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# Ethylene removal evaluation and bacterial community analysis of vermicompost as biofilter material

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ABSTRACT

Biofiltration of ethylene provides an environmentally friendly and economically beneficial option relative to physical/chemical removal, where selection of appropriate bed material is crucial. Here the vermicompost with indigenous microorganisms as bed material was evaluated for ethylene removal through batch test and biofilter experiment. Temporal and spatial dynamics of bacterial community in the vermicompost-biofilter under different ethylene loads were characterized by culture and denaturing gradient gel electrophoresis (DGGE) methods. The results showed that ethylene was effectively degraded by the vermicompost under conditions of 25–50% moisture content and 25–35 °C temperature. The vermicompost-biofilter achieved nearly 100% ethylene removal up to an inlet load of 11 mg $m^{-3}$ $h^{-1}$. Local nitrogen lack of the vermicompost in the biofilter was observed over operation time, but the change of pH was slight. DGGE analysis demonstrated that the bacterial abundance and community structure of vermicompost-biofilter varied with the height of biofilter under different ethylene loads. Pseudomonads and Actinobacteria were predominant in the biofilter throughout the whole experiment.



## 1. Introduction

Volatile organic compounds (VOCs) are emitted into the atmosphere in large quantities from chemical and petrochemical industries. Ethylene ($C_2H_4$) is included in this category and regarded as a hazardous gaseous pollution substance by UK environment agency [1]. Ethylene frequently generates from petrochemical plants and products, because of its usage as a raw material for the production of polythene, other plastics and synthetic rubbers [2]. Airborne ethylene reacts with nitrogen oxides to form ground-level ozone harmful to human health and damaging to materials such as rubber under high concentrations. On the other hand, as a well-known plant hormone, ethylene is also produced by plant life. Ethylene accumulation easily occurs in confined areas such as greenhouses and plant storage containers, thus affecting growth, development, ripening, and aging of many plants [3]. As such, ethylene adversely impacts commercial agriculture and marketing, for example, accelerating softening process of fruits and shortening shelf life of cut flowers [4,5]. Therefore, it is essential to take removal of such pollutant into consideration when dealing with gaseous waste in industry and maintaining plant normal growth and postharvest quality in commercial agriculture.

Biofiltration of ethylene provides an environmentally friendly and economically beneficial option compared to physical/chemical methods such as catalytic oxidation and adsorption. The bed material plays a crucial role in the performance and steady operation of biofilter; therefore, it is desirable to develop novel low-cost and high-efficiency filter media [6]. Furthermore, environmental factors such as temperature and water content, and microbial community of biofilter need to be monitored, as they also have effects on the efficacy of biofilter [8]. The vermicompost, as a new kind of bed material, has great potential to remove waste gas because of its superiority in bulk density, porosity, diverse indigenous microorganisms as well as rich nutrients for microbial growth [7]. Several studies have been conducted about the use of vermicompost in elimination of waste gases such as methane and odor from human feces [8–10]. However, there is no information available in literature on vermicompost as bed material to remove ethylene.

In this work, ethylene removal by the vermicompost as a filter material for a biofilter was evaluated, and the effects of temperature and moisture of the vermicompost on ethylene removal were investigated. Analysis of bacterial community dynamics in the biofilter was performed by culture and denaturing gradient gel electrophoresis (DGGE) methods. The dominant microorganisms in the vermicompost were also identified by sequencing. Our data show promise for creating strategies and developing biotechnology for ethylene emission of petrochemical industries and ethylene control of greenhouses and plant storage containers.

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**Table 1**
Physical and chemical characteristics of the vermicompost.

| | |
|---|---|
| Physical characteristics[a] | |
| pH | 7.96 |
| Density (kg m$^{-3}$) | 75.3 |
| Bulk density (g cm$^{-3}$) | 0.382 |
| Specific area (m$^2$ g$^{-1}$) | 3.07 |
| Porosity (%) | 81.3 |
| Water content (%) | 68.5 |
| Chemical characteristics[b] | |
| Organic substance (%) | 18.38 |
| Ash (%) | 24.95 |
| Total carbon (%) | 22.73 |
| C/N ratio | 17.6 |
| $NH_4$-N (mg kg$^{-1}$) | 698.5 |
| $NO_3$-N (mg kg$^{-1}$) | 1191.5 |
| P (mg kg$^{-1}$) | 435.2 |
| K (g kg$^{-1}$) | 18.41 |

[a] Value on a wet-weight basis.
[b] Value on a dry-weight basis.

## 2. Materials and methods

### 2.1. Vermicompost

Vermicompost, the physical and chemical characteristics of which are summarized in Table 1, was prepared from plant waste in our laboratory [11,12]. The vermicompost was air-dried at room temperature (RT) for 3 days until its moisture content dropped below 30%. A granulometric analysis was carried out to determine the diameter of the vermicompost particles (2–4 mm) using standard sieves.

### 2.2. Batch experiments

#### 2.2.1. Ethylene removal evaluation

Batch experiments were conducted to study ethylene removal using the vermicompost in a 1 L glass bottle with a butyl-rubber stopper. After adjusting moisture content to 30%, weighted portions of the vermicompost were respectively put into 3 sealed glass bottles (each containing 300 g, about 0.37 L). The ethylene in a gas cylinder (Haipu, Beijing, China) was sampled using a syringe and injected into each bottle to a final concentration of 0.5% (v/v, 5000 ppm). All glass bottles were incubated at 25 °C without shaking, and the gas in the headspace of bottles was regularly sampled using a gas-tight syringe to measure ethylene with a gas chromatography (GC) 7890 (Tianmei, Shanghai, China) according to previous methods [13]. When the concentration of ethylene dropped below 100 ppm, all bottles were opened in the laboratory for 1 h to replace the gases inside the bottle with air, and then were resealed and replenished ethylene to the concentration of ca. 0.5%. The bottles were reincubated at 25 °C, and the ethylene was remeasured periodically. The performance of replenishment of ethylene was repeated in the same way for 10 times. In addition, in order to investigate the ethylene adsorbed on the vermicompost, some of the vermicompost materials were sterilized by autoclaving, and placed into sealed glass with a final concentration of 0.5% ethylene, and then ethylene was measured.

