

RESEARCH ARTICLE

# Microbial Community in a Biofilter for Removal of Low Load Nitrobenzene Waste Gas

Jian Zhai<sup>1,2</sup>, Zhu Wang<sup>1</sup>, Peng Shi<sup>1</sup>, Chao Long<sup>1\*</sup>

**1** State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing, PR China, **2** Department of Applied Chemistry, Nanjing Polytechnic Institute, Nanjing, PR China

\* [clong@nju.edu.cn](mailto:clong@nju.edu.cn)



**OPEN ACCESS**

**Citation:** Zhai J, Wang Z, Shi P, Long C (2017) Microbial Community in a Biofilter for Removal of Low Load Nitrobenzene Waste Gas. PLoS ONE 12 (1): e0170417. doi:10.1371/journal.pone.0170417

**Editor:** Andrew C Singer, Natural Environment Research Council, UNITED KINGDOM

**Received:** August 24, 2016

**Accepted:** January 4, 2017

**Published:** January 23, 2017

**Copyright:** © 2017 Zhai et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** The authors confirm that all data underlying the findings are fully available without restriction. The high-throughput sequencing datasets have been deposited into the NCBI Short Reads Archive Database (accession number: SRP095067).

**Funding:** This study was financially supported by Natural Science Foundation of Nanjing Polytechnic Institute (Grant No. NHKY-2016-02, PI: JZ, URL: <http://menhu.njcc.edu.cn/>); Jiangsu Provincial Science and Technology Plan Project (No. BY2015060-03). The funder had no role in study

## Abstract

To improve biofilter performance, the microbial community of a biofilter must be clearly defined. In this study, the performance of a lab-scale polyurethane biofilter for treating waste gas with low loads of nitrobenzene (NB) ( $< 20 \text{ g m}^{-3} \text{ h}^{-1}$ ) was investigated when using different empty bed residence times (EBRT) (64, 55.4 and 34 s, respectively). In addition, the variations of the bacterial community in the biofilm on the longitudinal distribution of the biofilters were analysed by using Illumina MiSeq high-throughput sequencing. The results showed that NB waste gas was successfully degraded in the biofilter. High-throughput sequencing data suggested that the phylum *Actinobacteria* and genus *Rhodococcus* played important roles in the degradation of NB. The variations of the microbial community were attributed to the different intermediate degradation products of NB in each layer. The strains identified in this study were potential candidates for purifying waste gas effluents containing NB.

## Introduction

Nitrobenzene (NB) is a typical hydrophobic (Henry's law constant  $2.41 \times 10^{-6} \text{ MPa m}^3 \text{ mol}^{-1}$  and other salient properties on the [S1 Table](#)) and refractory biodegradable volatile organic compound. NB is emitted mainly from explosives, aniline, dyes, pesticides, pharmaceutical manufacturing processes and waste water treatment plants. Because NB is relatively toxic and persistent in the environment, NB emissions are facing increasingly stringent environmental regulations. Biofilters were originally developed for odor pollution control due to their high efficiency, low energy consumption, low operating costs, and environmental-friendliness [1] and have recently been proven to be a promising technology for the treatment of volatile organic compounds (VOCs) at moderately high flow rates and low concentrations ( $< 1000 \text{ ppm}$ ) [2–3].

Biofilters have been successfully applied to treat hydrophilic VOCs, such as styrene [4], ethanol [5], methanol [6], xylene isomers [7], hexane [8], toluene [9], phenol [10], methylamine [11], triethylamine [12], dimethylsulfide [13], and dichloromethane [14]. However, the low mass transfer of hydrophobic VOCs from the gas phase to the biofilm phase, which limits the supply of substrates to the microorganisms, results in low biodegradation rates of these

design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

compounds. Several researchers have attempted to enhance the removal of hydrophobic VOCs in biofilters by adding surfactants, applying fungal biocatalysts, and by using biofiltration with pretreatment, innovative bioreactors, and hydrophilic compounds [15].

However, to the best of our knowledge, only Oh and Bartha reported an NB-elimination capacity (EC) of  $50 \text{ g m}^{-3} \text{ packing h}^{-1}$  in a trickling air biofilter [16]. In addition, few studies have investigated the relation between biofilter performance and the microbial community in NB-degrading biofilters. Microbial communities are sensitive to changes in environmental conditions; therefore, understanding the microbial ecology of biofilms in gas phase biofilters helps to optimize the design and operation of such biological treatment systems [17].

Culture-dependent conventional isolation and identification methods only provide information on relatively few microbial communities. It remains unclear whether the isolates were of any quantitative or functional significance *in situ* [18]. With the rapid development of analysis technologies, some advanced analysis methods, such as confocal laser scanning microscopy (CLSM), microautoradiography (MAR), polymerase chain reaction (PCR) techniques, quantitative reverse transcriptase real-time PCR (qRT-PCR), denaturing gradient gel electrophoresis (DGGE), fluorescent *in situ* hybridization (FISH), environmental scanning electron microscopy (ESEM), microprobe sensor techniques, laser triangulation sensor (LTC) techniques, and BIOLOG ECO-plates, have been successfully used for studying biofilms [19]. Compared with the above methods, high-throughput sequencing provides researchers with a deeper understanding of the population dynamics or functions of microorganisms in microbial communities and a new perspective for understanding the degradation processes occurring in bioreactors in their native habitat without the need of isolation [20].

