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Petrochemical wastewater odor treatment by biofiltration

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ABSTRACT

The treatment of odorous pollutants by microorganisms on packed waste straw and cortex was investigated at the wastewater treatment plant of the Shanghai petrochemical factory. The removal efficiency of H_2S, NH_3 and VOCs (volatile organic compounds) reached 98%, 91% and 90%, respectively after operation for one month at an empty bed retention time (EBRT) of 120 s. The heterotrophic bacteria were found to be the dominant microorganism in the biofilter, while fungi and actinomycetes were also present. The bacteria were mostly identified as the members of the genus *Bacillus* (62.5% of cultured bacteria). The single strand conformation polymorphism (SSCP) results revealed that the genus *Bacillus* and *Pseudomonas* were the predominant bacteria. The microbial diversity gradually increased as the treatment progressed, which indicated that the microbial community in the biofilter became more stable upon pollutant removal. The scanning electron microscopy (SEM) was performed to evaluate the microorganism growth on the media. It was found that the waste straw and cortex were suitable for microorganism attachment and growth, and may have potential application in odor treatment.

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1. Introduction

The odors generated from wastewater are secondary pollutants that can cause nuisance to adjacent residents and contribute significantly to atmospheric pollution (Burgess et al., 2001; Pandey et al., 2007). Biological treatment processes are promising techniques and have been widely applied to odor pollution control (Chen et al., 2005) due to their advantages in high efficiency, eco-friendliness, low energy consumption and low operating cost (Dennis and John, 2003; Rappert and Müller, 2005).

Biofiltration is the most common biological odor treatment method (Tsang et al., 2007), in which the odorous components are removed by the indigenous microbes attached on the filter media (Langolf and Kleinheinz, 2006; Pandey et al., 2009). The performance of the biofilter is dependent on the nature of the filter media (Kennes and Veiga, 2001). The properties of both the microbial growth and the pollutant adsorption are essential for the filter media, as the pollutants have to be adsorbed on the filter media to be available for biological transformation. Although common filter media such as pall rings, activated carbon and polyurethane foam have the advantage of low head loss, large specific surface area and excellent solid phase adsorption of pollutants, the high investment and regeneration cost has limited their application (Martin et al., 2002). On the other hand, biomedia such as compost and soil are prone to clogging and channeling over long-term operation to cause high resistance and channeling flow (Leson and Winter, 1991; Abumaizar et al., 1998). Therefore, it is necessary to develop new types of biomedia for the effective and economic removal of odorous pollutants by biofiltration.

Microorganism plays a major role in the removal of odorous pollutants, and the understanding in the microbial structure and diversity is very important. Currently, a lot of molecular methods have been developed to study microbial communities, such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and SSCP. These new technologies have allowed deeper understanding in the population dynamics and the microorganism function of microbial communities in both natural and artificial systems (Muyzer, 1999; Sabine et al., 2000; Ogino et al., 2001). However, only limited information is available on the microbial communities involved in the biofiltration of odorous pollutants (Chung, 2007). Compared with other molecular methods, PCR-SSCP is more cost efficient (only the inexpensive electrophoresis equipment is needed) and operationally simple (Stach et al., 2000; Zumstein et al., 2000).

In the present study, the use of waste straw and cortex as the biomedia was investigated, and the performance of this biomedia in the removal of petrochemical odorous pollutants was systematically analyzed. The bacterial communities of the biofilter were examined by conventional microbial methods and the PCR-SSCP technique, and the growth of the microorganisms during the odor treatment was evaluated by SEM.

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2. Methods

2.1. Biofiltration system

A pilot scale biofiltration system was set up at the wastewater treatment plant of the Shanghai petrochemical factory. The wastewater discharged from the factory contained benzene, phenol, sulphate, ammonia, oil, etc. The offensive odor of the wastewater mainly came from the VOCs (0.5–10.4 mg m^{-3}), H_2S (0.01–2.78 mg m^{-3}) and NH_3 (0.89–6.06 mg m^{-3}).

The capacity of the biofiltration system was 500 m^3 h^{-1}, and the bed volume of the biofilter was 18 m^3. Therefore, the empty bed retention time (EBRT) was about 120 s. The biofilter was operated in the downflow mode and equipped with sampling points at the inlet and the outlet of the filter bed. The odorous gas was pumped by an air blower from the sealed well into the humidifier tower to moisturize the gas, and then passed through the biofilter bed to remove the pollutants. The exhaust was discharged to the atmosphere. The nutrient solution was sprayed from a nozzle at the top of the biofilter to maintain the bed moisture, prevent biofilter dehydration, and provide the essential nutrients for the microbial activity. The recirculation liquid in the humidifier was discharged to the wastewater treatment plant periodically.