#### 2.2.2. Effects of temperature and moisture on ethylene removal

The effects of temperature and moisture of vermicompost on ethylene removal were investigated as follows. Ten glass bottles with each containing 300 g of vermicompost (moisture content of 30%) were prepared. As described above, after ethylene was injected to the final concentration of 0.5%, the bottles were incubated at 25 °C without shaking. When the ethylene concentration in each bottle dropped below 100 ppm, ethylene was replenished after replacing the inner gases of bottle with air. Replenishing ethylene 10 times over, all of the vermicompost were taken out and manually mixed well. The mixed sample was air-dried at RT for 3 days until its moisture content dropped below 30%. After adjusting moisture content to 30%, each weighed portion equal to 30 g was placed into a 100 ml serum bottle with butyl-rubber stopper. Ethylene removal at several temperatures (5, 10, 20, 25, 30, 35, and 40 °C) was evaluated with the same methods as described above. To investigate the effect of moisture, the vermicompost samples were placed into 9 serum bottles of 100 ml with butyl-rubber stoppers (each containing 30 g), and the moisture contents of the samples were respectively adjusted to 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55% and 60% by air drying at RT or addition of distilled water. Ethylene removal of each bottle was evaluated at 25 °C by the same methods as described above. All these experiments were carried out in triplicate.

**Table 2**
Experimental scheme for continuous ethylene degradation experiments.

| Phases of biofilter operation | Operating time (day) | Inlet concentrations (mg m$^{-3}$) | Empty bed residence time (min) |
|---|---|---|---|
| Phase I | 0–20 | 24.6–57.3 | 2.26 |
| Phase II | 21–50 | 45.8–91.6 | 1.31 |
| Phase III | 51–80 | 81.2–137.4 | 0.50 |
| Phase IV | 81–110 | 84.6–136.9 | 1.31 |
| Phase V | 111–135 | 25.7–55.9 | 1.31 |

### 2.3. Biofilter experiment

#### 2.3.1. Biofilter set-up and operation

The three-layer biofilter used in this study is the same as that described in our previous study [13] (see Supporting Information Fig. S1). The vermicompost was packed into the biofilter after pretreatment described in Section 2.2.2. The synthetic waste gas stream was prepared by mixing compressed air and ethylene via two mass flow controllers. Before reaching the inlet, the waste gas was humidified by being bubbled through a flask containing distilled water. The moisture content of the bed was maintained by continuously pre-humidifying the inletting waste gas and periodically sprinkling water from the top of the vermicompost-biofilter (VCBF). The biofilter was operated in up-flow mode. Experiments were carried out by varying flow rates of the ethylene and air independently to get different initial concentrations and empty bed retention time (EBRT) in the VCBF. Biofilter operation was divided into five consecutive phases as summarized in Table 2.

#### 2.3.2. Gas and bed material analysis

Gaseous samples were periodically collected at the inlet and outlet of each layer of the VCBF by 0.5 L Tedlar gas sampling bags (Cole-Parmer, USA), and then directly injected into the injector port of GC 7890 using a 1 ml gas syringe (Agilent, USA) with a push-button valve to analyze ethylene. Three quantitative parameters including the level of ethylene inlet load (IL), the corresponding removal efficiency (RE) and elimination capacity (EC) have been used to describe performance of the VCBF [13]. The vermicompost samples were obtained from each layer of the VCBF in different phases. The pH was measured with a pH meter (METTLER TOLEDO) after mixing 0.5 g of vermicompost with 5 ml of distilled water within 20 min [14]. To analyze $NH_4^+$-N and $NO_3^-$-N contents of the vermicompost, an extract was prepared by mixing 5 g of sample with 25 ml 1 M KCl [14]. After shaking for 1 h, the vermicompost was separated from the liquid phase by filter membrane. The concentrations of $NH_4^+$-N and $NO_3^-$-N in liquid were determined according to standard methods [15].

### 2.4. Bacteria number determination

0.5 g of vermicompost mixed with 5 ml saline (0.9%, w/v NaCl) was vortexed for 3 min, and the bacterial numbers were enumerated by serial dilute plate counting method [16] using tryptic soy agar (TSA). The containers holding culture plates were placed in incubator for 4 days at 30 °C. A colony count was recorded from each replicate.

### 2.5. Analysis of bacterial community by DGGE

DGGE technique was employed to monitor the change of the bacterial community in the VCBF. The structure and abundance of the bacterial community were analyzed by a DGGE apparatus (Bio-Rad, Hercules, CA) and Bio-Rad's image program (Quantity One 4.5.2). The DGGE gels consisted of 8% acrylamide gel with a 35–60% denaturant gradient. DNA extraction and PCR amplification were carried out as described in our previous study [13]. The cluster analysis and dendrogram generation were performed by SPSS 13.0 (SPSS Inc., USA) with the un-weighted pair group method (UPGMA) according to the presence or absence of individual bands in all lanes [17]. PCR primers for F968GC and R1378 were used to amplify the V6 variable region of 16S rRNA between positions 968 and 1378 resulting in a 433-bp product for total bacteria [18]. The different bands (=strains of bacteria) were identified by excising the bands from DGGE gel, eluting, re-amplifying and sequencing. Sequences were submitted for comparison with the GenBank databases using BLAST algorithm.