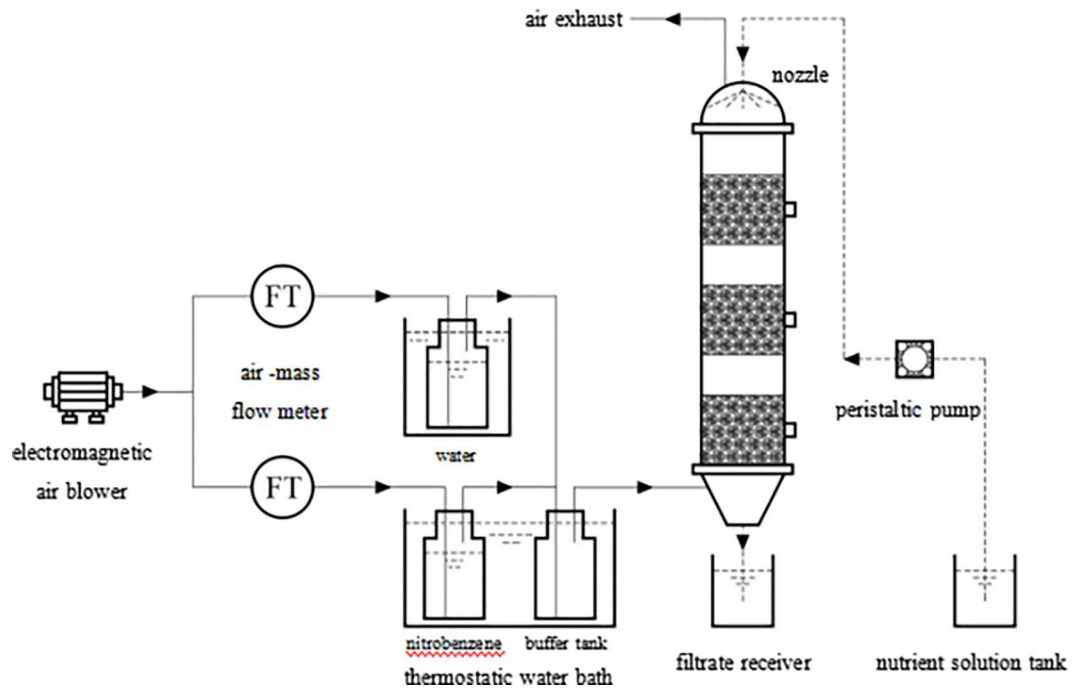
In this study, gas-phase NB was treated in a biofilter, and the microbial community was analysed by high-throughput sequencing techniques. The primary objective was to clearly understand the vertical distribution of the bacterial community in a biofilter for removal of low load nitrobenzene waste gas. The present research will help enhance the degradation performance of biofilters for treating NB waste gas.

## Materials and Methods

### Biofilter setup

A schematic of the experimental setup is illustrated in Fig 1. The biofilter reactor (inner diameter = 7 cm, effective bed height = 60 cm), which was packed with 2.3-L polyurethane foam cubes with low head loss and high porosity, was operated in a up-flow mode [21–22]. The packing parameters are summarized in Table 1. NB waste gas passed through the biofilter at a flow rate of 129.4, 149.5 and  $243.5 \text{ L h}^{-1}$ , respectively. Gas sampling ports sealed with rubber septa were provided at equal intervals along the biofilter height to estimate the changes in the NB concentrations and microbial communities.

The biofilter was inoculated using aerobic activated sludge from the secondary sedimentation tank of an NB production waste water treatment plant (WWTP). The mixed culture was re-circulated through the packed bed bioreactor using a peristaltic pump (WT600-1F, Baoding longer Pump Ltd., CHN) at a rate of  $0.5 \text{ L min}^{-1}$  until a visible biomass was noticed on the support material surface. The moisture content of the bed was maintained at 75%–85% by periodically adding (once a day) fresh mineral salt media. The trickling density was maintained at  $0.52 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$  throughout the experiments. The mineral salt nutrient solution contained the following components (per litre in distilled water): 0.5 g  $\text{K}_2\text{HPO}_4$ , 0.1 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 4.5 g  $\text{KH}_2\text{PO}_4$ , 2 g  $\text{NH}_4\text{Cl}$ , and 2 mL of vitamins and trace minerals [23]. The original pH of the nutrient solution was adjusted to 7.0.



**Fig 1. Schematic representation of the experimental setup.**

doi:10.1371/journal.pone.0170417.g001

### Analytical methods

The NB concentrations in the gas samples were measured by gas chromatography (GC) (2014, SHIMADZU, USA) using a Rtx-5 column (30 m, 0.32 mmID, 0.5  $\mu\text{m}$ ) and an FID. The flow rates were 30 mL  $\text{min}^{-1}$  for  $\text{H}_2$  and 300 mL  $\text{min}^{-1}$  for air. Nitrogen was used as the carrier gas at a flow rate of 2 mL  $\text{min}^{-1}$ . The temperatures located at the GC injection, oven and detection ports were 150°C, 220°C and 250°C, respectively. The pH value of the filter liquor was measured by a pH metre (FEP20, Mettler-Toledo, CHE). The nitrite concentration was monitored according to the Chinese National Standard GB 7493–87 water quality-determination of nitrogen (nitrite)- spectrophotometric method [24].

### Abiotic experiment

The NB abiotic experiment was performed with a biofilter containing a clean packing medium (2.3 L) to determine the amount of NB removed by abiotic means.

**Table 1. The packing parameters.**

parameters	value
shape	cube
size	per side 4–6 mm
bulk density	0.015 g/cm <sup>3</sup>
water holding capacity	55 g-H <sub>2</sub> O/g
porosity	95%
average pore size	0.8 mm
specific surface area	73.65 m <sup>2</sup> /g

Values provided by the manufacturer.

doi:10.1371/journal.pone.0170417.t001

## Sample collection, DNA extraction and polymerase chain reaction (PCR)

The representative biological samples were collected from the sampling port within the biofilter at the end of the experiment (EBRT 55.4 s). The performance of the biofilter was stable during this stage ( $ILR < 20 \text{ g m}^{-3} \text{ h}^{-1}$  in accordance with engineering practice). Each sample was immersed in 20 mL of sterilized water and sonicated for 20 s to strip the biomass from the packing material. At room temperature, 2 mL samples were then centrifuged at 13,000 rpm for 2 min using an Eppendorf centrifuge 5417R (Eppendorf, Hamburg, GER). The DNA template was extracted using a FastDNA<sup>®</sup> Spin Kit for Soil (MP Biomedicals, CA, USA) according to the manufacturer's instructions.

