Research Square

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Metagenomic analysis of microbial community structure and function in a improved biofilter with odorous gases

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Research Article

Keywords: Biofilter, dominant microbes, metagenome, microbial community, odorous gas

Posted Date: February 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-229916/v1

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Abstract

Background

Biofilters have been broadly applied to degrade the odorous gases from industrial emissions. To explore biofilter potentials, the microbial community structure and function must be well defined. Using of improved biofilter, changes in microbial community structures and functions were investigated by metagenomic analysis.

Results

Odorous gases have the potential to alter the microbial community structure in the sludge of biofilter. A total of 90,016 unigenes assigned into various functional metabolic pathways were identified. In the improved biofilter, the dominant phyla were *Proteobacteria*, *Planctomycetes*, and *Chloroflexi*, and the dominant were *Thioalkalivibrio*, *Thauera*, and *Pseudomonas*. Several xenobiotic biodegradation-related pathways showed significant changes during the treatment process. Compared with the original biofilter, *Thermotogae* and *Crenarchaeota* phyla were significantly up-regulated in the improved biofilter, suggesting its important role in nitrogen-fixing. Furthermore, nitrogen metabolic pathway-related genes, such as *nirA* and *nifA*, and sulfur metabolic pathway-related genes, such as *fccB* and *phsA*, were considered to be efficient genes involved in removing odorous gases.

Conclusions

Our findings can be used for improving the efficiency of biofilter and helping the industrial enterprises to reduce the emission of waste gas.

Background

Odorous gases are typical outdoor air pollutants that have effects on the environment and health of human beings [1, 2]. With the development of economy, odorous gases from industrial emissions becomes a major factor to haze contamination in the world [3]. As a public health concern, odorous gases is a significant threat to personal health and comfort [4, 5]. The major components of odorous gases are nitrogen containing compounds, such as NH3 and NO_x, sulfur containing compounds, such as H_2S and SO_2, and volatile organic compounds (VOCs) [6, 7]. Most of these nitrogen- or sulfur-containing compounds have pungent odor. In addition to strong offensive smell, odorous gas need to be solved due to their long-term health effects [8]. Thus, controlling odorous gas emissions is essential for reducing air pollution.

In past years, a number of technologies have been developed to control air pollution caused by odorous gases and VOCs. Among these technologies, most of them can be classified into three different groups, including physical methods, chemical methods, and biological methods [9]. Although physical-chemical methods have high efficiency to treat odorous gases, several adverse factors, such as high cost, high

concentration and secondary pollution, greatly limit their application [10]. Biological technologies are recently considered to be environment friendly methods for the process of low concentrations of odorous gases, compared with physical-chemical methods [11]. Three major categories, including biofilter, bioscrubbers, and biotrickling filters, were widely applied as biological technologies [9].

Biofilter is commonly used for harmlessness treatment of industrial odorous gases by forcing waste gases to rise through a layer of packed material [12]. Several key parameters, such as nutrients, temperature, pH value, and microbial community, determined the efficiency of the biofilter [13]. The packing materials in biofilters contain specific microorganisms, which are the key characteristics of different biofilters and largely varied to treat different pollutants [14]. A greatly effective microbial community plays an important roles in degrading pollutants into no harmful small molecules [15]. It is therefore important to take improvement of microbial community into consideration.

Studies on microbial community and function of biofilter packing is a shortcut to optimize the management of this biological treatment system [16]. Most previous works have used isolation and identification techniques to get high efficient microbial species in interesting pathways [17]. For example, an *Acidithiobacillus* and a *Thiobacillus* species were considered to play a dominant role in novel horizontal flow biofilm reactors under H_2S treatment [18]. The functions of *Sphingomonas* sp. in the degradation of ethylbenzene, *Thiomonas* sp. in the degradation of carbon disulfide, and *Acidithiobacillus* sp. in the degradation of hydrogen sulfide have been well uncovered [19–21]. With the application of high-throughput sequencing, the traditional method is thought to be very inefficient.

High-throughput studies have focused on the structural and functional responses of microbial communities during the treatment of contaminants [17, 22, 23]. Analysis of samples from N_2O emission area showed significantly different microbial communities and structures between high N_2O emission area and low N_2O emission area [24]. In anaerobic ammonium oxidation systems, high-throughput sequencing results revealed that *Kuenenia* was the dominant species of anammox bacteria [25]. In addition to microbes, a number of functional genes were considered to play important roles in metabolism of nitrogen- and sulfur-containing compounds. For examples, *nirK* and *nirS* were treated as maker genes of bacterial community under the nitrous oxide treatment [25]. Analysis of bacterial ammonia monooxygenase (*amoA*) gene was applied to evaluation of bacterial communities in different types of biofiltration technologies [26]. Although many efficient bacteria and functional genes have been identified, affection of odorous gases on the microbial community and structure in a biofilter system are largely unknown.

