



Technical Note

Efficient bio-deodorization of aniline vapor in a biotrickling filter: Metabolic mineralization and bacterial community analysis

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ABSTRACT

A biotrickling filter inoculated with commercial mixed microorganisms B350 was employed to treat N-containing odorous vapor – aniline. Results indicated no aniline could be detected when empty bed residence time (EBRT) was larger than 110 s at inlet concentration of 0.30 g m⁻³. The variation of inlet concentration did not change removal efficiencies when concentration is less than 0.21 g m⁻³ at fixed EBRT 110 s. Biodegradation mechanism of aniline was tentatively proposed based on identified intermediates and predicted biodegradation pathway as well as final mineralized products. Aniline was firstly biodegraded to catechol, and then to levulinic acid and subsequently to succinic acid. Finally, about 62% aniline carbon was completely mineralized to CO₂, while about 91% aniline nitrogen was converted into ammonia and nitrate. Bacterial community in biotrickling filter was found that at least seven bands microbes were identified for high efficiencies of bioreactor at stable state. In all, biotrickling filter seeded with B350 would be a better choice for the purification odorous gas containing high concentration aniline.

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1. Introduction

Odoriferous compounds are a special group of air pollutants, including phenols, hydrogen sulfide, ammonia, amines, volatile fatty acids, volatile organic sulfur or nitrogen compounds (Zhu, 2000; Ho et al., 2008; Tsang et al., 2008). They can enter atmosphere and cause nuisance to adjacent residents from industrial process or plants, such as agriculture and food industry (Sironi et al., 2007), paper making plant (Yoon et al., 2001), and sewage treatment process (Gostelow et al., 2001). Aniline is also widely used as an intermediate in the production of dye, raw material for the manufacturing of synthetic organic chemicals and polymers including polyurethanes, rubber additives, and pharmaceuticals (Emtiazzi et al., 2001). Because of its volatile and potent toxicity to human and animals (Patnaik, 2007), it is urgent to remove aniline from polluted air.

Biological technology is the most environmental friendly and cost-effective method for odorous pollutants control (Friedrich et al., 2002; Luo and Lindsey, 2006). Among bioreactors used, biotrickling filter has attracted considerable interests due to its high capability to purify waste gas containing biodegradable volatile organic compounds, toxic and odorous compounds by seeded microorganisms (Deshusses, 1997; Smet et al., 1998; Chan and Lin, 2006; An et al., 2010; Mudliar et al., 2010). In particular, a

liquid mobile phase was forced to flow in the inner of biotrickling filter. Thus, environmental conditions, such as pH, humidity and mineral nutrient can be better controlled during the process via cycled trickling liquid (Cox and Deshusses, 1998). In biotrickling filter, pollutants are adsorbed onto carrier subsequently degraded by biofilms immobilized on carrier material with release of CO₂ (Cox and Deshusses, 1998). Thus, microorganisms, which are the catalysts for biodegradation of organics, are expected to play an important role in the removal of pollutants.

Currently, a number of single strains capable of degrading aniline were isolated and identified (Aoki et al., 1984; Liu et al., 2002; Xiao et al., 2009). However, besides target compound, various other pollutants will be coexisted in the real waste gas with aniline vapor. Hence, using different mixture microorganisms with various organic degrading abilities will be usually superior to using single strain for industrial application in aniline treatment, although some single strain possesses broad degradation spectra for a group of compounds. Among mixture microorganisms, B350 which containing 28 species of microorganisms and various enzymes are not only widely-used but also efficient for the biodegradation of organics in water (Zhao et al., 2006, 2009) and air (An et al., 2010; Wan et al., 2011a). Furthermore, most of researches mainly focused on the biodegradation performance of bioreactor (Ding et al., 2007; Jiang et al., 2009) rather than microbial community development in biotrickling filter using B350 group microorganisms. However, a better understanding of microbial community structure is important to explain why the high efficiencies achieved in biotrickling filter at its stable state. Nevertheless, only limited information is available

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on the analysis of microbial community composition involved in biofiltration or biotrickling filtration treatment of N-containing odorous gas, for example trimethylamine (TMA) (Chung, 2007). No studies have been dedicated to industrial treatment of waste gas containing aniline using B350 group microorganisms, even with the microbial community development in biotrickling filter.

Therefore, a biotrickling filter immobilized with B350 group microorganisms was assessed its potential capability for aniline vapor treatment. The influence of inlet concentration and empty bed residence time (EBRT) on removal efficiencies (REs) and elimination capacities (ECs) of aniline were investigated in detail. The biodegradation mechanism of aniline by B350 was also tentatively attempted based on identified intermediates and final mineralized products, CO_2 , $\text{NH}_3/\text{NH}_4^+$ and NO_3^- . In addition, to gain further insights into the relationship between the efficient decontamination efficiencies of B350 and the microorganism domain in the biotrickling filter at steady state, the sequences of dominant microorganisms and the bacterial community composition were also analyzed by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

2. Materials and methods

2.1. Microorganisms and culture medium

A mixed culture B350 was obtained from Bio-System USA. Aniline (>99.5%, Tianjin, China) was selected as the representative odorous organic pollutant. An inflow medium was provided from a nutrient tank which contained mineral medium (MM) as described previously (An et al., 2010).