DNA samples (10 mg each) were employed to generate amplicons that covered the V3-V5 hypervariable regions of the bacterial 16S rRNA. PCRs were performed in 20  $\mu\text{L}$  reaction mixtures that each contained 1  $\mu\text{L}$  of the DNA template with approximately 10 ng of DNA, 0.8  $\mu\text{L}$  (5  $\mu\text{M}$ ) of the forward (338 F(5' - ACT CCT ACG GGA GGC AGC A-3')) and reverse (806 R (5' - GGA CTA CHV GGG TWT CTA AT-3')) primers, 2  $\mu\text{L}$  (2.5 mM) of deoxynucleoside triphosphate (dNTPs), 0.4  $\mu\text{L}$  of Fast Pfu Polymerase, 4  $\mu\text{L}$  of 5 $\times$ FastPfu Buffer and complement ddH<sub>2</sub>O. The DNA was amplified by using a GeneAmp<sup>®</sup> (9700, ABI, USA). PCR amplification was performed using initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s and a final extension at 72°C for 10 min. Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and were quantified using QuantiFluor<sup>™</sup>-ST (Promega, USA).

## Illumina MiSeq high-throughput sequencing

Purified amplicons were pooled in equimolar and paired-end sequences (2 $\times$ 250) on an Illumina MiSeq platform (PE250, Illumina, USA) according to the standard protocols. Corresponding sequences with specific barcodes inside each sample were selected using Mothur (<http://www.mothur.org/>). Then, data denoising was conducted as described in a previous study [25]. After noise reduction, 23,600 reads were drawn randomly from the samples to compare all the samples at the same sequencing depth. Downstream taxonomic assignment was performed using the Ribosomal Database Project (RDP) (<http://rdp.cme.msu.edu/>) classifier with confidence threshold of 50%. Mothur was applied to calculate the richness and diversity indices, including the operational taxonomic units (OTUs), Chaos index, and Shannon index [26]. A cluster analysis of the microbial community was conducted using PAleontological STatistics (PAST, v.3.01) software with an unweighted pair-group average method.

## Calculations

The empty bed residence time (EBRT, s), removal efficiency of NB (RE, %), inlet loading rate of NB ( $ILR, \text{g m}^{-3} \text{ h}^{-1}$ ) and the elimination capacity of NB ( $EC, \text{g m}^{-3} \text{ h}^{-1}$ ) of the reactor were estimated by using the following equations:

$$\text{Empty bed residence time, } EBRT = V/Q \quad \text{s} \quad (1)$$

$$\text{Removal efficiency, } RE = (C_i - C_o) / C_i * 100\% \quad \% \quad (2)$$

$$\text{Inlet loading rate, } ILR = QC_i / V \quad \text{g m}^{-3} \text{ h}^{-1} \quad (3)$$

$$\text{Elimination capacity, } EC = Q(C_i - C_o) / V \quad \text{g m}^{-3} \text{ h}^{-1} \quad (4)$$

where  $V$  is the volume ( $m^3$ ) of the packed bed section,  $Q$  is the gas flow rate ( $m^3 h^{-1}$ ), and  $C_i$  and  $C_o$  are the inlet and outlet concentrations ( $mg m^{-3}$ ) of NB, respectively.

## Results and Discussion

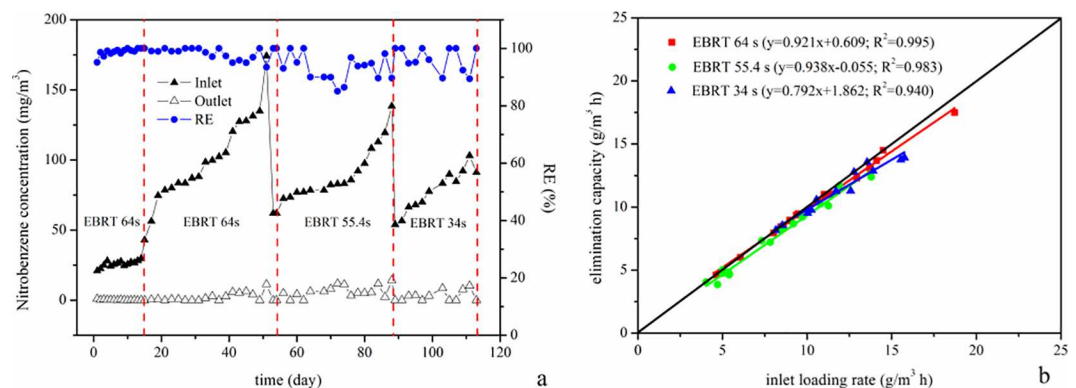
### NB removal by abiotic means

During the abiotic experiment,  $100 mg m^{-3}$  gas-phase NB was introduced to the biofilter at a flow rate of  $149.5 L h^{-1}$  for a period of 7 days, and the NB removal efficiency was measured under the trickling density of a mineral salt nutrient solution of  $0.52 m^3 m^{-2} h^{-1}$ . The packing medium removed less than 2.47% of the inlet NB from the gas stream (data not shown), which showed that the effect of the NB abiotic degradation was negligible in the biofilter.