Our previous study have discovered the shifts in microbial community structures in a industrial scale biofilter [27]. After a year of adjustment, a improved biofilter showed more efficient role in assimilating H_2S and NH_3. In the present study, metagenomics sequencing was used to screen novel microbial species and functional genes involved in degradation of odorous gases. Comparison of the original and improved biofilters will provide useful targets for improving the efficiency of biofilter.

Materials And Methods

Materials and sampling

Waste gas treatment plant used in the present study is the same to our previous study [27]. Sample materials were isolated from improved sludge in this equipment after a 3-week acclimation period with clean airflow, followed by a 2-week period of odor contaminated airflow. The sample isolated before the treatment of clean airflow was named as control sample (CS) and the sample isolated after the treatment of odor contaminated airflow was named as treated sample (TS). The main components of odorous gases are H_2S and NH_3. For H_2S, the initial concentration is 19.2 $mg.m^{-3}$ and the emission rate is about 0.2 $kg.h^{-1}$. For NH_3, the initial concentration is 9.3 $mg.m^{-3}$ and the emission rate is about 0.2 $kg.h^{-1}$. The NH_3 concentration was determined using the Nessler's reagent colorimetry method [28]. The H_2S concentration was determined using the methylene blue spectrophometry method (GB/T11742-89, China).

DNA isolation and library construction

Microbial genomic DNA was extracted using a Omega DNA kit (D4015-02) following the producer's procedures. Then, the DNA samples were purified by 1% agarose gel electrophoresis. The quality of DNA samples was analyzed by a NanoDrop spectrophotometer according to the criterion of A260/A280 between 1.7 to 1.8 and A260/A230 > 1.7. Afterwards, DNA samples were cut into small fragments of about 250 bp to construct paired-end sequencing libraries. DNA templates were then processed using the TruseqTM kit according to the manufacture's instruction.

Illumina sequencing and raw data uploading

High-throughput sequencing was processed on a HiSeq4000 platform and the mode was set at PE150. The metagenomic sequence reads were processed to remove invalid reads. Low quality reads, including adapters, taqs and N > 5% reads, were removed using softwares Cutadapt v1.9 and Fqtrim v0.94 with sliding-window algorithm. The sequences without sequencing tags and adapters were subjected to quality control using the Galaxy FastX software with a minimum size 100 bp and minimum quality score 20. The raw sequence data has been submitted to the NCBI as a BioProject with accession number PRJNA699130.

Sequence assembly and unigene identification

To get the metagenomic contigs, *de novo* assembly was performed using the clean reads. For taxonomic affiliations and functional annotations, generated metagenomic contigs larger than 500 bp in length were subjected to the MG-RAST server with related metadata files. The result sequences were used to predict their protein coding sequences (CDS) using MetaGeneMark v3.26 software. Then, the CDSs were clustered by CD0HIT v4.6.1 software to produce unigenes. The functional annotation of unigenes was performed by the Reduced Alphabet based Protein similarity Search tool against the Nr, Gene Onotology

(GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Carbohydrate-Active enzymes (CAZy) databases with default parameters.

Taxonomic profile analysis

For species classification, all unigenes were searched against the Nr_meta database using the DIAMOND software with evalue < $1e^{-5}$. Different species classification levels, including Phylum, Class, Order, and Family, were analyzed using the NCBI Taxonomy system with a Lowest Common Ancestors (LCA) algorithm. The diversity of microbial communities in the CS and TS samples was evaluated using the Chao1 richness index and Shannon diversity index from the unigene data at different classification levels [29]. A lineage without classified information in the database was set as 'unclassified'. We classified the microorganisms with a relative abundance lower than 0.10% as 'Others'.

GO and KEGG enrichment analysis

For each GO or KEGG category, a two-tailed Fisher's exact test was employed to calculate the enrichment of the differentially expressed genes (DEGs) in different comparisons. Correction for multiple hypothesis testing was carried out using standard false discovery rate (FDR) control methods. The GO or KEGG term with a corrected *P* value < 0.05 is considered significant. The MeV software was used to visualize the enrichment results.

Statistical analysis

Three parallel experiments were carried out for sequencing. All data on the diversity indexes, the relative abundances of unigenes, and the H_2S and NH_3 levels were processed using SPSS 18.0. One-way analysis of variance (ANOVA) was applied to analyze the differences between the CS and TS sample groups. Wilcoxon tests were conducted to detect differences in microbial community structures between two groups. The *P* value was produced by the false discovery rate (FDR) analysis and adjusted using the Benjamini and Hochberg's method [30]. A significant difference was indicated by a probability value (*P*) less than 0.05.