## 3. Results and discussion

### 3.1. Ethylene removal by the vermicompost

To get information that whether the vermicompost is capable of removing ethylene, we performed batch test firstly. As shown in Fig. 1, ethylene was removed effectively by the vermicompost in the glass bottles. Note that a lag period of 1 day occurred before ethylene degradation in the first injection, but in subsequent injections, the lag period of ethylene removal disappeared. In contrast, the vermicompost samples that were sterilized were unable to result in ethylene removal (data not shown). These results indicate that the vermicompost does have the capacity of ethylene removal,

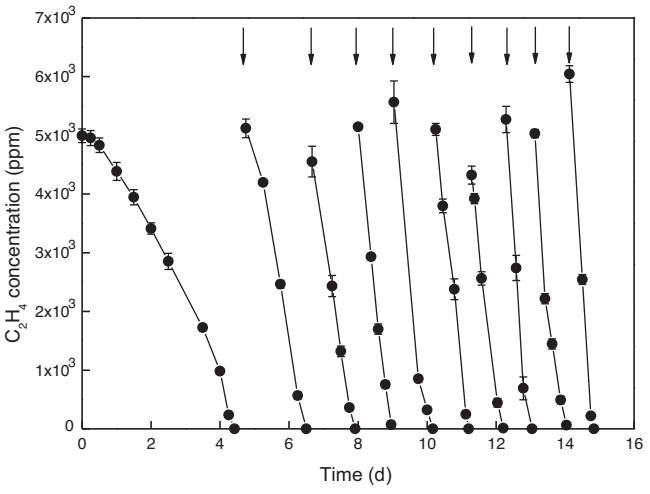


**Fig. 1.** Change of $C_2H_4$ concentrations with incubation time in bottles under condition of 30% moisture content and 25 °C temperature. Arrows indicate the re-injection time points of ethylene.

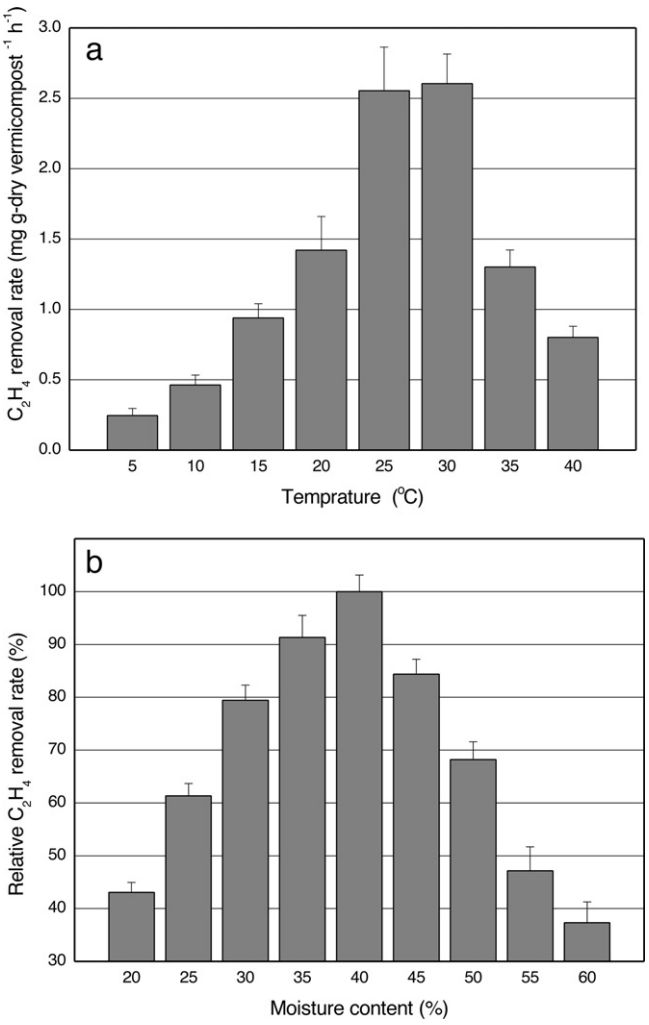


**Fig. 2.** Effects of moisture content (a) and temperature (b) on $C_2H_4$ removal rate by the vermicompost.

which is attributed to microbial activity of the vermicompost but not physicochemical property.

Next, we investigated the effect of temperature on ethylene removal rate of the vermicompost. Fig. 2a shows that the vermicompost exhibited the highest ethylene removal rate of 2.6 μg g-dry weight$^{-1}$ h$^{-1}$ at 30 °C in batch study under 5–40 °C. The ethylene removal rates at 20, 25 and 35 °C were all higher than 50% of the maximum. However, they rapidly declined to 0.46 and 0.8 μg g-dry weight$^{-1}$ h$^{-1}$ at 10 °C and 40 °C, respectively. These data suggest that, like peat-soil [19], the ethylene removal of the vermicompost was carried out by mesophilic bacteria, whose ethylene-degrading activity was effective over a range of temperatures from 20 to 35 °C. This finding is consistent with the report that no appreciable uptake of ethylene by microorganisms in soil samples at 45 °C under batch conditions was observed relative to that at 37 °C [20]. Furthermore, an alternative application of ethylene removal is frequently required in horticultural storage facilities, where temperatures ≤10 °C are often obligatory. Although the ethylene removal rate of the vermicompost at 10 °C was relatively low in our present work, it is still higher than that of peat-soil (0.28 μg g-dry weight$^{-1}$ h$^{-1}$) [19].

Except for temperature, water content is another important factor affecting the removal capacity of bed materials. Thus, we analyzed the effect of moisture of the vermicompost on ethylene removal. As illustrated in Fig. 2b, the removal rate of ethylene for

each condition of moisture content was expressed as a value relative to the maximum obtaining at 40% moisture content. The values of ethylene removal rate at moisture contents of 25%, 30%, 35%, 45% and 50% were 60–90% of the maximum, indicating that the vermicompost displays its ethylene removal ability in a relatively broad range of moisture contents (25–50%). However, when the vermicompost was either too wet (>55% of moisture content) or too dry (<25% of moisture content), the removal rate was decreased to below 50% of the maximum. A prior study demonstrates that drying of the biobed to <40–45% achieved a twofold improvement in the ethylene removal capacity in granular activated carbon [21]. Obviously, the optimal moisture contents are different for different bed materials, undoubtedly depending on their moisture holding capacity.