### NB removal by the biofilter

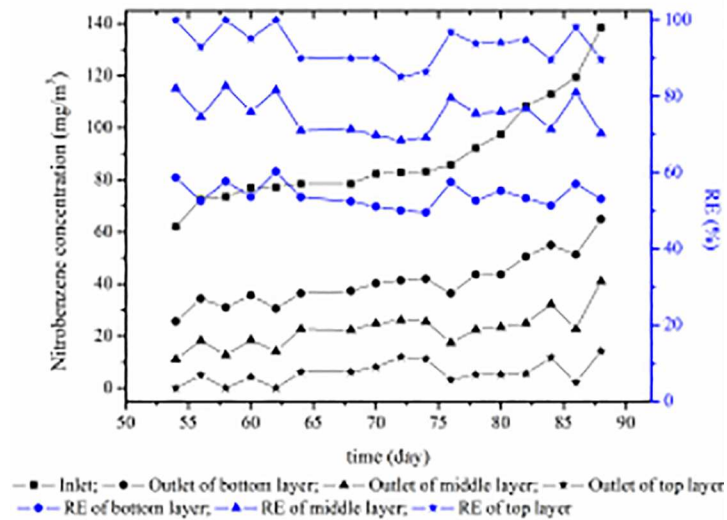
Generally, most laboratory-scale biofilters were fed with only low concentrations of pollutants during acclimation. In this study,  $20\text{--}30 mg m^{-3}$  gas-phase NB was introduced to the biofilter with an empty bed residence time (EBRT) of 64 s. The biofilter achieved a nearly stable RE value of 98.8% after 14 days (Fig 2A), which indicated that the biofilter inoculated with aerobic activated sludge from the NB waste water treatment plant required a shorter acclimation time. After the acclimation period, steady-state experiments were carried out at different EBRTs and inlet NB concentrations, which are important parameters that affect the efficiency of biofiltration units. As depicted in Fig 2A, when the inlet concentrations of NB gradually increased from  $43.0$  to  $174.2 mg m^{-3}$  with an EBRT of 64 s during days 15–53, the RE values of NB fluctuated between 93.5% and 100%. The EBRT decreased from 64 s to 55.4 s during the next period from the 54th day to the 88th day (Fig 3), the inlet concentrations of NB were maintained in the range of  $62.0\text{--}138.4 mg m^{-3}$  and the outlet concentrations of NB in the bottom layer, middle layer and top layer were  $25.6\text{--}64.9 mg m^{-3}$ ,  $11.1\text{--}41.1 mg m^{-3}$ , and  $0\text{--}14.3 mg m^{-3}$ , respectively. RE values of 49.5%–60.3%, 68.5%–82.7%, and 85.1–100% were achieved in the bottom layer, middle layer and top layer, respectively. Finally, the biofilter was subjected to a much lower EBRT (34 s) and RE values of 89.4%–100% were maintained during days 89–113 (Fig 2A). These data indicate that the biofilter performed well and that the RE was hardly relevant to the EBRT with low NB concentrations.

In this study, maximum EC values of 17.5, 12.4 and  $13.9 g m^{-3} h^{-1}$  were achieved with EBRT values of 64, 55.4 and 34 s and ILR values of 18.7, 13.8 and  $15.7 g m^{-3} h^{-1}$ , respectively (Fig 2B). The slope of the linear fitting equation of NB degradation with an EBRT of 64 s (0.921) was



**Fig 2.** Performance of the biofilters for the removal of NB: effect of the EBRT (a) and the elimination capacity vs. the inlet loading rate (b).

doi:10.1371/journal.pone.0170417.g002



**Fig 3. Outlet concentrations of NB and REs in different layers with an EBRT of 55.4 s.**

doi:10.1371/journal.pone.0170417.g003

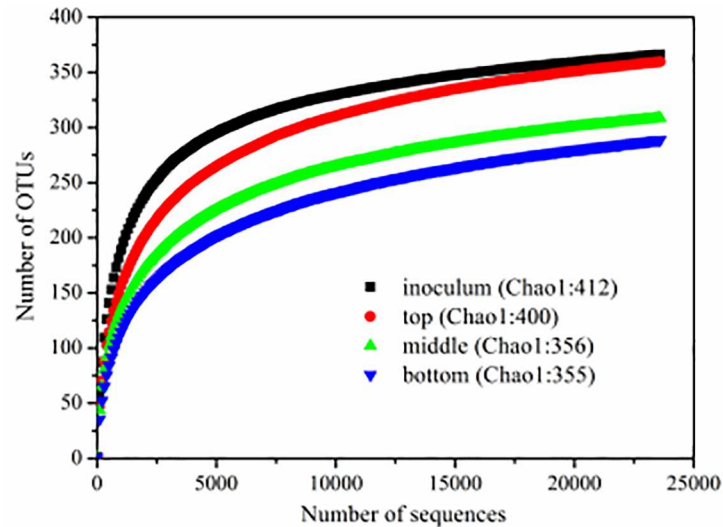
higher than that with an EBRT of 34 s (0.792) and was similar to that with an EBRT of 55.4s (0.938), which was near 1.0 under low NB loads. In this region, the EC of the biofilter is highly dependent on the EBRT. Increasing the given EBRT enhanced the transfer rate of NB from the gas-phase to the biofilm and improved the EC [27].

### Overall microbial community diversity

Samples were collected from the initial inoculum in the top, middle and bottom layers of the biofilter with an EBRT of 55.4 s, respectively. The microbial community diversities and phylogenetic structures of these samples were analysed by using Illumina MiSeq high-throughput sequencing. Over 20000 sequences were obtained for each sample. The Good's coverages of these samples ranged from 99.76% to 99.89%, indicating that the sequences generated at this sequencing depth represented the bacterial communities of these samples well [26]. For fair comparison, the library size of each sample was normalized to the same sequencing depth (23,600 reads) by randomly removing the redundant reads. Relatively higher OTUs were found in the initial inoculum (366) than the samples of the top (360), middle (309) and bottom layers (289). The estimated species richness Chao1 for each sample was also consistent with this trend (Fig 4). Based on the Shannon index, the initial inoculum (4.60) also had a relatively higher diversity than the sample of the top layer (4.12), followed by the samples of the middle (3.84) and bottom layer (3.43). The biodiversity variation trends were attributed to the NB waste gas acting as the sole carbon and energy source to the biofilter, which decreased the biodiversity of each layer in the biofilter. During the continuous biodegradation of NB, the increment of intermediate product types may have resulted in the increment of biodiversity from the bottom layer to the top layer in the biofilter.