Results

Improved removal performance for NH_3 and H_2S

Our study evaluated the improved performance in removing odorous gases containing NH_3 and H_2S. After treatment, the emission concentrations of H_2S decreased from 35.3 $mg.m^{-3}$ to 0.32 $mg.m^{-3}$ in original version and to 0.14 $mg.m^{-3}$ in improved version; the emission rate of H_2S were decreased from 0.26 $kg.h^{-1}$ to 0.0032 $kg.h^{-1}$ in original version and to 0.0019 $kg.h^{-1}$ in improved version. After treatment, the emission concentrations of NH_3 decreased from 16.1 $mg.m^{-3}$ to 2.2 $mg.m^{-3}$ in original version and to 1.1 $mg.m^{-3}$ in improved version; the emission rate of NH_3 were decreased from 0.12 $kg.h^{-1}$ to 0.022 $kg.h^{-1}$ in original version and to 0.009 $kg.h^{-1}$ in improved version.

Detail information of the metagenomes

A total of 6.18E + 08 and 5.53E + 08 raw reads were obtained from the CS and TS samples, respectively. Then, 5.90E + 08 clean reads from CS and 5.30E + 08 clean reads from TS were used to assemble metagenomes (**Additional file 1**). After filtering out the low quality sequences, 143,441 contigs (N50: 1689 bp) were obtained from the CS sample and 155,721 contigs (N50: 1476 bp) were obtained from the TS sample (Fig. 1a and **b**). Correlation coefficient analysis indicated the sequencing data had good repeatability (**Additional file 2**). To get an overview of the metagenomic variations, a PCA was performed, and the percentages of explained value in the analysis of PC1 and PC2 were 99.12% and 0.44%, respectively (Fig. 1c).

Functional prediction and classification of unigenes

Based on the assembled contigs, a total of 901,016 unigenes with an average length of 660 bp and GC content of 58% were predicted. All the unigenes were detected in the CS sample and only 875,737 unigenes were detected in the TS sample (Fig. 1d). Length distribution of all predicted unigenes was showed in **Additional file 3** and expression abundances of unigenes were showed in **Additional file 4**.

In total, 726,022 unigenes were assigned to different functional KEGG pathways. In the 'metabolism' category, the most typical pathways were 'carbohydrate metabolism' (59,872 unigenes), 'amino acid metabolism' (49,584 unigenes), and 'cofactors and vitamins metabolism' (30,315 unigenes). In the 'genetic information processing' category, most unigenes were classed into the 'translation' (18,585 unigenes), 'replication and repair' (15,850 unigenes), and 'folding, sorting and degradation' (11,594 unigenes) pathways. In the 'environmental information processing' category, most unigenes were grouped into the 'signal transduction' (27,557 unigenes) and 'membrane transport' (26,101 unigenes). In the 'cellular processes' category, the major terms were 'cell motility' (19,559 unigenes) and 'cellular community' (9,011 unigenes) (**Additional file 4**).

Taxonomic profile of the two metagenomes

Based on the predicted ORFs, the taxonomy annotation and abundance of microbial species derived from the two sample groups was analyzed. At phylum level, 184 taxa were summarized from the two sample groups (**Additional file 5)** According to their annotation, *Proteobacteria* (37.7%), *Planctomycetes* (8.7%), *Chloroflexi* (2.5%), *Bacteroidetes* (2.2%), *Cyanobacteria* (1.6%), and *Actinobacteria* (1.0%) were considered to be the dominant phyla, accounting for more than 1% of the total population. At the phyla level, the significantly up-regulated taxa are *Methanosarcinaceae* (3.55 fold) and *Ichthyobacteriaceae* (4.78 fold), and the significantly down-regulated taxa are *Morchellaceae* (-2.26 fold), *Nautiliaceae* (-2.28 fold) and *Sterolibacteriaceae* (-1.75 fold).

At the genus level, a total of 3619 genera were obtained from the two sample groups. Among these genera, there are 112 genera with a relative abundance more than 0.1% of the total microbes. Microbial compositions for both CS and TS sample groups at genus level were showed in Fig. 2a. According to their

annotation, *Thioalkalivibrio* (2.73%), *Thauera* (1.94%), and *Pseudomonas* (0.1%) were considered to be the dominant genera, accounting for more than 0.1% of the total population (**Additional file 6**). Several dominant genera, including *Methanomethylovorans* (3.91 fold), *Stappia* (0.83 fold), *Mesorhizobium* (0,2 fold), *Desulfovibrio* (0.32 fold), *Mesotoga* (0.87 fold), and *Defluviicoccus* (0.31 fold), were significantly up-regulated during the treatment process. Contrarily, several other dominant genera, such as *Methyloversatilis* (-1.82 fold), *Elioraea* (-0.66 fold), *Rhodovulum* (-0.82 fold), and *Amaricoccus* (-0.92 fold), were significantly down-regulated during the treatment process **(**Fig. 2b**)**.