2.2. Biotrickling filter system

A biotrickling filter with an inner diameter of 140 mm, six layers (each layer has a sampling ports) packed with ceramic particles and inoculated with B350 was used to treat gaseous aniline and shown in our previous work (Wan et al., 2011a). After incubation, air and aniline gas were continuously fed into biotrickling filter by an air-pump. Aniline inlet concentrations were controlled with flow rate of mixed aniline gas and air with a mass flow controller. Simultaneously, MM was trickled from top of biotrickling filter at a rate of 7.5 L h^{-1} for 10 min each, 16 times per day. Meanwhile, the pH value of MM was adjusted regularly to 7.0 with 1 M HCl or NaOH to obtain the optimal range of pH value for microorganisms. Biotrickling filter performance was analyzed according to the changes of REs and ECs of aniline after adjusting gas flow rate and inlet concentration. RE (%), inlet loading ($\text{g m}^{-3} \text{ h}^{-1}$) and EC ($\text{g m}^{-3} \text{ h}^{-1}$), were determined and calculated according to our previous paper (An et al., 2010). The biotrickling filter was usually allowed to stabilize for at least 12 h after adjusting the inlet concentration of pollutants or changing EBRT, and then gas sample of different ports was taken to determine the performance of the biotrickling filter in the next 48 h, or longer period of time. In addition, all studying parameters and operating conditions are listed in Table 1. CO_2 production (P_{CO_2} , $\text{g m}^{-3} \text{ h}^{-1}$) was calculated according to our previously published reference (Wan et al., 2011b).

2.3. Analytical methods

Aniline concentration was determined as follows: 300 μL gas sample collected from the inlet and outlet of biotrickling filter at regular intervals using an airtight syringe (Agilent 500 μL) was injected into an HP 5890 gas chromatography (GC) (Hewlett-Packard, USA) equipped with an HP-5MS capillary column ($30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$) and a flame ionization detector in the splitless mode. The

injector and detector temperatures were set at 280 and 300 $^\circ\text{C}$, respectively, the column temperature was maintained at 180 $^\circ\text{C}$. CO_2 concentrations in gas sample, ammonia, nitrate and nitrite in re-circulating liquid were determined according to our previous reference (Wan et al., 2011b). Thickness of biofilm on ceramic particles was calculated according to method described by An et al. (2010). The experimental details are shown in Supplementary material (SM). In brief, the ceramic particles (ca. 50 g) were sampled from biotrickling filter, and the mass of the biofilm (expressed in mg g^{-1} dry ceramic particles) was determined by weight loss. The average diameter of ceramic particles is $4.4 \pm 0.9 \text{ mm}$ with 1 g containing average of 10 particles. On the assumption that the average density of biofilm was 62.5 mg cm^{-3} , the thicknesses of biofilm was calculated according to the biomass, density of biofilm and diameter of ceramic particles.

2.4. Identification of metabolites

To identify metabolites in gas phase, 300 μL gas samples were directly injected into an Agilent 7890A GC–5975C mass spectrometer detector (GC/MSD) with an HP-5MS silica fused capillary column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ film thickness) using full scan model (m/z : 30–300). To identify metabolites in re-circulating liquid, 50 mL sample was extracted consecutively with 50 mL ethyl acetate two times at pH 7.0 and 2.0, respectively. The mixed extract was concentrated and intermediates were determined using GC/MSD directly and after N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) derivatization. The experimental details are shown in SM.

2.5. Microbial community analysis

The morphology and microbial community diversity on ceramic particles was observed by scanning electron microscope (SEM, JSM-6360, Japan), and DGGE, respectively. The detail analytical procedure of microbial community is shown in detail in the SM. As a parameter for the structural diversity of microbial community, the Shannon–Weaver index (H') measuring proportional abundances of species in a community, emphasizing community richness, was calculated according to Konstantinov et al. (2003).

3. Results and discussion

3.1. Start-up of biotrickling filter

The biotrickling filter performance from day 1 to 23 is shown in Fig. SM-1. On day 1, 0.52 g m^{-3} gaseous aniline was fed into biotrickling filter at fixed EBRT 110 s. After aniline passing through all bioreactor layers, no aniline could be detected in outlet. This may partially attribute to the adsorption by ceramic particles and medium liquid, which could be confirmed by RE decrease on day 2 (37%). As inlet concentrations further decreased to 0.11 g m^{-3} on day 4, total RE increased to 74%. When inlet concentrations increased slightly from 0.11 to 0.16 g m^{-3} , total REs also increased slightly from 74% to 85% from day 4 to 9, which revealed slow formation of biofilm. However, total RE first slightly and dramatically decreased to 81% and 58% as inlet concentration increased to 0.18 and 0.38 g m^{-3} on day 10 and 11. When concentrations were maintained at about 0.31 to 0.42 g m^{-3} (day 11–23), REs firstly increased steadily, and finally were higher than 90% at EBRT 110 s. Compared with start-up process with TMA (Wan et al., 2011b), B350 is much difficult to acclimate for aniline vapor.