### Shifts in microbial community structure and composition

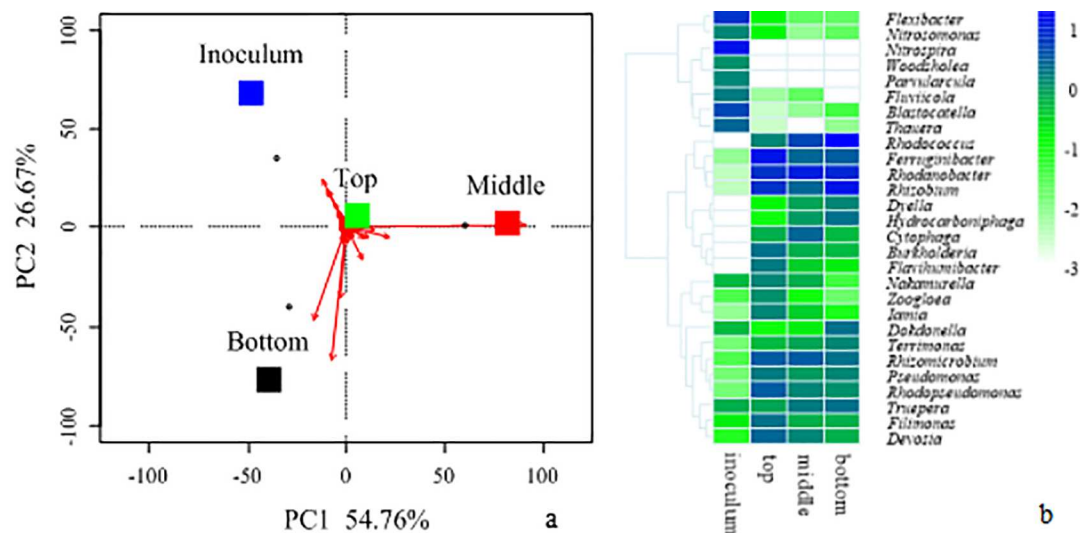
The small differences between the microbial community structures in the three layers of the biofilter were distinguished by using principal component analysis (PCA) and from the abundance of major genera (Fig 5). PCA revealed that the bacterial community structure varied



**Fig 4. Rarefaction curves based on 16S rRNA gene sequencing of the samples (from top, middle and bottom layers) and the initial inoculum.** The Chao1 index is also shown. The OTUs were defined by a 97% cutoff.

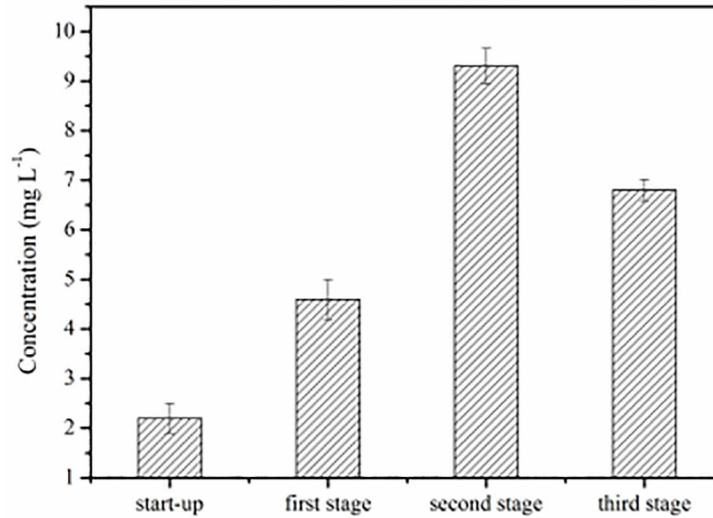
doi:10.1371/journal.pone.0170417.g004

significantly among the layers of the biofilter. Principle component 1 (PC1) and principle component 2 (PC2) accounted for 54.76% and 26.67% of the total variance, respectively. As demonstrated by PCA (Fig 5A), the initial inoculum was located in the first quadrant, samples of the top and middle layer were located in the second quadrant, and samples of the bottom layer were located in the third quadrant. Interestingly, the samples of the top layer were not near the samples of the middle and bottom layers or the initial inoculum. The heat map shows that the major genera of each layer were different from each other (Fig 5B). In this study, nitrite was detected in the filter liquor (Fig 6). In addition, nitrite and catechol were



**Fig 5. Principal component analysis (PCA) of bacterial communities from the initial inoculum and samples of the top, middle and bottom layers based on the classified OTUs (a). Heat map illustrating the abundances of all the major genera (with a relative abundance of more than 1% in at least one sample).** The color intensity (log scale) in each panel indicates the relative abundance of the genus in each sample (b).

doi:10.1371/journal.pone.0170417.g005



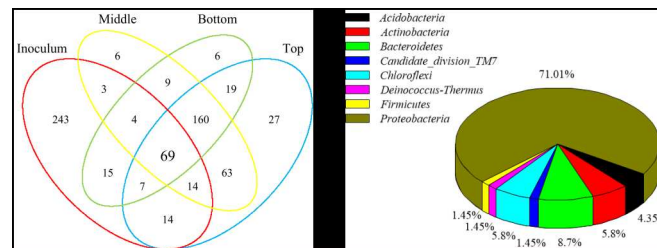
**Fig 6. Concentrations of nitrite released.**

doi:10.1371/journal.pone.0170417.g006

characterized as the metabolic products of NB oxidation under aerobic conditions [28]. This result suggests that the subtle variations of the bacterial community structure are attributed to the accumulation of NB metabolites.

The total classified number of OTUs in all four bacterial communities was 659, and 69 OTUs (10.47%) of the OTUs were shared by the four communities. More than 71.01% of the shared OTUs belonged to *Proteobacteria* according to the phylum-level taxonomic distribution of the four samples (inoculum and top, middle and bottom layers), and the OTUs belonging to *Bacteroidetes* (8.70%), *Actinobacteria* (5.80%), *Chloroflexi* (5.80%) and *Acidobacteria* (4.35%) were shared at similar levels (Fig 7). The data showed that the microbes in the phylum *Proteobacteria* were key during the removal process of NB from waste gas with low amounts of NB.