Analysis of the DEGs between CS and TS samples

A large number of DEGs, including 166,011 up-regulated and 151,567 down-regulated genes, were identified in our study (Fig. 3a). According to their annotations, most of the DEGs were assigned into different categories. For the GO classification, the top five significant enriched GO terms were 'symporter activity', 'phosphorelay signal transduction system', 'serine-type carboxypeptidase activity', 'peptide metabolic process', and 'metallocarboxypeptidase activity' (Fig. 3b). Most of the DEGs were grouped into 187 KEGG metabolic pathways (**Additional file 7**). Based on their KEGG classification, the top five significant enriched KEGG terms were 'Two-component system', 'Other glycan degradation', 'Sphingolipid metabolism', 'Galactose metabolism', and 'Bacterial chemotaxis' (Fig. 3c).

Analysis of xenobiotic biodegradation pathway-related KEGGs

Previous studies have reported several xenobiotic biodegradation pathways in different microbes [31]. In our study, a large number of DEGs involved in 15 typical xenobiotic biodegradation pathways were identified (**Additional file 8**). The benzoate degradation pathway (map00363) contained the largest number of DEGs, including 310 up- and 550 down-regulated genes. The second largest xenobiotic biodegradation pathway was the chloroalkane and chloroalkene degradation pathway, including 252 up- and 228 down-regulated genes. Aminobenzoate degradation pathway was the third largest xenobiotic biodegradation pathway, containing 125 up- and 198 down-regulated genes (Fig. 4).

Analysis of the genes involved in the nitrogen metabolic and sulfur metabolic pathways

A number of genes involved in the nitrogen metabolic and sulfur metabolic pathways have been reported in the past years [32]. For the nitrogen metabolism, the genes encoding five key enzymes, including nirK, nirB, nrfA, hao, and nirA, were identified in our study (Fig. 5a). For the sulfur metabolism, the genes encoding seven key enzymes, including suoX, dsrA, sir1, asr1, glpE, phsA, and fccB, were identified in our study (Fig. 5b). For the nitrogen metabolic pathway, most of the nirA and hao encoding genes were significantly up-regulated during the treatment (Fig. 5c). For the sulfur metabolic pathway, most of the

phsA encoding genes were up-regulated and most of the suoX encoding genes were down-regulated during the treatment (Fig. 5d).

Comparison of the microbial community between original and improved biofilters

In our study, comparison of the microbial community between original and improved biofilters has been performed. Firstly, we analyzed the changes between original and improved biofilters at phyla level. In the original biofilter, the significantly up-regulated phyla were *Proteobacteria*, *Euryarchaeota* and *Nitrospirae*, and the significantly down-regulated phyla were *Ignavibacteriae*, *Bacteroidetes*, and *Planctomycetes* (Fig. 6a). In the improved biofilter, the significantly up-regulated phyla were *Deferribacteres*, *Tenericutes*, and *Microsporidia*, which showed opposite responses in the original version. While in the improved biofilter, the significantly down-regulated phyla were *Elusimicrobia*, *Fibrobacteres*, and *Verrucomicrobia*, which showed similar responses in the original version (Fig. 6b).

Then, we analyzed the changes between original and improved biofilters at genera level. In the original biofilter, the significantly changed genera were *Ferroplasma* and *Cetobacterium*, which showed no responses in the improved version (Fig. 6c). In the improved biofilter, the most up-regulated genera was *Arcanobacterium*, which was siginificantly down-regulated in the original version, and the most down-regulated genera was Oleispira, which showed similar response in the original version (Fig. 6d).

Comparison of the functional genes between original and improved biofilters

Comparison of the functional genes between original and improved biofilters has been also performed. For the nitrogen metabolic pathway, average expression levels of the nirA, nifA and nirB encoding genes were significantly up-regulated in the improved biofilter and only the nirB encoding genes were up-regulated in the original version (Fig. 7a). For the sulfur metabolic pathway, most of the key genes were up-regulated in both of two biofilters, except for phsA and fccB. The average expression levels of the phsA and fccB encoding genes were significantly increased in the improved version (Fig. 7b).