### 3.2. Ethylene removal by the VCBF

In order to further confirm the capacity of ethylene removal of the vermicompost, a VCBF study based on different inlet concentrations and EBRT was conducted under optimal temperature (25–30 °C) and moisture content (35–45%) conditions. The performance of the VCBF was firstly evaluated by measuring RE of ethylene. As shown in Fig. 3a, on days 0–20 (Phase I), ethylene RE greater than 99% was achieved immediately after start-up of the VCBF under inlet concentrations of 22.9–57.3 mg $m^{-3}$ with an EBRT of 2.26 min. The relatively short acclimation period in the VCBF was presumably due to the great presence of indigenous ethylene-degrading microorganisms in the vermicompost after re-injecting ethylene pretreatment. The steady-state REs were maintained between 90% and 100% up to an inletting concentration of 91.6 mg $m^{-3}$ even when the EBRT was decreased to 1.13 min (Phase II). A further increase in inletting concentration to 137.4 mg $m^{-3}$ with a further decrease in EBRT to 0.50 min resulted in lowering of the RE to around 50% (Phase III). In the next 30 days (Phase IV), although the inletting ethylene concentration was maintained at the level of Phase III, the RE sharply increased because of prolonged EBRT (1.13 min). During Phase V, ethylene RE recovered up to near 100% while only inletting concentrations were returned to the values on days 0–20. These results support the view of prior study that decrease of EBRT usually leads to lowering in RE due to insufficient contact time between pollutant and microbial population [22]. In addition, RE of biofilter is negatively correlated to IL of

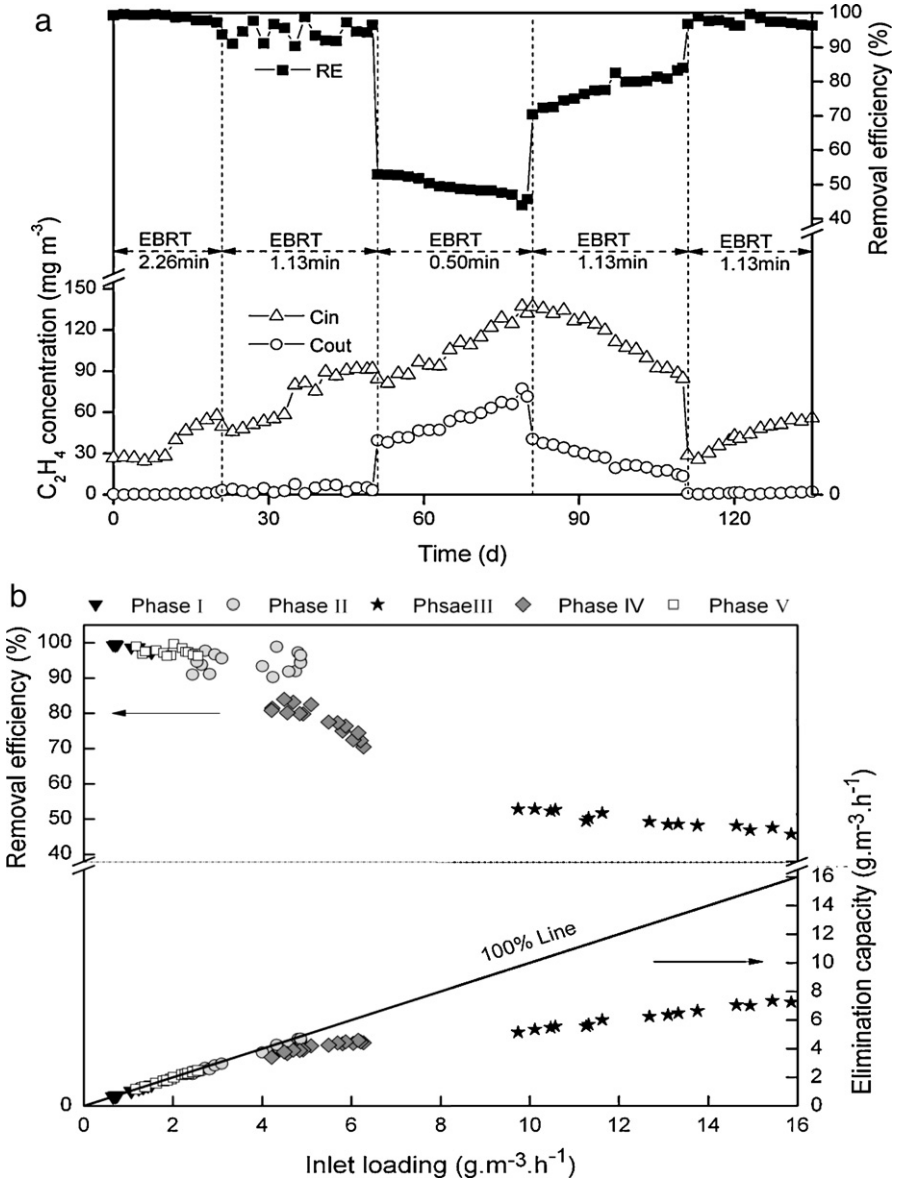


**Fig. 3.** (a) Variation in RE of the VCBF with changes of gas flow rate and inlet $C_2H_4$ concentration and (b) influence of inlet load on RE and EC of the VCBF.

pollutant [23]. Fig. 3b suggests that RE decreased with increasing of ethylene IL throughout the whole experimental period. Substrate or metabolic product inhibition for microbial activity at high IL of ethylene may be responsible for the observed decrease of RE [13].