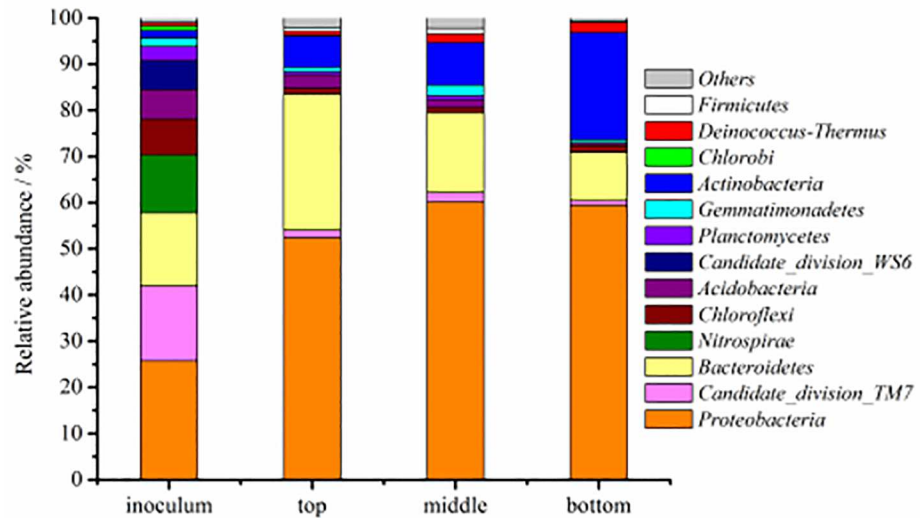
A total of 14 phyla were identified, among which 14, 12, 12 and 12 were detected from the initial inoculum and the samples of the top, middle and bottom layers, respectively. *Proteobacteria* were a dominant part of the bacterial community in the initial inoculum (25.73%), top layer (52.36%), middle layer (60.16%) and bottom layer (59.45%), respectively. The relative abundance of *Bacteroidetes* was 29.52% in the top layer, which was higher than those in the middle layer (17.23%), bottom layer (10.46%) and the initial inoculum (15.86%). The relative abundance of *Actinobacteria* decreased from 23.24% (bottom layer) to 9.18% (middle layer),



**Fig 7. Venn diagram illustrating the overlap of the four bacterial communities from the initial inoculum and the samples of the top, middle and bottom layers. The shared OTUs (69) were analysed at the phylum level.**

doi:10.1371/journal.pone.0170417.g007





**Fig 8. Abundances and distributions of the different phyla in the four biofilm samples.**

doi:10.1371/journal.pone.0170417.g008

6.69% (top layer) and 1.63% (the initial inoculum) (Fig 8). From Fig 3, NB was removed mainly in the bottom layer (49.5%-60.3%) and middle layer (68.5%-82.7%); therefore, it can be concluded that the phylum *Actinobacteria* and *Proteobacteria* had major contributions to the degradation of NB, and the main function of the phylum *Bacteroidetes* was to degrade the NB metabolic intermediates.

### Potential functions and relative abundances of the dominant genera

A total of 89 genera were classified among the test samples. Twelve genera with a relative abundance >1% in the initial inoculum and samples of the top, middle and bottom layer communities are shown in Table 2. *Nitrospira* (12.56%), *Flexibacter* (7.08%) and *Blastocatella* (5.05%) were obviously dominant in the initial inoculum.

In the bottom layer, genus *Rhodococcus* (22.18%), *Rhizobium* (12.86%) and *Rhodanobacter* (8.36%) had obviously higher relative abundance than in the initial inoculum (Table 2). The

**Table 2. Genera (relative abundance>1% at genus levels) in the initial inoculum and the samples of the top, middle and bottom layers.** The dominant genera in the different layers are shown in bold.

Phylum	Class	Genus (%)	Initial	Top	Middle	Bottom
<i>Nitrospirae</i>	<i>Nitrospira</i>	<i>Nitrospira</i>	12.56	0.00	0.00	0.00
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Flexibacter</i>	7.08	0.17	0.02	0.02
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Blastocatella</i>	5.05	0.00	0.01	0.04
<i>Deinococcus-Thermus</i>	<i>Deinococci</i>	<i>Truepera</i>	0.65	0.83	1.87	2.16
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Devosia</i>	0.08	2.59	1.46	0.72
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Rhizomicrobium</i>	0.04	3.09	3.22	2.32
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Rhodopseudomonas</i>	0.02	3.62	1.35	1.21
<i>Proteobacteria</i>	<i>γ-proteobacteria</i>	<b><i>Pseudomonas</i></b>	0.02	1.92	0.93	1.58
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<b><i>Ferruginibacter</i></b>	0.01	14.74	2.98	3.22
<i>Proteobacteria</i>	<i>γ-proteobacteria</i>	<b><i>Rhodanobacter</i></b>	0.00	7.70	10.35	8.36
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Rhizobium</i>	0.00	8.01	2.45	12.86
<i>Actinobacteria</i>	<i>Actinobacteria</i>	<b><i>Rhodococcus</i></b>	0.00	1.43	4.48	22.18

doi:10.1371/journal.pone.0170417.t002

genus *Rhodococcus* belonged to the phylum *Actinobacteria*, which is known to be capable of NB biodegradation [29–31]. According to Fig 3, the RE of NB was 49.5%–60.3% in the bottom layer; thus, it was concluded that the genus *Rhodococcus* was the major NB-degrading genus. This finding may explain why *Rhodococcus* was dominant in the inlet of the biofilter. In the middle layer, the genus *Rhodanobacter* was obviously enriched (10.35%); however, the relative abundance of *Rhodococcus* in the initial inoculum was low (0.004%). *Rhodococcus* belongs to the phylum *Proteobacteria*, which is capable of denitrification [32–33]. Nitrite is a type of metabolite that is used for NB oxidation, which explains the predominance of *Rhodanobacter* in this layer [34]. In the top layer, the genera *Ferruginibacter* (14.74%), *Rhizobium* (8.01%) and *Rhodanobacter* (7.70%) exhibited exceedingly higher abundance than those in the initial inoculum. The genus *Ferruginibacter* belongs to the phylum *Bacteroidetes*, which has frequently been detected at wastewater treatment plants [35–36]. A previous study indicated that members of *Ferruginibacter* are capable of hydrolysing some organic matter [37]. It was asserted that the genus *Ferruginibacter* predominated in the top layer because of the degradation of the NB metabolic intermediates. Although *Pseudomonas* possessed a fairly lower abundance in the initial inoculum (0.02%) and top (1.92%), middle (0.93%) and bottom (1.58%) layers, respectively, *Pseudomonas* has also been reported to have the ability to degrade high concentrations of NB [38].