Discussion

Microbial community structures were largely affected by odorous gas treatment [33, 34]. Based on a industrial scale biofiltration system, our previous study have revealed the changes in microbial communities and identified a number of functional genes involving in removing odorous gases [27]. Recently, this biofiltration system has been improved and higher effective in treating odorous gases was achieved by extending the acclimation period. Our present study has been detected 901,016 unigenes, which was larger than the unigenes detected in the previous study (496,718 unigenes), giving us an opportunity to screen more microbes and functional genes involved in removing odorous gases.

For many biofiltration systems, *Proteobacteria* was the most abundant phylum during the treatment process. For examples, *Proteobacteria* occupies 58–92% share in two butyric acid biofiltration systems [17], 3.55–51.03% share in a series of laboratory-scale landfill reactors [35], and 51.9% share in a drinking water biofiltration [36]. Addition to *Proteobacteria*, *Planctomycetes* and *Chloroflexi* also were reported to be the dominant phyla in various biofilter reactors [37, 38]. Our data also showed that *Proteobacteria*, *Planctomycetes* and *Chloroflexi* phylum possessed the largest shares in the improved biofilter (**Additional file 5**), suggesting the consistency in matters of microbial community. Previous studies have pointed out that microbes belonging to *Proteobacteria* participated in blackening and odor formation processes of odorous gases [39]. During the treatment process, microbes belonging to *Proteobacteria* were significantly reduced, indicated a close relationship between *Proteobacteria* and odorous gas removal.

At genera level, a number of microbes were reported to be involved in biological degradation. For examples, *Methanomethylovorans* was the dominant genus for degrading various types of polycyclic aromatic hydrocarbon [40]. For methanogenic degradation of tetraethylammonium hydroxide, *Methanomethylovorans* was the dominant genus isolated from the methanogenic degradation bioreactor [41]. *Mesorhizobium* was another genus associated the emission of sulfur-containing odors such as hydrogen sulfide, methyl mercaptan and dimethyl disulfide [42]. *Desulfovibrio* sp. SB8 was reported to be serve as core player for sulfate-reduction coupling polycyclic aromatic hydrocarbon degradation [43]. In our study, *Methanomethylovorans*, *Desulfovibrio* and *Mesorhizobium* were the most significantly up-regulated dominant genera during the treatment process, suggesting their important roles in removing odorous gases. Our study provided important guidance for screening and isolation of dominant microbes in odorous gas biofilter.

Elimination of NH_3 and H_2S is an important objective for the removal of odorous gases using biofilters [44]. Complex metabolic networks containing a number of key genes have been revealed in different microbes [45]. NH_4^- ion assimilation involves a nitrogen-cycling consisted of several key genes [46]. Meanwhile, the reaction of sulfur cycle that occurs in bio-system is essential for sulfur-containing odorous gases elimination [47]. In our study, a large number of homologous genes referring to each key gene were identified by metagenomic sequencing, suggesting the diversity of nitrogen-cycling microbial genes [46, 48]. Significantly changes in relative abundance of nitrogen- and sulfur-cycling related genes indicated an activated nitrogen and sulfur metabolisms in our biofilter.

Investigation of the differences between original and improved biofilters will help us to screen efficient microbial species and functional genes. Analysis of microbial communities in Nakabusa hot springs indicated that several possible nitrogen-fixing bacteria belonged to phylum *Thermotogae* [49]. Another study has reported that *Crenarchaeota* is closely related to the ammonia-oxidizer '*Nitrosopumilus maritimus*', indicating its potential contribution to nitrification in the biofilter [50]. Our study showed that *Thermotogae* and *Crenarchaeota* phyla were significantly up-regulated in the improved biofilter, suggesting its important role in nitrogen-fixing. A number of nitrite metabolism-related functional genes have been identified in the past years. For examples, several nitrite reductases encoded by *nirA*, *nirB* and *nirK* genes are important enzymes involved in fungal denitrification [51]. The nif-specific activator NifA

was reported to be involved in the regulation of nitrogenase activity during the nitrogen fixation process [52]. The expression of *hao* gene was highly correlating nitrogen removal and N_2O emission characteristics [53]. Compared with the original version, the average expression levels of *nirA* and *nifA* were significantly up-regulated the improved biofilter, suggesting their roles in assimilating ammonia-containing odorous gases. In the sulfur metabolic pathway, FccB genes encoding flavocytochrome c sulfide dehydrogenases oxidized endogenous or exogenous H_2S [54]. *PhsA* encoding the putative thiosulfate reductase participated in an intraspecies sulfur cycle [55]. Both of *fccB* and *phsA* were significantly up-regulated in the improved biofilter, suggesting an activated oxidization of H_2S during the odorous gas treatment. Our findings can be used for improving the efficiency of biofilter and helping the industrial enterprises to reduce the emission of waste gas.