Biofiltration performance was also evaluated in terms of EC reflecting the capacity to remove the pollutant. Ethylene EC of the VCBF was plotted as a function of the IL of ethylene in Fig. 3b. A near 100% removal among three variables (corresponding in Phase I, II and V) was observed up to an IL of 5 g $m^{-3}$ $h^{-1}$. With further increase in the IL during Phases III and IV, EC of the VCBF increased at a gradual slower rate with nearly constant at IL beyond 13 g $m^{-3}$ $h^{-1}$. The maximal EC of 7.3 g $m^{-3}$ $h^{-1}$ of the VCBF was achieved at an IL of 15.4 g $m^{-3}$ $h^{-1}$. Compared to ethylene EC of biofilters packing with other bed materials, the EC maximum observed here was higher. For instance, Kim et al. reported the maximum EC of ethylene removal by a biofilter packed with activated carbon was 1.42 g $m^{-3}$ $h^{-1}$ at a loading rate of 1.42 g $m^{-3}$ $h^{-1}$ [24]. Elsgaard obtained the maximum EC less than 1 g $m^{-3}$ $h^{-1}$ at inlet ethylene concentration of approximately 140 mg $m^{-3}$ by employing a peat-soil biofilter [25]. Furthermore, the maximum EC values of bioreactors packed with compost [26] and perlite [27] were all lower than that observed in the present study. The combination of high RE and good EC in the VCBF substantiates that the vermicompost appears to be a promising bed material for ethylene removal.

### 3.3. Removal profiles along column of the VCBF

The pollutant-removal profiles along the biofilter column and their changes with operational time can be used as an indicator of biofilter performance [23]. Concentration profiles of ethylene in the VCBF during different phases were measured along the column (Fig. 4a). The VCBF achieved active ethylene degradation during the initial start-up (Phase I) and the first elevation of ethylene IL (Phase II) periods, with greater than 70% of the inlet ethylene being degraded in the bottom layer. More ethylene was removed in the bottom layer of the biofilter due to the more active microbial growth in this section of the filter bed [13,24]. With further increase of inlet ethylene IL, the removal ethylene proportions of the upper two layers rapidly increased (Phases III and IV). Although inlet ethylene IL was reduced to the values near to those of the first two phases during Phase V, the performance of ethylene removal decreased in the bottom layer compared to that in Phases I and II. Nutrient limitation and pH change of the vermicompost are possible reasons for the performance loss of the bottom layer.

In addition to temperature and moisture, nutrient and pH were the two key factors in the performance of biofilter. Of all the nutrients, nitrogen makes up the largest fraction of microorganism and is essential for sustaining microbial growth and activity [17]. The forms of nitrogen readily utilized by microorganisms are available inorganic nitrogen sources such as $NH_4^+$ and $NO_3^-$. Thus, the concentrations of $NH_4^+$ and $NO_3^-$ are usually used as indicators of nitrogen available in packing media in different biofilters [27]. Changes in available nitrogen ($NH_4^+$-N and $NO_3^-$-N) of the VCBF during operation are presented in Fig. 4b. The average concentration of available nitrogen along the biofilter column was gradually declined from an original value of 1410 on day 0 to 213.5 mg $kg^{-1}$ on day 135. Moreover, the available nitrogen was lower at the bottom layer than those at the other two layers of the column on days 55 and 135. In particular, the available nitrogen concentration at the bottom layer was declined to only 78.5 mg $kg^{-1}$ on day 135. We believe that this low level of nitrogen availability resulted in lack of nutrient at the bottom layer of the VCBF. This is also the reason for the difference of the ethylene removal profiles along the column between first two phases and Phase V. In order to improve purification performance, the filter bed should be irrigated by means of a

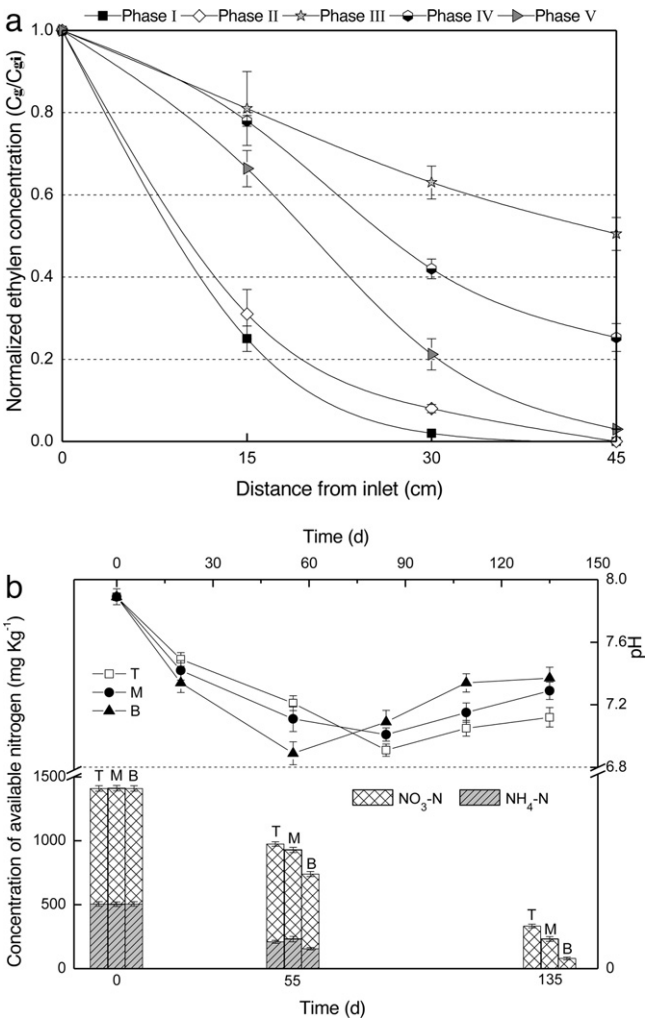


**Fig. 4.** (a) $C_2H_4$ concentration profiles along the column of the VCBF. (b) Changes in available nitrogen and pH along the column with time. B, M, T: samples in bottom, middle and top layer of the VCBF.

continuous recirculation of the nutrient solution. In pH test, by the end of operation the pH of the vermicompost was dropped from an initial value of 7.89 to 7.37 in the bottom layer, 7.29 in the middle layer and 7.12 in the top layer, respectively (Fig. 4b). The slight change in pH might be the result of assimilation of $NH_4^+$-N and $NO_3^-$-N. Consistent with this view, previous study conducted using organic bed material demonstrates that the slight change in pH was associated with microbial assimilation of $NH_4^+$-N and $NO_3^-$-N in the biofilter [27]. Taken together, our data indicate that the pH in the VCBF remains at a near-neutral range during the entire operational period, thereby providing further support for the use of the vermicompost in ethylene removal.