The waste straw and cortex collected from the farmland were immersed and cultured in the aeration tank of the wastewater treatment plant for 24 h, and then packed into the biofilter bed. The initial bacteria counting of the biofilter was 10^8–10^9 CFU g^{-1} media.

2.2. Odorous gas collection and analysis

The gas concentration at the sampling points, the pressure drop in the columns, and the gas flow rate of each column in the biofilter system were monitored periodically. The gas samples were collected in Tedlar bags at the sampling points and analyzed immediately to avoid sample deterioration. The H_2S concentration was measured by a Jerome 631-X hydrogen sulfide analyzer (Arizona Instruments, USA). The NH_3 concentration was measured by a Dräger detector. The VOCs were determined by a pocket VOC detecting equipment (PGM-50).

2.3. Microorganism counting and identification

The cortex samples were labeled as "C" and the straw samples were labeled as "S". The cortex and straw samples were collected after the system had operated for one week (C1 and S1), one month (C2 and S2), two months (C3 and S3) and three months (C4 and S4). The biofilm was removed gently from the skin of the straw and the cortex with a disinfected knife. The fresh biofilm was put into a 100 mL flask with distilled water, then vortexed and swirled under sonication for 3 min. The cell number of heterotrophic bacteria, fungi and actinomycetes were determined by serial dilution. The culture media of heterotrophic bacteria, fungi and actinomycetes were Lurina-Bertani agar (LB), potato dextrose agar (PDA) and Gause's No.1, respectively (APHA, 1998; Chung, 2007). The density of the nitrifying bacteria and the sulfur oxidizing bacteria were estimated by the most probable number (MPN) method (Takaya et al., 2003; Gallagher et al., 2004). About 10 distinct bacterial and 10 fungal colonies in each plate were isolated, cultured in pure form, and subjected to 16S rRNA and 18S rRNA sequencing for identification.

2.4. Analysis of the bacterial community by PCR-SSCP

The total DNA of the biofilm was extracted by the hot phenol method. The biofilm sample (ca. 100 mg) was lysed in the CTAB buffer (500 μL) with proteinase K (10 μL, 10 mg/mL) and RNAse (10 μL, 10 mg/mL), then extracted with a preheated (60 °C) mixture of 25:24:1 phenol/chloroform/isoamyl alcohol. The upper aqueous layer was extracted until no debris was visible at the interface. After addition of cold absolute ethanol, the DNA was precipitated at 4 °C overnight, collected and air-dried. The genomic DNA was suspended in the TE buffer (100 μL, containing RNAse) and stored at 4 °C for later use. The integrity of the DNA was examined by 1% agarose gel.

The DNA was amplified with the primers SRV3-1 and SRV3-2, which corresponded to the *Escherichia coli* 16 S rRNA genes 330–348 bp and 533–515 bp (Lee et al., 1996). Phosphate was labeled on the 5′ end of the reverse primer SRV3-2. Each 50 μL PCR reaction mixture contained 20 pmol of each primer, 5 mmol of each deoxynucleoside triphosphate (dNTP), 5 μL of Taq DNA polymerase, and the extracted total crude DNA (2 μL, about 10 ng). The PCR amplifications were performed on an automatic thermocycler (PCR Express, Long Gene, China) with the following programming parameters: 5 min at 95 °C, a total of 30 denature cycles at 94 °C for 40 s, annealing at 50 °C for 30 s and extension at 72 °C for 40 s, followed by a final extension at 72 °C for 10 min.

The PCR products were digested by the λ exonuclease for 2 h (Subramanian et al., 2003), then mixed with loading-dye (95% formamide, 20 mmol NaOH, 0.05% bromophenol blue and 0.03% xylene cyanole, Sigma–Aldrich), denatured for 10 min at 95 °C, transferred onto ice for 10 min and finally loaded onto the SSCP gel. The SSCP was then carried out on a Macrophor sequencing apparatus. The DNA was visualized for 30 min in the $AgNO_3$ solution, and the picture was taken by the Smartview system (Shanghai Furi Company, China). The bands from the SSCP profiles were excised with disposable sterile scalpels, transferred into reaction tubes, crushed, soaked in the buffer solution (20 μL), and used as the DNA templates for re-amplification under the same condition as of the first PCR. The re-amplified products were sequenced (Shanghai Sangone Company, China), and the sequences were deposited in the Genbank under Accession Numbers FJ372971 to FJ372979.