Biomass immobilized on ceramic particles was also determined during the start-up process of biotrickling filter (Fig. SM-2). Initially, no bacteria were grown on ceramic particles (day 0).

Table 1
Biotrickling filter operating conditions.

Filtering medium	Ceramic particles (moisture content: 15–25%; pile density: 0.75–1.10 g cm ⁻³ ; particle diameter: 4–6 mm; BET surface area: 2–5 × 10 ⁴ cm ² g ⁻¹ ; maximum porosity volume for pile: no less than 36%)
Pollutant	Aniline
Packing bed height	100 mm × 6 layers
Column diameter	Inner diameter of 140 mm
Volume of the packing materials	9.23 L
Microorganisms	B350 group microorganisms
Inlet aniline concentration	0.1–0.6 g m ⁻³
Airflow rate	200–800 L h ⁻¹
EBRT	42–166 s

However, the bacteria attached onto ceramic particles increased gradually with the increase of operational time. For instance, on day 10, the biomass increased to 2.3 mg g⁻¹, the number of microorganisms and thickness of biofilm also increased to 5.7 × 10⁷ Colony Forming Unit (CFU) g⁻¹ and 60 μm, respectively. On day 30, the corresponding data increased to 7.0 mg g⁻¹, 1.7 × 10⁸ CFU g⁻¹, and 171 μm, respectively. All results indicated that B350 were gradually successfully immobilized onto ceramic particles, which is the reason why biotrickling filter exhibited a high performance during the start-up process.

3.2. Effect of EBRT and inlet aniline concentration

The effect of EBRTs (from 166 to 42 s, corresponding to gas flow rate from 200 to 800 L h⁻¹) on biotrickling filter performance inoculated with B350 was investigated at fixed concentration of 0.30 g m⁻³ (Fig. 1a). No aniline was detected at an EBRT of 166 s, and more than 68% aniline was degraded after passing through 1–3 layers of biotrickling filter bed. With further shortening of EBRT to 110 and 42 s, total REs decreased slightly to 91% and sharply to 42%, respectively. Additionally, the bioreactor performance was also evaluated in terms of EC of aniline with various flow rates (Fig. 1b). Total ECs increased gradually and peaked as EBRT shortened from 166 (EC = 6.9 g m⁻³ h⁻¹) to 55 s (12.6 g m⁻³ h⁻¹), and then decreased to 12.2 and 11.0 g m⁻³ h⁻¹ with EBRT further decreased to 47 and 42 s, respectively. It is worth mentioning that, the majority of aniline was eliminated by layers 1–3, for instance, total ECs of layers 1–3 and 4–6 achieved the maximum 7.6 and 5.0 g m⁻³ h⁻¹ at EBRT 55 s, respectively.

ECs plotted against inlet loadings with different flow rates at fixed inlet concentration of 0.30 g m⁻³ are illustrated in Fig. 2. No aniline was detected as inlet loading was 6.9 g m⁻³ h⁻¹. With the increase of gas flow rate to 600 L h⁻¹, the inlet loading increased to 19.6 g m⁻³ h⁻¹ accordingly, and total ECs increased gradually and peaked at 12.6 g m⁻³ h⁻¹ (RE = 64%). As flow rate increased further to 700 and 800 L h⁻¹, ECs slightly decreased to 12.2 (inlet loading = 24.6 g m⁻³ h⁻¹; RE = 49%) and 11.0 g m⁻³ h⁻¹ (inlet loading = 26.5 g m⁻³ h⁻¹; RE = 42%). Comparing with the performance of biotrickling filter to purify TMA (Wan et al., 2011b) and aniline by B350 at identical condition possessed, the similar trend can be obtained for REs and ECs, although specific data are different. For instance, no aniline and TMA could be detected at inlet concentration 0.30 g m⁻³ as EBRT no less than 166 s for aniline and 110 s for TMA, respectively. It can also observe that REs dropped with the increase of flow rate or with the decrease of EBRT as described in previous papers (He et al., 2009). The possible reason is that the biodegradation process is both controlled by mass transfer of aniline from air to biofilm (diffusion limitation) and by biodegradation reaction (reaction limitation) in biotrickling filter (Jorio et al., 2000).