### 3.4. Bacterial counts of the VCBF

Except for physicochemical factors, microbial biomass in the bed materials plays a critical role in biofilter performance. The results of bacterial counts in the vermicompost of the VCBF during different phases are presented in Fig. 5. The initial cell numbers of the vermicompost were about $7.8 \times 10^7$ cell/g of dry-weight in each layer. After 10 days of exposure to low ethylene IL (Phase I), the bacterial number decreased slightly at all layers and tended to increase with the increase of IL in Phase II. In the first two phases, the cell numbers in the bottom layer were higher than that in the other two layers, probably due to that the inlet ethylene load was

**Table 3**
Nucleotide sequence similarity and intensity of sequenced DGGE bands.

| Bands[a] | Phylogeny | Closest relatives | Similarity | Relative abundance of DGGE bands | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Day 10 | Day 35 | Day 65 | Day 95 | Day 124 |
| 1 | γ-*Proteobacteria* | *Xanthomonas campestris* | 100% | 3% | 1% | 0% | 0% | 0% |
| 2 | γ-*Proteobacteria* | *Xanthomonas fuscans* | 98% | 1% | 2% | 0% | 0% | 0% |
| 3 | γ-*Proteobacteria* | *Pseudomonas poae* | 97% | 3% | 2% | 13% | 3% | 4% |
| 4 | γ-*Proteobacteria* | *Pseudomonas fluorescens* | 99% | 2% | 1% | 2% | 3% | 1% |
| 5 | γ-*Proteobacteria* | *Pseudomonas syringae* | 99% | 2% | 7% | 1% | 3% | 4% |
| 6 | γ-*Proteobacteria* | *Pseudomonas putida* | 100% | 3% | 10% | 2% | 4% | 9% |
| 7 | γ-*Proteobacteria* | *Pseudomonas aeruginosa* | 98% | 37% | 45% | 37% | 53% | 55% |
| 8 | *Actinobacteria* | *Mycobacterium neoaurum* | 100% | 6% | 6% | 9% | 10% | 7% |
| 9 | *Actinobacteria* | *Rhodococcus erythropolis* | 97% | 2% | 2% | 0% | 0% | 0% |
| 10 | *Acidobacteria* | Uncultured *Acidobacteria bacterium* | 98% | 31% | 17% | 32% | 20% | 19% |
| 11 | *Sphingobacteria* | *Rhodothermus*.sp | 96% | 2% | 3% | 2% | 3% | 2% |

[a] The bands are designated as shown in Fig. 6a.

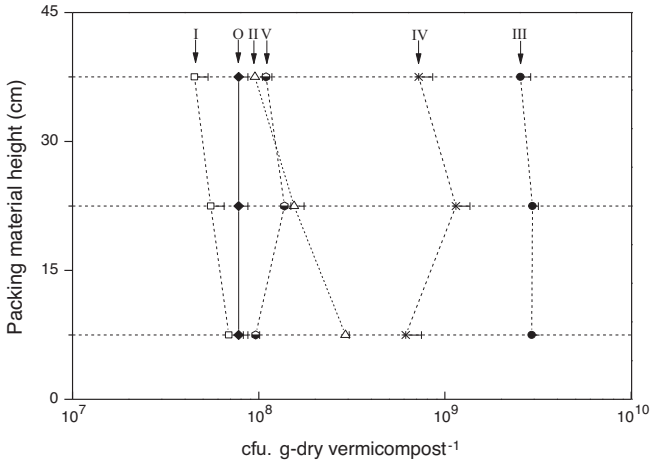


**Fig. 5.** Bacterial plate counts in the vermicompost before the column packing (O, day 0) and in the vermicomposts taken at different heights of the VCBF in the different phases (I, day 10; II, day 35; III, day 65; IV, day 95; V, day 124).

gradually decreased from the bottom to top. During Phase III, in parallel with a strong increase in IL, the microbial colonization of the vermicompost showed a sharp increment as demonstrated by the increased number of colonies (approximately $3 \times 10^9$ cell/g of dry-weight vermicompost) in all layers of the VCBF. In the last two phases, the bacterial counts in all layers decreased with IL decrease. These results show that the microbial biomass in the VCBF sharply changed with the change of ethylene IL. Similar microbial dynamics were reported in the benzene removal using compost as a filter medium [28]. In addition, the phenomenon that the microbial cells in the middle layer were more than those in the bottom layer in the last two phases also indicates that the nutrient at the bottom layer was insufficient during the late period.

### 3.5. Bacterial community in the VCBF

DGGE was used to determine the bacterial community for (1) different operating conditions over time for the VCBF and (2) different spatial portions of the VCBF at the early and last phases of the operation. Fig. 6a shows the DGGE fingerprints of amplified 16S rDNA gene fragments of the vermicompost samples from the VCBF during five operating phases and the original vermicompost without ethylene treatment. Some bands observed in the prevailing pattern in the original vermicompost disappeared completely in biofiltration process; however, some new dominant bands appeared. Cluster analysis reveals that microbial community structure in the VCBF showed low similarity with that of the original vermicompost at the coefficient of 48% (Fig. 6b). Only 5 out of the initial 15 bands in the original vermicompost remain till the end, which must represent the totally acclimated species. This suggests that microbial community in the vermicompost was greatly altered from the original condition to the environment enriched ethylene. A change was observed in the bacterial community during different operating periods of the biofilter. In general, the complexity of DGGE bands decreased over operating time. During Phases I and II (days 10 and 35), the microbial population was relatively unstable in the VCBF. Eight bands disappeared by 10th day, and 8 new bands appeared on the 10th day but did not remain after 35th day. It is expected that the change of conditions from the batch experiment to biofilter environment or intermediates formation may affect the bacterial community. After further acclimation to biofilter environment, the bacterial community structure presented stable over time despite ethylene IL and EBRT being varied (from Phase III to V). In ethylene biofiltration system based on natural zeolite with a specific inoculum, microbial community was highly affected by different ILs of pollutant and shifted throughout the experimental period [13].