The DNA diversity of the microorganisms in the biofilms was characterized by the Shannon–Wiener diversity index by the equation of

$$H = -\sum_{i=1}^{s} Pi \ln Pi$$

where '*H*' was the Shannon–Wiener diversity index, '*Pi*' was the percentage of each species, and '*s*' was the content of the species in the sample. In the SSCP fingerprint, each band represents a species, and the brightness of the band reflects the content of the species.

2.5. SEM observation

Light microscopy and SEM was performed to identify the biofilm development. The samples were coated with a 25 nm layer of gold–palladium mixture and examined with the SEM (JSM-5610LV) at 4000× to 5000× magnification.

3. Results and discussion

3.1. The performance of the biofiltration treatment

According to Fig. 1a, the inlet H_2S concentration was 0.014–21.8 mg m^{-3}, and the H_2S removal rate was >90% throughout the operation. The H_2S removal rate was >99% at the start of the operation, declined temporarily in the first week, then recover to >98% at the end of the first month. On the other hand, it is seen that the H_2S loading increased line with the inlet concentration, however, the removal rate increased with the loading increase as well. After

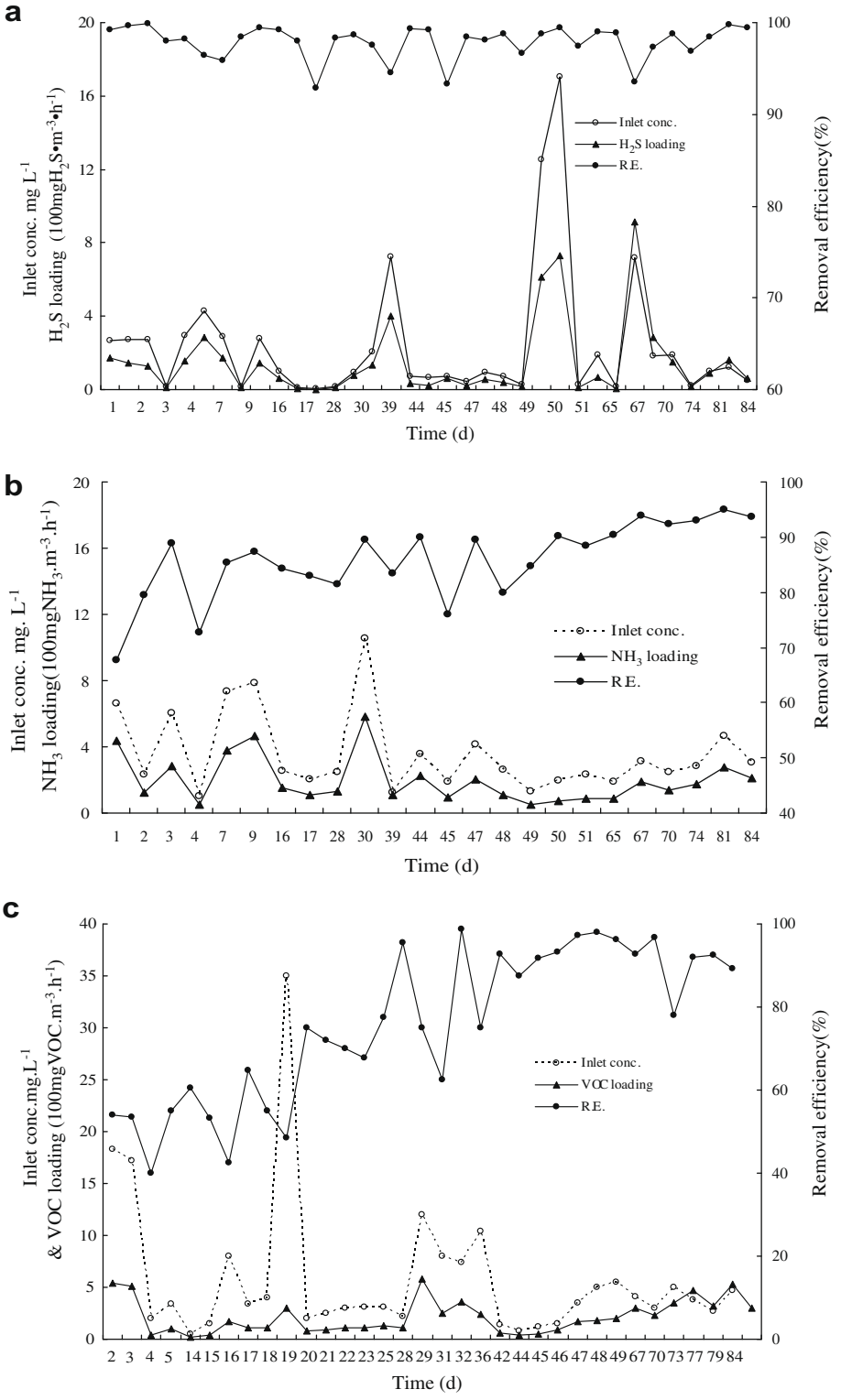

Fig. 1. The profile of inlet gas concentration, gas loading and removal efficiency. (a) H_2S, (b) NH_3 and (c) VOCs.

48 days of operation, the H_2S removal rate reached 99% at a H_2S loading of 1.6 g m^{-3} h^{-1}. The H_2S removal rate remained stable regardless of the changes in the H_2S loading, which indicated that the H_2S loading was relatively low. Kim et al. (2008) have reported a H_2S removal rate of >99% at a H_2S loading of 6 g m^{-3} h^{-1}. The pattern of H_2S removal rate suggested that the