The inlet concentration of pollutant is also a key parameter in a biofiltration process. The effect of aniline concentration ranged from 0.11 to 0.60 g m⁻³ on the performance of bioreactor was carried out

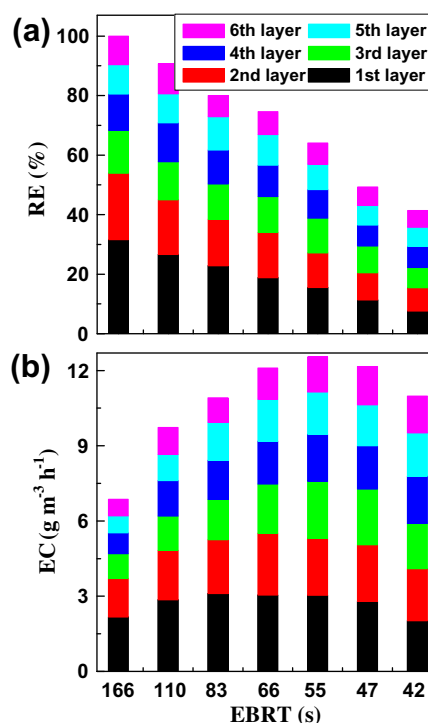


Fig. 1. (a) Removal efficiencies and (b) elimination capacities of aniline at different EBRTs at fixed inlet concentration 0.30 g m⁻³.

at fixed EBRT 110 s (Fig. 3a). No aniline could be detected at layers 1–4 and even 1–6 when inlet concentrations were 0.11 and 0.21 g m⁻³, respectively. With further increase of concentrations to 0.31 and 0.42 g m⁻³, total REs dropped slightly and then swiftly to 95% and 83%. It was worth noting that REs of layers 1–3 and 4–6 had different trend. That is, REs of layers 1–3 decreased always, while 4–6 gradually increased, peaked at 35% (aniline = 0.31 g m⁻³), and then slightly decreased to 27% as inlet aniline concentration further increased to 0.60 g m⁻³. This is because the biotrickling filter was operated as a co-current down-flow mode. Aniline was firstly degraded by microorganisms in layers 1–3 and then 4–6. At lower inlet concentration, a diffusion limitation occurred in layers 4–6, because majority of aniline was already removed by layers 1–3 before it reach to 4–6 and adequate microorganisms existed in layers 4–6 to eliminate the rest aniline. However, the reaction limitation may occur in layers 4–6 as concentration further increased.

The performance of the biotrickling filter was also evaluated in terms of ECs at same treatment conditions (Fig. 3b). Total ECs had a similar trend as showed in Fig. 1b, that is, total ECs firstly increased to its maximum and then dropped slightly with a rise of aniline concentration. Nevertheless, no aniline could be detected only as

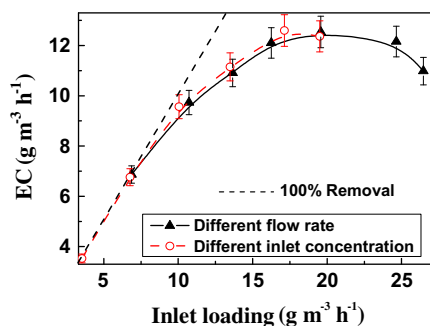


Fig. 2. ECs of biotrickling filter plot against inlet loadings with different flow rates at inlet concentration of 0.30 g m^{-3} and with different inlet concentrations at EBRT of 110 s.

inlet loading was less than $6.8 \text{ g m}^{-3} \text{ h}^{-1}$ (corresponding to inlet concentration 0.21 g m^{-3} at EBRT of 110 s) (Fig. 2b). Only 74% of aniline was removed as EC up to its maximum ($12.6 \text{ g m}^{-3} \text{ h}^{-1}$) when inlet loading increased to $17.1 \text{ g m}^{-3} \text{ h}^{-1}$. As inlet loading increased further, EC slightly decreased to $12.4 \text{ g m}^{-3} \text{ h}^{-1}$ at the concomitance with further decrease of RE (63%). Similar findings were also found for xylene vapors treatment in a biofilter (Jorio et al., 2000). The possible reason can also be interpreted as the diffusion limitation and the reaction limitation as described previously (Wan et al., 2011b).

3.3. Aniline metabolism and mineralization

GC/MSD was employed to analyze aniline biodegradation intermediates during biotrickling filtration process. However, no any by-products were detected in gas phase besides aniline (Fig. SM-3), indicating that aniline vapor could be completely removed or converted to more polar intermediates and then dissolved in re-circulating liquid when passing through biotrickling filter bed. As predicted, besides original aniline, two intermediates catechol (1) and very small quantity of acetanilide could be directly detected in re-circulating liquid (Fig. SM-4). It is worth mentioning that acetanilide is only a reversible acylation product of aniline (Lyons et al., 1984). After derivatization, catechol and aniline were also detected in re-circulating liquid (Fig. SM-5). In addition, the chromatography with their spectra demonstrated that small amount of levulinic acid (2) and succinic acid (3) were also indirectly detected in re-circulating liquid after derivatization with BSTFA by using extract ion chromatography, and their spectra were demonstrated in Fig. SM-6.