The DGGE banding pattern of the samples along the column of the VCBF on days 10 and 124 is presented in Fig. 7. In early period (day 10), the microbial populations in the bottom layer were more diverse than those in the other two layers. Moreover, the highest intensities of the common bands (i.e., those identified at the same relative position in different lanes) among three layers were observed in the bottom layer. However, on day 124, the bacterial species and intensities of common bands in the bottom layer were less than those in the middle layer. A reasonable explanation for this observation was twofold: (1) A concentration gradient of ethylene along the column could form a sufficiently large selective pressure on influencing the bacterial community and abundance at each layer in early period. (2) In last period, the low nitrogen availability became a more important factor influencing the bacterial community and abundance at the bottom layer relative to the concentration gradient of ethylene. In any case the differences of concentration of introduced pollutant and nutrient level over the biofilter height could have been contributed to the fact that bacterial community and abundance of the biofilter differed significantly as a function of height along the biofilter, as has been observed previously [29,30].

### 3.6. Identification of strain, its potential function, and relative abundance

The major bands from DGGE fingerprints of the VCBF samples from different operating conditions were excised, reamplified, purified and sequenced. Eleven bands were individually identified as members of different eubacterial classes as shown in Table 3, and the closest relative was identified by comparison with the GenBank

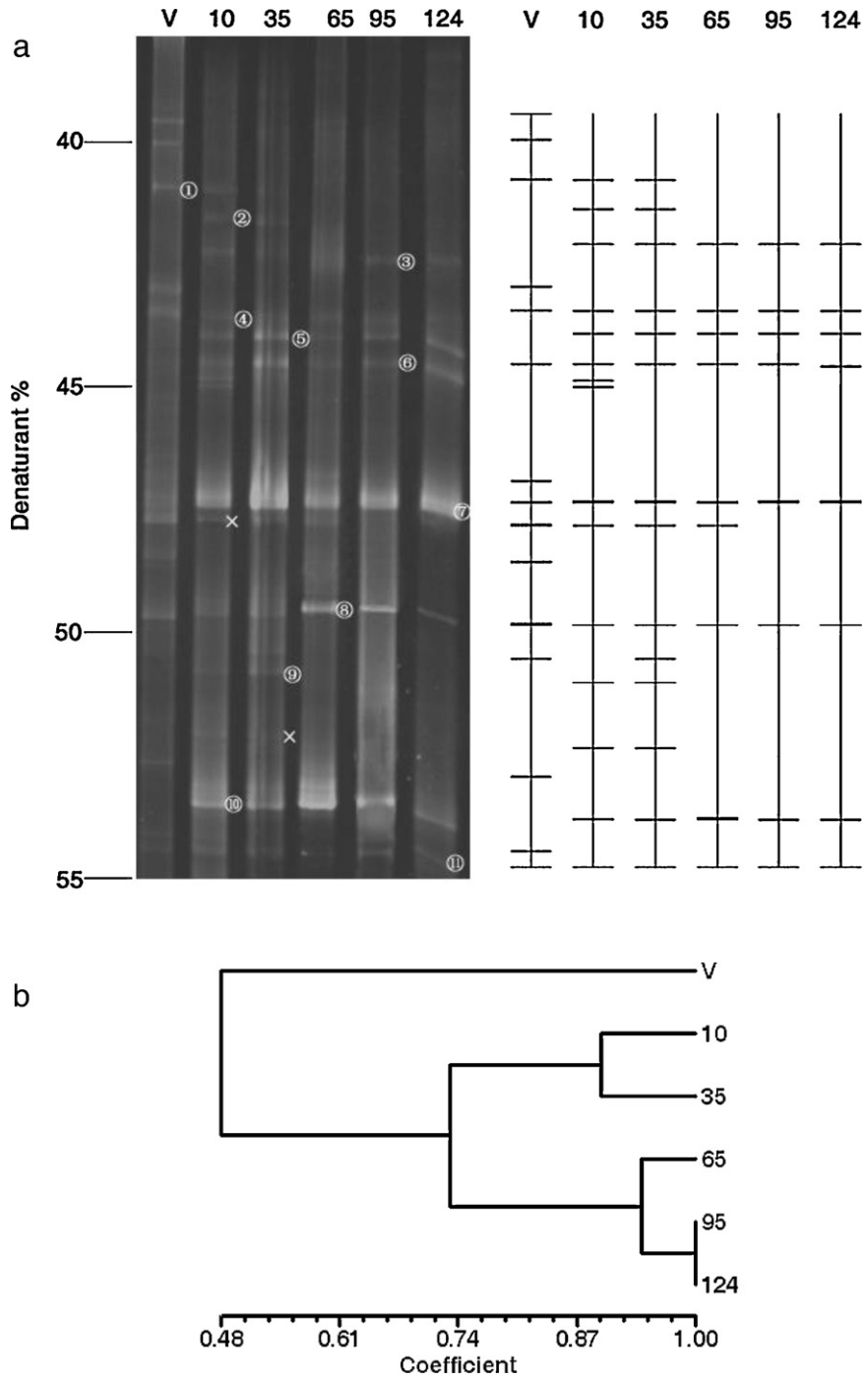


**Fig. 6.** (a) DGGE profiles of bacterial community in the vermicompost. V: original vermicompost; and the day when the sample was taken from the VCBF is denoted by the corresponding number at the top of other five lanes. Marked bands were excised, reamplified and purified for sequencing. Numbers in the circles correspond to the numbers used in phylogenetic analysis, while X corresponds to bands unable to be reamplified or purified. (b) UPGMA dendrograms revealing the similarity of DGGE profiles.

database. A relative quantitative evaluation of abundance of these 11 bacteria by PCR-DGGE is also presented in Table 3. Seven bands were grouped with the class γ-Proteobacteria, in which most bands (3–7) belonged to genus *Pseudomonas*, and the other two bands (1 and 2) showed homology to *Xanthomonas campestris* and *X. fuscans*. Three bands (8–10) were clustered within the class *Actinobacteria*, namely *Mycobacterium neoaurum*, *Rhodococcus erythropolis* and *Acidobacteria bacterium*, respectively. Only band 11 affiliated *Rhodothermus* sp., was a member of the class *Sphingobacteria*. These results indicate that Proteobacteria phylum was predominant in the VCBF.