initial stage was probably dominated by the biofilter adsorption. The microbial transformation gradually took over as the operation proceeded, which was demonstrated by the growth of the sulfur oxidated bacteria (SOB) (Fig. 2). Kim's (2008) study also showed that the biofilter system was sensitive to H_2S shock loading, as the removal efficiency would drop to 64–78% immediately if a H_2S shock loading of 10 g m^{-3} h^{-1} took place. Since the petrochemical wastewater contained many pollutants, it is beneficial to perform the

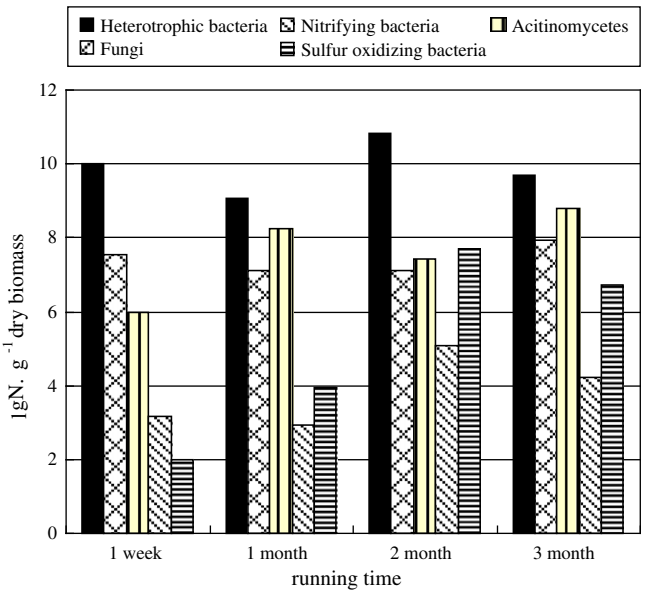

Fig. 2. The microbial counting in the biofilter.

biofiltration at low pollutant loading in order to have high removal efficiency.

The NH_3 inlet concentration was 0.89–10.54 mg m^{-3}. The outlet NH_3 concentration varied from 0.049 to 2.13 mg m^{-3}, and the mean outlet NH_3 concentration was 0.51 mg m^{-3} (Fig. 1b). The NH_3 removal rate remained 80% in the first few days of operation, decreased briefly to around 70% one week later, then gradually recovered to 90% after one month. The removal rate remained above 90% regardless of the fluctuations in the NH_3 inlet concentration, which indicated that the system had a good capacity to NH_3 under present loading.

The mechanism of NH_3 removal was similar to that of H_2S removal. Both the nitrifying bacteria (NB) and the SOB were autotrophic bacteria with long life generation cycle and slow growth (Madigan et al., 2002). In the initial stage, the removal of H_2S and NH_3 mostly depended on the biofilter adsorption, as the biomass of the bacteria was too low to allow active microbial transformation. With the growth of NB and SOB, the microbial transformation improved, and the biofilter exhibited steady removal efficiency for the pollutants.