Based on the identified key intermediates and previously published literatures, a possible biodegradation mechanism of aniline was tentatively proposed (Fig. 4). The amino-group rather than benzene ring of aniline was firstly attacked with the release of ammonia by B350, which agreed well with the earlier reports (Aoki et al., 1983). Simultaneously, benzene ring of aniline was converted to catechol, which might be the contribution of aniline dioxygenase as described (Liu et al., 2002). Catechol was also suggested to be the first intermediate during biodegradation of aniline in aqueous medium using single bacterial strains (Zhang et al., 2008). Subsequent degradation might go as follows: catechol (1) was attacked with two atoms of oxygen, the dihydroxylated aromatic ring was opened by oxidative ortho cleavage, and converted to *cis,cis*-muconic acid, which was further metabolized to β -keto adipic acid, and then to levulinic acid (2) and succinic acid (3); or directly to succinic acid (3). Finally, these small molecule metabolites could be completely mineralized into $\text{NH}_4^+/\text{NO}_3^-$, CO_2 and H_2O . In this study, *cis,cis*-muconic acid and β -keto adipic acid were not detected because that the degradation can be directly converted into catechol (1), and then rapidly to levulinic acid (2) and succinic acid (3) without accumula-

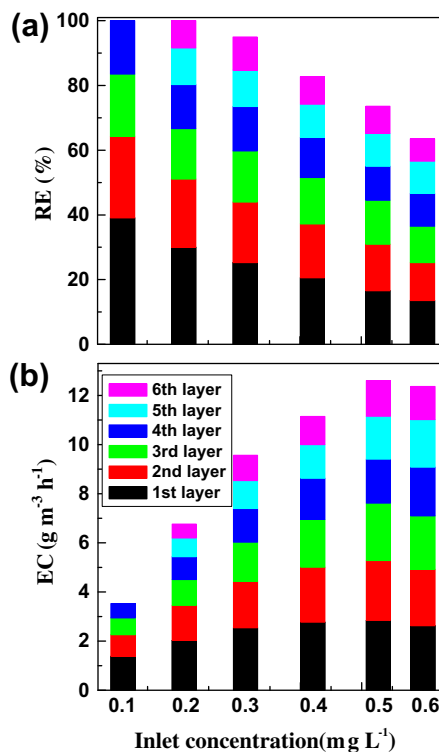


Fig. 3. (a) Removal efficiencies and (b) elimination capacities of aniline at different inlet concentrations at fixed EBRT of 110 s.

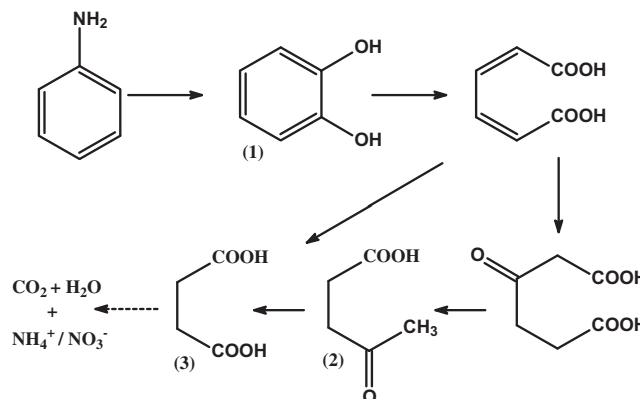


Fig. 4. Proposed biodegradation pathway for aniline by B350.

tion of them. Aoki et al. (1984) observed the accumulation of *cis,cis*-muconic acid and β -keto adipic acid in aniline metabolism by a *Frateria* sp. ANA-18. In addition, based on the predicted biodegradation pathway of aniline (Fig. SM-7) using University of Minnesota pathway prediction system (UM-PPS, <http://umbbd.msi.umn.edu/predict/>) (Ellis et al., 2008), the first step from aniline to catechol is aerobic likelihood value of neutral, which can confirm our proposed first step of biodegradation in the experiments. However, unlike our proposed route, two routes were predicted by UM-PPS for further degradation of catechol with aerobic likelihood. One route is the same as described above by our proposed mechanism that catechol was converted to *cis,cis*-muconic acid and then directly to succinic acid, the other is that catechol was directly converted to levulinic acid and then to 2-oxopropanoic acid (not detectable in this work). The difference is probably due to the fact that various microbial species were employed in this work with B350 mixture and led to different aniline metabolic pathways.