Based on the presence of DGGE bands, five species of genus *Pseudomonas* were consistently presented from day 10 to 124 in biofilter experiment. Moreover, *Pseudomonas aeruginosa* was proportionally the most prevalent in all the operating phases. The result supports that *Pseudomonas* species were able to effectively degrade ethylene [24]. *X. campestris*, *X. fuscans* and *R. erythropolis* appeared only in Phases I and II, while IL of ethylene was relatively low. *X. campestris* and *R. erythropolis* have been reported to be prevailing in microbial composition of the vermicompost [31]. *R. erythropolis* and *Xanthomonas* sp. were found to grow well with ethylene as sole substrate [32]. An uncultured *A. bacterium* seemed typical for

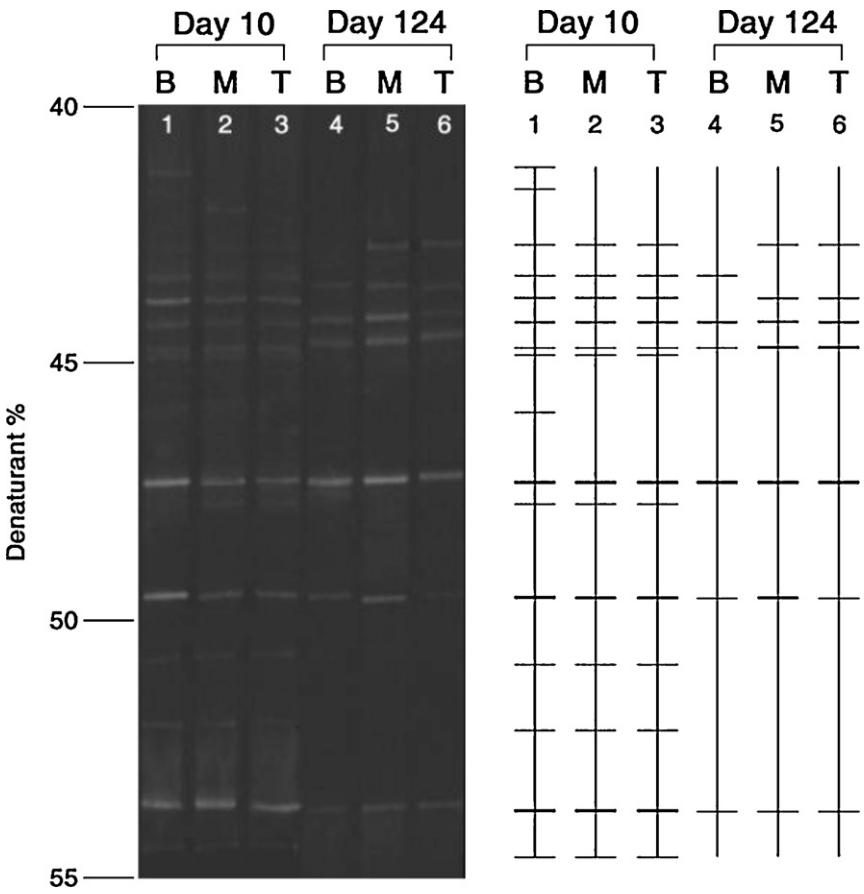


**Fig. 7.** DGGE profiles of bacterial community in the vermicompost samples taken at different column heights on days 10 (lanes 1–3) and 124 (lanes 4–6). B, M, T: samples in bottom, middle and top layer of the VCBF.

the vermicompost in the VCBF, which was accounted for 17–31% of the total bacteria community during the biofiltration period. A lot of strains of Acidobacteria are widely distributed in soils and other environments and difficultly cultivated [33], thereby in our system it is not sure if *A. bacterium* possesses the ability to utilize ethylene. *M. neoaurum* was also presented in the vermicompost throughout the biofiltration period, and stably accounted for 6–11% of the total bacteria quantity in the VCBF. As a member in genus *Mycobacterium* being able to metabolize ethylene [32], *M. neoaurum* could be considered to possess the ability to degrade ethylene. *Rhodothermus sp.* can product stable cellulases to degrade cellulose during aerobic compost process [34]. This mechanism may result in *Rhodothermus sp.* accounting for 2–3% of the total bacteria abundance in our system from day 10 to 124. Overall, a relatively high bacterial diversity is presented in the VCBF, contributing to the good performance in ethylene removal.

## 4. Conclusions

Our experimental data here demonstrate that the vermicompost is capable of successfully purifying air containing ethylene. Ethylene was effectively degraded by the vermicompost under a range of 25–50% moisture content and 25–35 °C temperature. Under optimal moisture content (35–45%) and temperature (25–30 °C), the VCBF achieved nearly 100% ethylene removal up to an IL of 11 mg $m^{-3}$ $h^{-1}$. The maximum EC of the VCBF was 16.6 mg g $m^{-3}$ $h^{-1}$ at an IL of 34.9 mg $m^{-3}$ $h^{-1}$. The pH in the VCBF remained at a near-neutral range during the entire operation period. However, the lack of nitrogen availability in the bottom layer after long time operation should be considered to be further improved by means of a continuous recirculation of the nutrient solution that would replenish N and other nutrients in the vermicompost. The sharp changes in microbial dynamics of the VCBF were found with change of ethylene IL. The results of the DGGE analyses provide basal information on the roles or functions of different bacterial species during the ethylene removal processes. The valuable strains identified in this study may be useful in the biofiltration of contaminated ethylene environments.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.05.064.

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