The removal rate of VOCs exhibited a different pattern compared with that of H_2S and NH_3. The inlet VOC concentration was 0.5–10.4 mg m^{-3}, and the outlet VOC concentration was about 0–3 mg m^{-3}. The removal rate of VOCs increased gradually from 50–60% to 90% after one month (Fig. 1c). The initial removal rate was relatively low compared with that of H_2S and NH_3. The reason might be that the mechanism of VOCs removal was different with that of H_2S and NH_3. Due to the less adsorption of VOCs, the removal of VOCs which depended mostly on adsorption in the beginning was relatively lower compared with that of the H_2S and NH_3. Nevertheless, the VOCs were more biodegradable and readily transformed by the heterotrophic bacteria, and the heterotrophic bacteria could use the VOCs as their food source quickly upon the adsorption of VOCs to the biofilm, therefore the microbial transformation became more available and the removal rate increased with the treatment progressed. Meanwhile, it is noticed that the removal rate of VOCs decreased sharply upon VOCs shock loading (on days 16, 19 and 29–32), which suggested the loading was a key parameter in the treatment of VOCs. High VOCs loading would result in reduced VOCs removal efficiency, especially at the initial stage of the operation.

3.2. The Shift of the cultural microbial community in the biofilter during operation

According to the plate counting and MPN analysis, the most abundant microorganism in the biofilm of the biofilter were found to be the heterotrophic bacteria (>10^9 CFU g^{-1}, dry media). The amount of each microorganism varied differently in the operation (Fig. 2). The NB and SOB increased sharply as the operation proceeded. Before the operation, the amount of NB and SOB were significantly smaller than that of the heterotrophic bacteria. However, after the operation of three months, the NB and SOB increased rapidly and became more abundant. The community structure changed substantially, and the elevated ratio of actinomycetes to heterotrophic bacteria indicated increased bio-diversity and stabilization. The amount of fungi increased and the pH decreased in the liquid of the biofilter (data not shown), indicating enhanced nitrification and sulfuration. Fungi could generally function in more demanding condition, such as low pH or low water activity (Sheridan et al., 2003; Duan et al., 2006).

The identification of distinct pure culture colonies showed that most bacteria colonies were members of the *Bacillus* and *Pseudomonas* genus, such as *Bacillus sphaericus* and *Pseudomonas putida*, which were common bacterial species involved in the sulfur or ammonia odor treatment system (Moller et al., 1996; Chung et al., 2001; Shim et al., 2005; Pandey et al., 2007). The ratio of the *Bacillus* and *Pseudomonas* in the bacteria were 62.5 ± 11% and 12.5 ± 8%, respectively. With regard to the fungi colonies, the genus of *Peniciall*, *Aspergillus* and *Trichoderma*, such as *Penicillium citrinum*, *Aspergillus flavus* and *Trichoderma saturnisporum* were identified. These microorganisms belonged to common fungi widely spread in the general environment (soil, water and dry plant), and could live on the odorous pollutants. The ratio of the *Peniciall* was 25 ± 7%, and the ratio of the *Aspergillus* and *Trichoderma* was 12.5 ± 6%.

3.3. Microbial community structure analysis by PCR-SSCP

The microbial community structure of the biomedia during the odor treatment was analyzed by PCR-SSCP (Fig. 3). The relative diversity of the bacterial community was represented by the number of bands on the gel, and the abundance of each bacterial group was represented by the intensity of the specific band. The appearance or disappearance of bands indicated the changes in the bacterial community of the biofilter as the deodorization proceeded. In

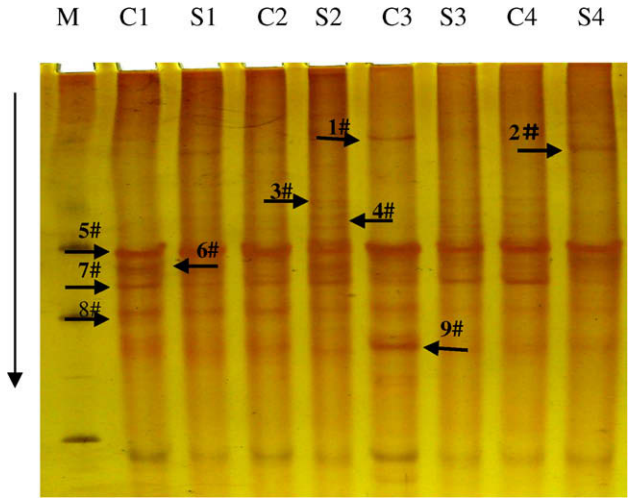

Fig. 3. The SSCP profile of the bacterial microbial community in different media.

Table 1
SSCP bands blasting.