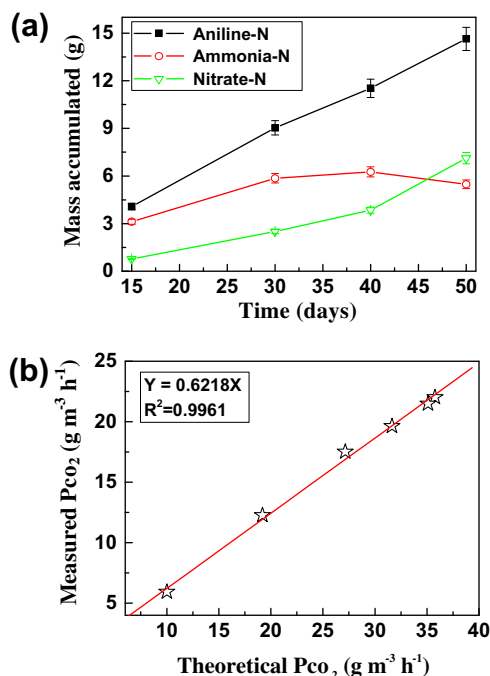


Fig. 5. (a) Analysis of nitrogen balance and (b) correlation between measured P_{CO_2} and theoretical P_{CO_2} .

To further validate final metabolites and mineralization degree of aniline, the production of final mineralization, such as NH_4^+ , NO_3^- and CO_2 were measured during biotrickling filtration process without considering biomass generated. The balance from the accumulation of NH_4^+ , NO_3^- in re-circulating liquid and CO_2 in gaseous phase were conducted quantitatively (Fig. 5). As shown in Fig. 5a, the accumulated amount of ammonia-N increased from 3.1 to 5.9 g and nitrate-N from 0.8 to 2.5 g from day 15 to 30 as the feed aniline-N increased from 4.1 to 9.0 g, indicating that ammonia-N was the main final metabolite of aniline-N during this period. As operational time increasing from day 40 to 50, the amount of ammonia-N slightly increases at first to 6.3 g and then decrease to 5.5 g; while nitrate-N firstly slightly to 3.9 g and then swiftly increased to 7.1 g as aniline-N further increased to 11.5 and 14.6 g, indicating that ammonia-N was further converted to nitrate-N by B350. It must note that the amount of nitrite-N in re-circulated liquid was notably low (less than 1%) during whole operational period, suggesting rapid transformation from nitrite to nitrate. Overall, 76%, 66%, 55% and 38% of aniline-N were converted into ammonia-N, and 20%, 28%, 34% and 49% into nitrate-N on day 15, 30, 40 and 50, respectively. All results indicated that aniline nitrogen was mainly converted to ammonia and nitrate, and total mineralization efficiency was up to 91%.

Meanwhile, the mineralization degree of aniline carbon was also investigated, and the theoretical and measured P_{CO_2} are plotted against ECs (Fig. SM-8). Linear relationships between P_{CO_2} and ECs were achieved and slope values of 2.84 and 1.77 P_{CO_2}/EC were obtained theoretically and experimentally, respectively. The discrepancy between theoretical and experimental values indicated that only 62% of aniline carbon was mineralized into final product CO_2 during the biofiltration process. This can be further evidenced by plotting measured (experimental) P_{CO_2} against theoretical P_{CO_2} as shown in Fig. 5b. That means that there are 38% aniline carbon remaining in other various forms, such as biomass and degradation intermediates. Thus, all above results indicated that the aniline nitrogen and carbon were mainly converted to NH_4^+/NO_3^- and CO_2 , respectively.

3.4. Microbial community analysis

To obtain crucial qualitative information about biofilm formation, the morphology of ceramic particles before and after cultivation was characterized by SEM. As shown in Fig. SM-9a, the surface of a clean ceramic particle is coarse and porous, which is favorable for microbial immobilization. After 24 d start-up, an abundance of rod and zoogloea bacteria were adhered onto ceramic particle surface (Fig. SM-9b–d).

To further probe the reason why the biotrickling filter seeded with B350 possessed excellent removal capability to aniline, the microbial community structure and diversity were analyzed as biotrickling filter achieved its stable state for aniline treatment. Fig. 6a shows gel purified PCR product of DNA extraction from biofilm on ceramic particles. The corresponding DNA fragment was approximate 240 bp and suitable for DGGE analysis. Based on DGGE profile (Fig. 6b), the microbial community structure was influenced extraordinarily by aniline vapor feed during the formation and maintenance process of biofilm. At least seven clear-cut bands (species) on DGGE profile were detected. The Shannon–Wiener diversity index H' was calculated as 1.03 based on the band intensity. The value of H' indicated the microbial community composition diversity was abundance in this study. In addition, the populations represented by bands B1–B7 were dominant in the biotrickling filter by analysis the P_i in the total population. The values of P_i (where P_i is the importance probability of the bands in a lane) were accounted for 12%, 12%, 10%, 12%, 13%, 12% and 10% of the total intensity of all selected bands for band B1–B7, respectively. Comparatively, the abundance and intensity of microorganisms represented by band B2, B4 and B5 were slightly higher than the others. Total intensity of bands B1–B7 was accounted for 81% of the total all bands intensity, indicating that microorganisms represented by bands B1–B7 were the predominant populations for aniline removal.