## Conclusions

This study demonstrated an efficient system for NB removal at low ILR values. The REs were always higher than 93% at different phases, and the slopes of EC/ILR were close to 1.0. Illumina MiSeq high-throughput sequencing technology was successfully applied to analyse the variations of the biodiversity and microbial community structure in these biofilms along the longitudinal direction of the biofilter. The increase of biodiversity from the bottom up was attributed to the increase of NB metabolic intermediate types. The microbial community structure affected the ecological function significantly and further influenced the performance of the biofilter. The sequencing data showed that the phylum *Actinobacteria* and genus *Rhodococcus* played key roles in the degradation of NB in the bottom layer.

## Supporting Information

**S1 Table. Nitrobenzene properties.**  
(DOCX)

## Author Contributions

**Conceptualization:** CL JZ.

**Data curation:** JZ ZW.

**Formal analysis:** JZ PS CL.

**Funding acquisition:** JZ.

**Investigation:** JZ ZW CL.

**Methodology:** CL JZ.

**Project administration:** CL.

**Resources:** CL JZ.

**Supervision:** CL JZ.

**Validation:** CL.

**Writing – original draft:** JZ ZW.

**Writing – review & editing:** CL JZ.

## References

1. Xie B, Liang SB, Tang Y, Mi WX, Xu Y (2009) Petrochemical wastewater odor treatment by biofiltration. *Bioresour Technol* 100(7): 2204–2209. doi: [10.1016/j.biortech.2008.10.035](https://doi.org/10.1016/j.biortech.2008.10.035) PMID: [19056260](https://pubmed.ncbi.nlm.nih.gov/19056260/)
2. Kennes C, Rene ER, Veiga MC (2009) Bioprocesses for air pollution control. *J Chem Technol Biotechnol* 84(10):1419–1436.
3. Estrada JM, Kraakman NJRB, Munoz R, Lebrero R (2011) A comparative analysis of odour treatment technologies in wastewater treatment plants. *Environ Sci Technol* 45(3):1100–1106. doi: [10.1021/es103478j](https://doi.org/10.1021/es103478j) PMID: [21275373](https://pubmed.ncbi.nlm.nih.gov/21275373/)
4. Arnold M, Reittu A, von Wright A, Martikainen PJ, Suihko ML (1997) Bacterial degradation of styrene in waste gases using a peat filter. *Appl Microbiol and Biot* 48(6):738–744.
5. Auria R, Aycaguer AC, Deviny JS (1998) Influence of water content on degradation rates for ethanol in biofiltration. *J Air Waste Manage* 48(1):65–70.
6. Babbitt CW, Pacheco A, Lindner AS (2009) Methanol removal efficiency and bacterial diversity of an activated carbon biofilter. *Bioresour Technol* 100(24):6207–6216.
7. Jorio H, Kiared K, Brzezinski R, Leroux A, Viel G, Heitz M (1998) Treatment of air polluted with high concentrations of toluene and xylene in a pilot-scale biofilter. *J Chem Technol Biotechnol* 73(3):183–196.
8. Morgenroth E, Schroeder ED, Chang DPY, Scow KM (1996) Nutrient limitation in a compost biofilter degrading hexane. *J Air Waste Manage* 46(4):300–308.
9. Shareefdeen Z, Baltzis BC (1994) Biofiltration of toluene vapor under steady-state and transient conditions: theory and experimental results. *Chem Eng Sci* 49(24):4347–4360.
10. Zilli M, Converti A, Lodi A, Del Borghi M, Ferraiolo G (1993) Phenol removal from waste gases with a biological filter by *Pseudomonas putida*. *Biotechnol and Bioeng* 41(7):693–699.
11. Chou MS, Shiu WZ (1997) Bioconversion of methylamine in biofilters. *J Air Waste Manage* 47(1):58–65.
12. Tang HM, Hwang SJ, Hwang SC (1996) Waste gas treatment in biofilters. *J Air Waste Manage* 46(4):349–354.
13. Smet E, Van Langenhove H, Verstraete W (1997) Isobutyraldehyde as a competitor of the dimethylsulfide degrading activity in biofilters. *Biodegradation* 8(1):53–59.
14. Ergas SJ, Kinney K, Fuller ME, Scow KM (1994) Characterization of a compost biofiltration system degrading dichloromethane. *Biotechnol and Bioeng* 44(9):1048–1054.
15. Cheng Y, He HJ, Yang CP, Zeng GM, Li X, Chen H, et al. (2016) Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. *Biotechnol Adv* 34(6): 1091–1102. doi: [10.1016/j.biotechadv.2016.06.007](https://doi.org/10.1016/j.biotechadv.2016.06.007) PMID: [27374790](https://pubmed.ncbi.nlm.nih.gov/27374790/)
16. Oh YS, Bartha R (1997) Removal of nitrobenzene vapors by a trickling air biofilter. *J Ind Microbiol Biotechnol* 18(5): 293–296.
17. van Groenestijn JW, Hasselink PGM (1993) Biotechniques for air pollution control. *Biodegradation* 4(4):283–301.
18. 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(11): 721–734. PMID: [12460280](https://pubmed.ncbi.nlm.nih.gov/12460280/)
19. Yang CP, Chen H, Zeng GM, Yu GL, Luo SL (2010) Biomass accumulation and control strategies in gas biofilters. *Biotechnol Adv* 28(4): 531–540. doi: [10.1016/j.biotechadv.2010.04.002](https://doi.org/10.1016/j.biotechadv.2010.04.002) PMID: [20385223](https://pubmed.ncbi.nlm.nih.gov/20385223/)
20. Sanz JL, Kochling T (2007) Molecular biology techniques used in wastewater treatment: An overview. *Process Biochem* 42(2): 119–133.
21. Qi B, Moe WM, Kinney KA (2005) Treatment of paint spray booth off-gases in a fungal biofilter. *J Environ Eng-Asce* 131(2): 180–189.
22. Aizpuru A, Dunat B, Christen P, Auria R, Garcia-Pena I, Revah S. (2005) Fungal biofiltration of toluene on ceramic rings. *J Environ Eng-Asce* 131(3): 396–402.