List Of Abbreviations

ANOVA: one-way analysis of variance

amoA: ammonia monooxygenase

CAZy: Carbohydrate-Active enzymes

CDS: coding sequences

CS: control sample

DEG: differentially expressed gene

FDR: false discovery rate

GO: Gene Onotology

LCA: Lowest Common Ancestors

VOC: volatile organic compound

KEGG: Kyoto Encyclopedia of Genes and Genomes

TS: treated sample

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to publish

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files. The datasets generated and/or analysed during the current study are available in the NCBI as a BioProject with accession number PRJNA699130 (https://www.ncbi.nlm.nih.gov/search/all/?term=PRJNA699130).

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by the National Natural Science Foundation of China (Grant No. 21207028), Zhejiang Provincial Natural Science Foundation of China (Grant No. LY15B070013 and LY19C160001), and Hangzhou Science and Technology Development Foundation of China (Grant No. 20190101A02).

Authors' contributions

QL and JN conceived and designed the study. CS collected the samples. JN, LC, LZ, JX, and GX performed the experiments. JN, JX, and GX analyzed the data. CS and Qi wrote the manuscript.

Acknowledgements

We are also grateful to LC Sciences company (Hangzhou, China) for technical support.

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Figures

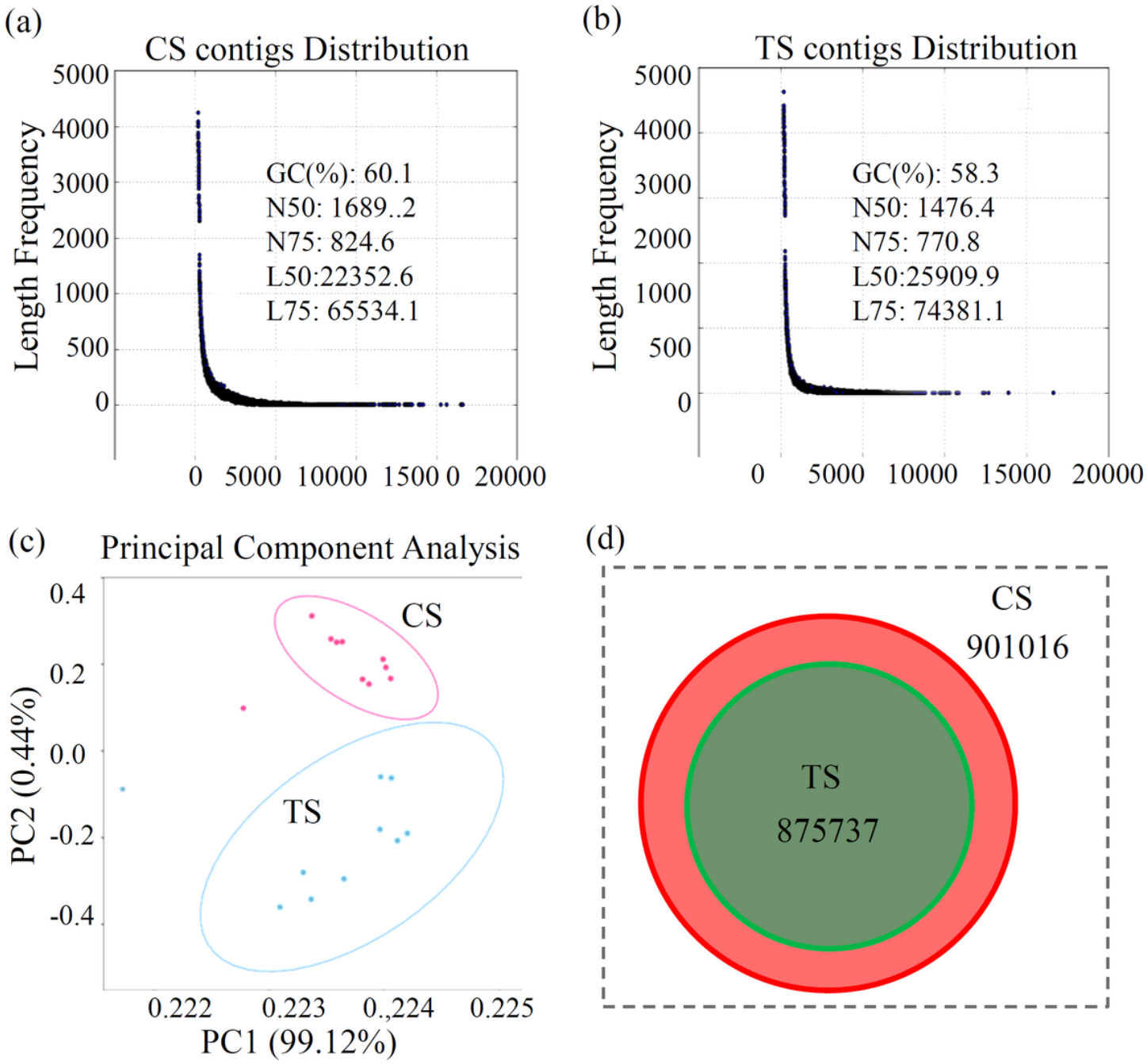

Figure 1

Overview of the metagenomes. (a) The detail information of all contigs from the sample group CS. (b) The detail information of all contigs from the sample group TS. (c) PC analysis of the data from two sample groups. (d) The numbers of unigene identified in the BT and AT sample groups are shown in a Venn diagram.

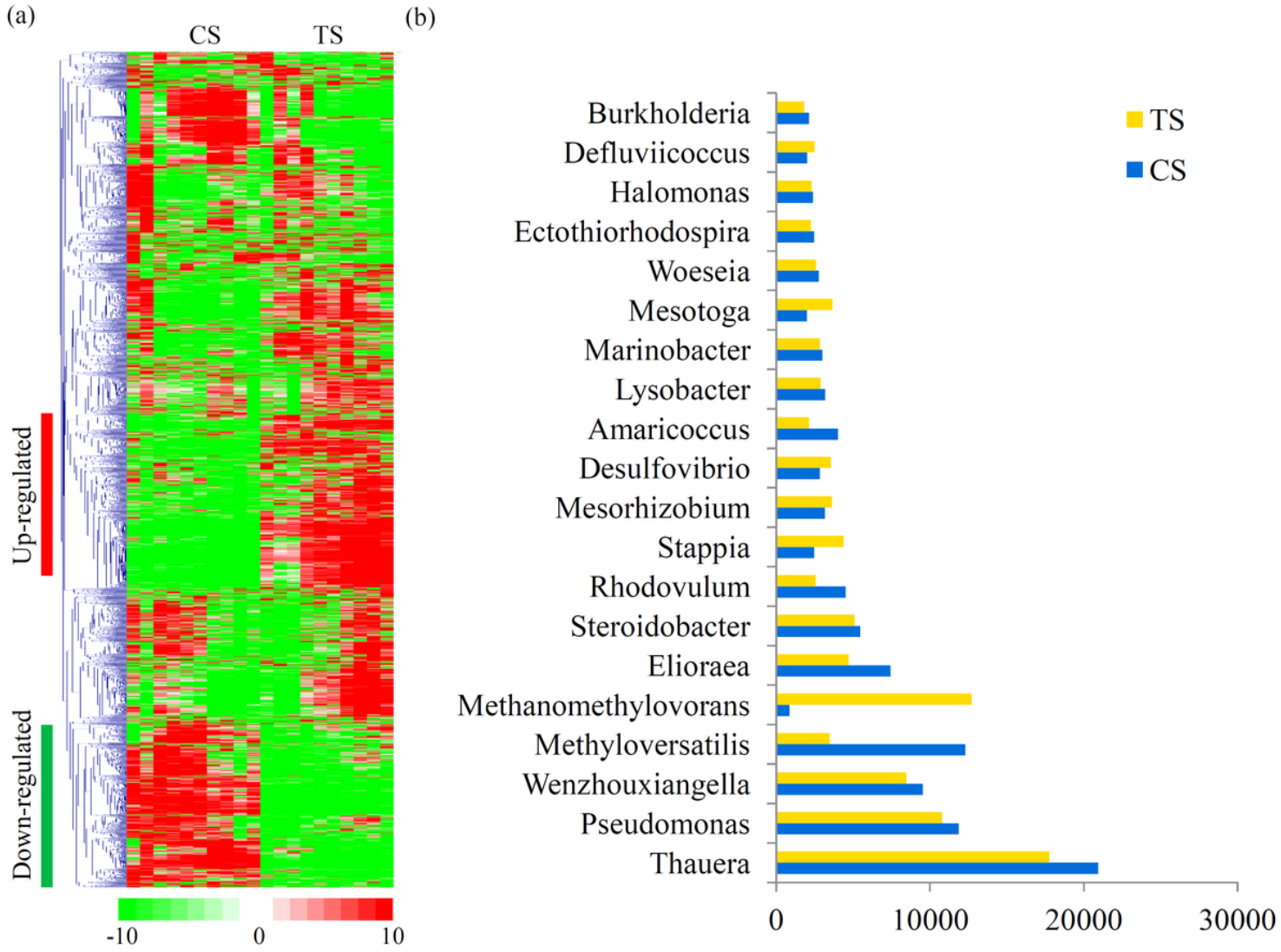

Figure 2

Comparative taxonomic profile of CS and TS metagenomes. (a) Microbial compositions for both CS and TS sample groups at genus level. Color intensity in each panel shows the relative abundances of each representative genus in the CS and TS sample groups. The heatmap scale ranges from −10 to +10 on a log2 scale. (b) Microbial compositions for both CS and TS sample groups at genus level.