Band number	Accession Number	Phylogeny	Closest relatives	Similarity (%)	Sources
1	FJ372972	*Firmicutes*	*Bacillus* sp. *YACN-9*	98	Soil, water
2	FJ372973	*γ-Proteobacteria*	*Pseudomonas* sp. *PD2*	95	Soil, water
3	FJ372974	*Firmicutes*	*Bacillus mycoides*	96	Soil, water
4	FJ372975	*Firmicutes*	*Uncultured Bacillus* sp.	95	Soil, water
5	FJ372976	*γ-Proteobacteria*	*Pseudomonas denitrificans*	93	Soil
6	FJ372977	*γ-Proteobacteria*	*Pseudomonas plecoglossicida*	97	Soil
7	FJ372978	*γ-Proteobacteria*	*Uncultured Enterobacter bacterium*	98	Soil, plant
8	FJ372979	*γ-Proteobacteria*	*Shigella* sp. *T47090*	98	Soil, water
9	FJ372971	*a-Proteobacteria*	*Mesorhizobium* sp.	100	Plant

order to better understand the differences in bacterial diversity among samples, discriminable bands were excised and sequenced, and the closest relative was identified by comparison with the Genebank database (Table 1). It was found that most bands of the operation samples were the genus of *Bacillus* and *Pseudomonas*, which belonged to the phylum *Firmicutes* and *Proteobacteria*. These species were also present in the pure culture, and their occurrence in the biofilter agreed with previous published results (Friedrich et al., 2002). According to SSCP, the bands 5 (*Paracoccus denitrificans*) and 7 (*Enterobacter*) were consistently present throughout the treatment. *P. denitrificans* has been previously found to be capable of removing sulfur-containing compounds and trimethylamine (Jordan et al., 1997; Kim et al., 2003). It is responsible for the removal of sulfur- and nitrogen-containing compounds. *Enterobacter* is seldom isolated from biofilter, it is capable of removing compounds such as *n*-butyric acid (Sheridan et al., 2003). Among all bands, two bands represented uncultured bacteria, which indicated that although some bacteria did not grow on the plate, they actually played an important role in the odor treatment.

The Shannon–Wiener diversity index (H) was calculated based on the PCR-SSCP result. The H value ranged from 1.5–2.5 for different biomedia at different sampling time. The diversity declined in the initial stage of operation, and then increased as the operation proceeded further. Samples S1 and C1 had high diversity ($H > 2$) because they were soaked in the activated sludge for some days, and a lot of activated sludge microorganisms could have been attached. The H value of the samples S2 and C2 decreased to 1.6 and 1.8, respectively, which indicated that some activated sludge bacteria disappeared as the operation proceeded, and fewer bacteria survived. Afterwards, some acclimated bacteria grew on the odorous pollutants and multiplied actively. The bacterial biomass and diversity improved gradually, and the diversity index recovered ($H > 2$) after operation of three months. The increase in the Shannon–Wiener diversity index indicated that the microbial community in the biofilter became more stable upon pollutant removal. In most cases, the diversity of the cortex (average $H = 2.2$) was higher than that of the straw (average $H = 2.0$), which suggested that the microorganisms were attached more readily to the cortex, probably due to the much harsher surface of the cortex.

3.4. SEM photos of the biofilter samples

The surface characteristics of the filter were examined by SEM. On both the cortex and the straw, the microorganisms exhibited steady growth as the operation progressed. The microorganisms had various shapes (long, short and rod), which demonstrated that the diversity and the biomass of the bacteria on the biofilter improved significantly through the treatment. Therefore, the waste straw and cortex were suitable media for the attachment and growth of microorganisms in the treatment of petrochemical odorous wastewater.

4. Conclusions

The waste straw and cortex were used as the packing material in a biofiltration system to remove the petrochemical odorous pollutants. The removal rate of H_2S, NH_3 and VOCs reached to 98%, 91% and 90%, respectively at an EBRT of 120 s after operation for one month. The removal mechanism of H_2S and NH_3 was different to that of the VOCs.

The most abundant microorganism in the biofilter was found to be the heterotrophic bacteria, while fungi and actinomycetes were also present. The bacteria and fungi were mostly identified as members of the *Bacillus* and *Penicillium* genus. The SSCP results also confirmed that the genus of *Bacillus* and *Pseudomonas* were the predominant bacteria in the biofilter. The increase in the Shannon–Wiener diversity index indicated that the microbial community in the biofilter became more stable upon pollutant removal. It was also found through SEM observations that the biomass and the biofilm on the biomedia increased as the treatment progressed.

This work suggested the potential application of the waste straw and cortex in the removal of odorous petrochemical pollutants.

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