent from the immobilized-cell process alone. Total removal rates of DOC and TMAH reached 78.8 and 100%, respectively, which were 11 and 1.2% higher than those of the single immobilized-cell process (Fig. 4(B)).

Fig. 5 shows the estimated microorganism concentration based on protein assay during the operation period. Due to the loss of microorganism during analyzing process, the initial microorganism concentration was 5,400 mg/L which was less than the expected value (7,000 mg/L). The microorganism concentration decreased in the initial period which might be due to adaptation to a new condition. Then the concentration increased to around 7,000 mg/L and maintained stable until 40 days. After 63 days of operation, the microorganism concentration increased to 10,436 mg/L and this is probably because a new biofilm was developed on the surface of carrier.

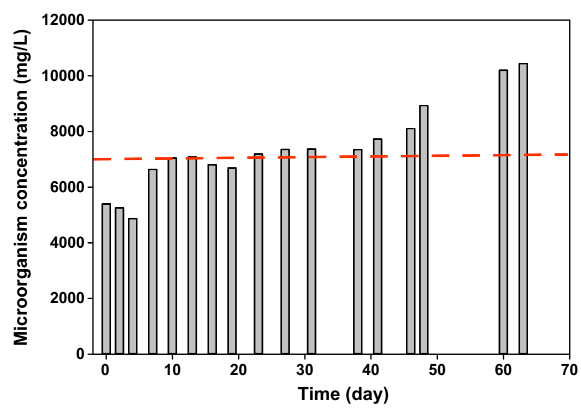

Fig. 5. Estimated microorganism concentration based on protein assay during the operation period.

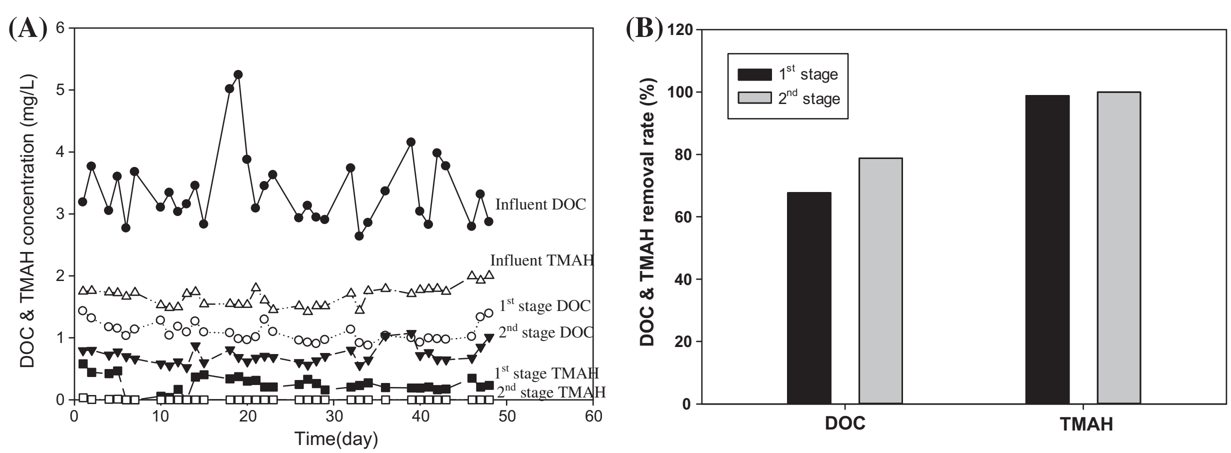

Fig. 4. Time course of concentrations of TMAH, DOC (A) and average removal rates during the operating period (48 days) (B).

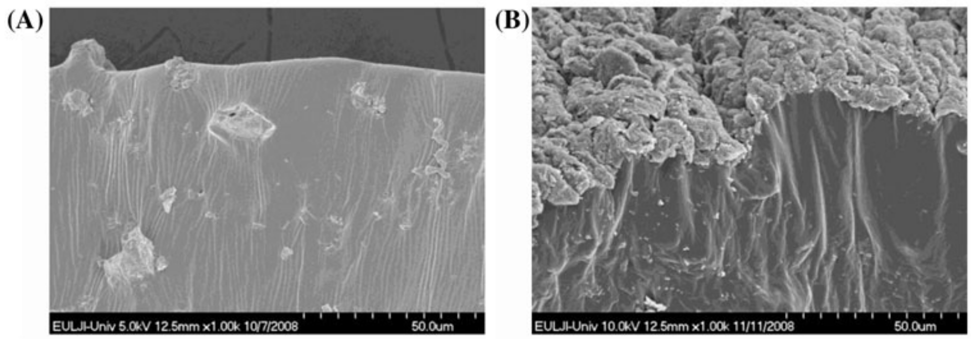

Fig. 6. SEM images of the immobilized-cell carrier after immobilization (A) and after eight weeks operation and (B) at 1,000× magnification.