Therefore, the bands B1–B7 were excised from DGGE gel, and the nucleotide sequences of them were analyzed and compared with other strains based on 16S rRNA gene sequence alignment and phylogenetic tree analyses (Figs. SM-10 and 11). Results indicated that the predominant population represented by bands B2, B4, B5 and B6 are much closer to uncultured bacterium (AB185009), (HM481235), (FJ674811) and (HQ015453) with 95%, 91%, 98% and

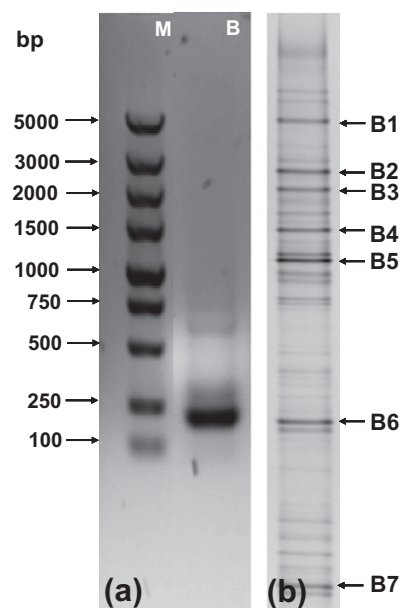


Fig. 6. (a) Agarose gel electrophoresis of PCR product from biofilm DNA. Line M: DNA Marker DS 5000, line B: biofilm DNA and (b) PCR-DGGE profile.

100% sequence similarity, respectively. In addition, the population represented by bands B1, B3 and B7 are much closer to uncultured bacterium (FJ416400), (AM259167) and (HM269827) with 96%, 87% and 98% sequence similarity, respectively. Comparatively, the bacterial communities in the biotrickling filter for aniline and TMA (Wan et al., 2011b) degradation system underwent quite different evolution trend with the continuous acclimation by different carbon source, although both of them originated from the same B350 group.

4. Conclusion

Biotrickling filter seeded with B350 showed high performance for degradation aniline. At fixed inlet concentration of 0.30 g m^{-3} , no aniline can be detected at EBRT 166 s, and the maximum EC of $12.6 \text{ g m}^{-3} \text{ h}^{-1}$ were achieved at EBRT 55 s. The biodegradation pathway of aniline by B350 could be proposed as: aniline was converted to catechol, and then was further to other small molecular compounds, and finally to ammonia/nitrate and CO_2 . Microbial community analysis demonstrated that seven populations represented by bands B1–B7 were the predominant species, which are mainly attributed to aniline mineralization at stable state in biotrickling filter.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.12.045.

References

- An, T.C., Wan, S.G., Li, G.Y., Sun, L., Guo, B., 2010. Comparison of the removal of ethanethiol in twin-biotrickling filters inoculated with strain RG-1 and B350 mixed microorganisms. *J. Hazard. Mater.* 183, 372–380.
- Aoki, K., Shinke, R., Nishira, H., 1983. Metabolism of aniline by *Rhodococcus erythropolis* AN-13. *Agric. Biol. Chem.* 47, 1611–1616.
- Aoki, K., Ohtsuka, K., Shinke, R., Nishira, H., 1984. Rapid biodegradation of aniline by *Frateuria* species ANA-18 and its aniline metabolism. *Agr. Biol. Chem.* 48, 865–872.
- Chan, W.C., Lin, Z.Y., 2006. A process to prepare a synthetic filter material containing nutrients for biofiltration. *Bioresour. Technol.* 97, 1927–1933.
- Chung, Y.C., 2007. Evaluation of gas removal and bacterial community diversity in a biofilter developed to treat composting exhaust gases. *J. Hazard. Mater.* 144, 377–385.
- Cox, H.H.J., Deshusses, M.A., 1998. Biological waste air treatment in biotrickling filters. *Curr. Opin. Biotechnol.* 9, 256–262.
- Deshusses, M.A., 1997. Biological waste air treatment in biofilters. *Curr. Opin. Biotechnol.* 8, 335–339.