23. Kennes C, Cox HHJ, Doddema HJ, Harder W (1996) Design and performance of biofilters for the removal of alkylbenzene vapours. *J Chem Technol Biotechnol* 66(3): 300–304.
24. Ministry of Environmental Protection the People's Republic of China. (1987) Water quality-Determination of nitrogen (nitrite)-Spectrophotometric method GB 7493. (In Chinese)
25. Guo X, Miao Y, Wu B, Ye L, Yu H, Liu S, et al. (2015) Correlation between microbial community structure and biofouling as determined by analysis of microbial community dynamics. *Bioresour Technol* 197(8): 99–105.
26. Wang Z, Zhang XX, Lu X, Liu B, Li Y, Long C, et al. (2014) Abundance and diversity of bacterial nitrifiers and denitrifiers and their functional genes in tannery wastewater treatment plants revealed by high-throughput sequencing. *PLoS One* 9(11) e113603. doi: [10.1371/journal.pone.0113603](https://doi.org/10.1371/journal.pone.0113603) PMID: [25420093](https://pubmed.ncbi.nlm.nih.gov/25420093/)
27. Li GW, Hu HY, Hao JM, Fujie K (2002) Use of biological activated carbon to treat mixed gas of toluene and benzene in biofilter. *Environ Technol* 23(4): 467–477. doi: [10.1080/09593332508618407](https://doi.org/10.1080/09593332508618407) PMID: [12088374](https://pubmed.ncbi.nlm.nih.gov/12088374/)
28. He Z, Spain JC (1999) Comparison of the downstream pathways for degradation of nitrobenzene by *Pseudomonas pseudoalcaligenes* JS45 (2-aminophenol pathway) and by *Comamonas* sp. JS765 (catechol pathway). *Arch Microbiol* 171(5): 309–316. PMID: [10382261](https://pubmed.ncbi.nlm.nih.gov/10382261/)
29. Warhurst AM, Fewson CA (1994) Biotransformations catalyzed by the genus *Rhodococcus*. *Crit Rev Biotechnol* 14(1): 29–73. doi: [10.3109/07388559409079833](https://doi.org/10.3109/07388559409079833) PMID: [8187203](https://pubmed.ncbi.nlm.nih.gov/8187203/)
30. Shen J, Zhang J, Zuo Y, Wang L, Sun X, Li J, Han W, He R (2009) Biodegradation of 2,4,6-trinitrophenol by *Rhodococcus* sp. isolated from a picric acid-contaminated soil. *J Hazard Mater* 163(2–3): 1199–1206. doi: [10.1016/j.jhazmat.2008.07.086](https://doi.org/10.1016/j.jhazmat.2008.07.086) PMID: [18762376](https://pubmed.ncbi.nlm.nih.gov/18762376/)
31. Lin H, Chen XJ, Ding HT, Jia XM, Zhao YH (2011) Isolation and characterization of *Rhodococcus* sp. NB5 capable of degrading a high concentration of nitrobenzene. *J Basic Microb* 51(4): 397–403.
32. Heuvel RNVD, Biezen EVD, Jetten MSM, Hefting MM, Kartal B (2010) Denitrification at pH 4 by a soil-derived *Rhodanobacter*-dominated community. *Environ Microbiol* 12(12): 3264–3271. doi: [10.1111/j.1462-2920.2010.02301.x](https://doi.org/10.1111/j.1462-2920.2010.02301.x) PMID: [20649643](https://pubmed.ncbi.nlm.nih.gov/20649643/)
33. Green SJ, Prakash O, Jasrotia P, Overholt WA, Cardenas E, Hubbard D, et al. (2012) Denitrifying Bacteria from the Genus *Rhodanobacter* Dominate Bacterial Communities in the Highly Contaminated Sub-surface of a Nuclear Legacy Waste Site. *Appl Environ Microb* 78(4): 1039–1047.
34. Li T, Deng XP, Wang JJ, Chen YC, He L, Sun YC, et al. (2014) Biodegradation of nitrobenzene in a lysogeny broth medium by a novel halophilic bacterium *Bacillus licheniformis*. *Mar Pollut Bull* 89(1–2):384–389. doi: [10.1016/j.marpolbul.2014.09.028](https://doi.org/10.1016/j.marpolbul.2014.09.028) PMID: [25440194](https://pubmed.ncbi.nlm.nih.gov/25440194/)
35. Wen Y, Jin YX, Wang JY, Cai LP (2015) MiSeq sequencing analysis of bacterial community structures in wastewater treatment plants. *Pol J Environ Stud* 24(4): 1809–1815.
36. Wang XH, Hu M, Xia Y, Wen XH, Ding K (2012) Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China. *Appl Environ Microb* 78(19):7042–7047.
37. Lim JH, Baek SH, Lee ST (2009) *Ferruginibacter alkalientus* gen. nov., sp. nov. and *Ferruginibacter lapsinensis* sp. nov., novel members of the family 'Chitinophagaceae' in the phylum *Bacteroidetes*, isolated from freshwater sediment. *Int J Syst Evol Micr* 59(Pt 10):2394–2399.
38. Liu N, Li HJ, Shi YE, Zhu BL, Gao S (2013) Biodegradation of high concentration of nitrobenzene by *Pseudomonas corrugata* embedded in peat-phosphate esterified polyvinyl alcohol. *World J Microb Biot* 29(10):1859–1867.