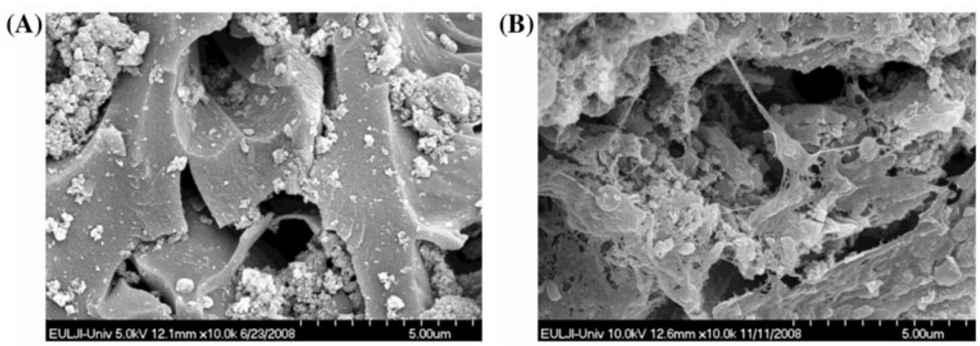

Fig. 7. SEM images of the activated carbon before operation (A) and after eight weeks operation and (B) at 10,000× magnification.

Fig. 6 shows the SEM images of the immobilized-cell carrier after immobilization (A) and after eight weeks operation (B). The SEM images show that the cells were effectively immobilized by polymerization method (Fig. 6(A)). By adding 30 g of carrier into 300 mL of R2A medium (glucose 0.5 g/L, yeast extract 0.5 g/L, casamino acid 0.5 g/L, peptone 0.5 g/L, soluble starch 0.5 g/L, K_2HPO_4 0.3 g/L, $MgSO_4{\cdot}7H_2O$ 0.05 g/L, sodium pyruvate 0.3 g/L) and TMAH medium (composition was described in cultivation part), separately, immobilized-cell activity was maintained constant during the operation period through measuring the decrease of DOC and TMAH concentrations. Interestingly, a thick biofilm was formed on the surface of carriers, indicating that the polymer not only effectively entrapped the cells in the polymer matrix, but also provided suitable environment for the attachment of microorganism (Fig. 6(B)). Lee et al. [11] observed that the microbial density of the outer part was relatively higher than that of the inner part of the carrier and the microbial density inside the cell-entrapped carrier during the early stage of operation was almost constant. It was suspected that microorganisms on the surface of the carrier could easily uptake a higher level of the substrate, but that cells inside the carrier center were subjected to starvation due to the substrate limitation [11].

The biological activity of activated carbon process was confirmed by observing the growth of microorganism on the surface of activated carbon granules. SEM images of the activated carbon showed a porous texture of the material, a biofilm, and organics covered structure before and after eight weeks operation (Fig. 7). Adsorption and biodegradation mechanisms are known to be the predominant factors contributing to organics removal during the activated carbon filtration process [12]. In our process, the surface of the activated carbon after eight weeks operation was thought to be covered by adsorbed DOC which provided nutrients for microorganisms.

4. Conclusions

The packing rate of 60% (v/v), M:S ratio of 2:3 (w/w), and total microorganism concentration of 7,000 mg/L were thought to be the optimal condition of immobilized-cell process for the removal of DOC and TMAH from low-strength electric wastewater. Granular activated carbon was used to further decrease the DOC and TMAH of the effluent from the immobilized-cell process. This two stages process of immobilized-cell reactor followed by activated carbon process effectively removed the DOC and TMAH with removal rates of 78.8 and 100%, respectively. Immobilization of cells was confirmed through protein assay and SEM observation. In conclusion, this immobilized-cell process followed by activated carbon process was thought to be a promising method for the treatment of low-strength electronic wastewater.

Acknowledgment

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