- Ding, Y., Shi, J.Y., Wu, W.X., Yin, J., Chen, Y.X., 2007. Trimethylamine (TMA) biofiltration and transformation in biofilters. *J. Hazard. Mater.* 143, 341–348.
- Ellis, L., Gao, J., Fenner, K., Wackett, L.P., 2008. The University of Minnesota pathway prediction system: predicting metabolic logic. *Nucleic Acids Res.* 36, W427.
- Emtiaz, G., Satarii, M., Mazaherion, F., 2001. The utilization of aniline, chlorinated aniline, and aniline blue as the only source of nitrogen by fungi in water. *Water Res.* 35, 1219–1224.
- Friedrich, U., Prior, K., Altendorf, K., Lipski, A., 2002. High bacterial diversity of a waste gas-degrading community in an industrial biofilter as shown by a 16S rDNA clone library. *Environ. Microbiol.* 4, 721–734.
- Gostelow, P., Parsons, S.A., Stuetz, R.M., 2001. Odour measurements for sewage treatment works. *Water Res.* 35, 579–597.
- He, Z., Zhou, L.C., Li, G.Y., Zeng, X.Y., An, T.C., Sheng, G.Y., Fu, J.M., Bai, Z.P., 2009. Comparative study of the eliminating of waste gas containing toluene in twin biotrickling filters packed with molecular sieve and polyurethane foam. *J. Hazard. Mater.* 167, 275–281.
- Ho, K.L., Chung, Y.C., Lin, Y.H., Tseng, C.P., 2008. Biofiltration of trimethylamine, dimethylamine, and methylamine by immobilized *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1. *Chemosphere* 72, 250–256.
- Jiang, X., Yan, R., Tay, J.H., 2009. Simultaneous autotrophic biodegradation of H_2S and NH_3 in a biotrickling filter. *Chemosphere* 75, 1350–1355.
- Jorio, H., Bibeau, L., Viel, G., Heitz, M., 2000. Effects of gas flow rate and inlet concentration on xylene vapors biofiltration performance. *Chem. Eng. J.* 76, 209–221.
- Konstantinov, S.R., Zhu, W.-Y., Williams, B.A., Tamminga, S., de Vos, W.M., Akkermans, A.D.L., 2003. Effect of fermentable carbohydrates on piglet faecal bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA. *FEMS Microbiol. Ecol.* 43, 225–235.
- Liu, Z., Yang, H., Huang, Z., Zhou, P., Liu, S.J., 2002. Degradation of aniline by newly isolated, extremely aniline-tolerant *Delftia* sp. AN3. *Appl. Microbiol. Biotechnol.* 58, 679–682.
- Luo, J., Lindsey, S., 2006. The use of pine bark and natural zeolite as biofilter media to remove animal rendering process odours. *Bioresour. Technol.* 97, 1461–1469.
- Lyons, C.D., Katz, S., Bartha, R., 1984. Mechanisms and pathways of aniline elimination from aquatic environments. *Appl. Environ. Microbiol.* 48, 491–496.
- Mudliar, S., Giri, B., Padoley, K., Satpute, D., Dixit, R., Bhatt, P., Pandey, R., Juwarkar, A., Vaidya, A., 2010. Bioreactors for treatment of VOCs and odours – a review. *J. Environ. Manage.* 91, 1039–1054.
- Patnaik, P., 2007. *A Comprehensive Guide to the Hazardous Properties of Chemical Substances*. Wiley-Interscience, New York.
- Sironi, S., Capelli, L., Céntola, P., Del Rosso, R., Grande, M.I., 2007. Odour emission factors for assessment and prediction of Italian rendering plants odour impact. *Chem. Eng. J.* 131, 225–231.
- Smet, E., Lens, P., Van Langenhove, H., 1998. Treatment of waste gases contaminated with odorous sulfur compounds. *Crit. Rev. Environ. Sci. Technol.* 28, 89–117.
- Tsang, Y.F., Chua, H., Sin, S.N., Chan, S.Y., 2008. Treatment of odorous volatile fatty acids using a biotrickling filter. *Bioresour. Technol.* 99, 589–595.
- Wan, S.G., Li, G.Y., An, T.C., Guo, B., 2011a. Co-treatment of single, binary and ternary mixture gas of ethanethiol, dimethyl disulfide and thioanisole in a biotrickling filter seeded with *Lysinibacillus sphaericus* RG-1. *J. Hazard. Mater.* 186, 1050–1057.
- Wan, S.G., Li, G.Y., Zu, L., An, T.C., 2011b. Purification of waste gas containing high concentration trimethylamine in biotrickling filter inoculated with B350 mixed microorganisms. *Bioresour. Technol.* 102, 6757–6760.
- Xiao, C., Ning, J., Yan, H., Sun, X., Hu, J., 2009. Biodegradation of aniline by a newly isolated *Delftia* sp. XYJ6. *Chin. J. Chem. Eng.* 17, 500–505.
- Yoon, S.H., Chai, X.S., Zhu, J.Y., Li, J., Malcolm, E.W., 2001. In-digester reduction of organic sulfur compounds in kraft pulping. *Adv. Environ. Res.* 5, 91–98.
- Zhang, T., Zhang, J., Liu, S., Liu, Z., 2008. A novel and complete gene cluster involved in the degradation of aniline by *Delftia* sp. AN3. *J. Environ. Sci.* 20, 717–724.
- Zhao, X., Wang, Y., Ye, Z., Borthwick, A.G.L., Ni, J., 2006. Oil field wastewater treatment in biological aerated filter by immobilized microorganisms. *Process. Biochem.* 41, 1475–1483.
- Zhao, G., Zhou, L., Li, Y., Liu, X., Ren, X., 2009. Enhancement of phenol degradation using immobilized microorganisms and organic modified montmorillonite in a two-phase partitioning bioreactor. *J. Hazard. Mater.* 169, 402–410.
- Zhu, J., 2000. A review of microbiology in swine manure odor control. *Agric. Ecosyst. Environ.* 78, 93–106.