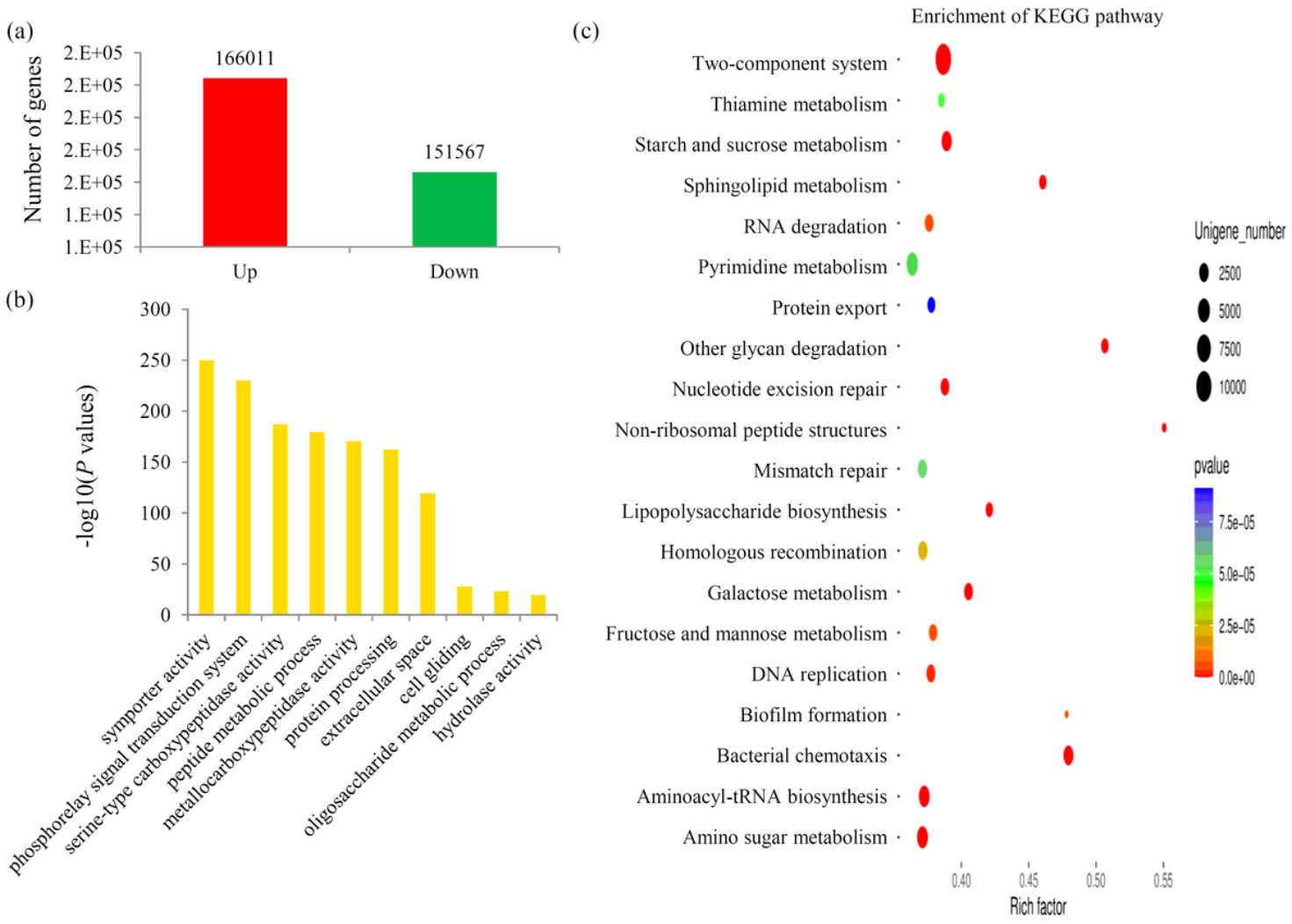

Figure 3

Analysis of differential expressed genes (DEGs) during the treatment. (a) The number of up- and down-regulated genes after treatment. (b) GO significance analysis of the DEGs between the CS and TS sample. (c) KEGG significance analysis of the DEGs between the CS and TS sample.

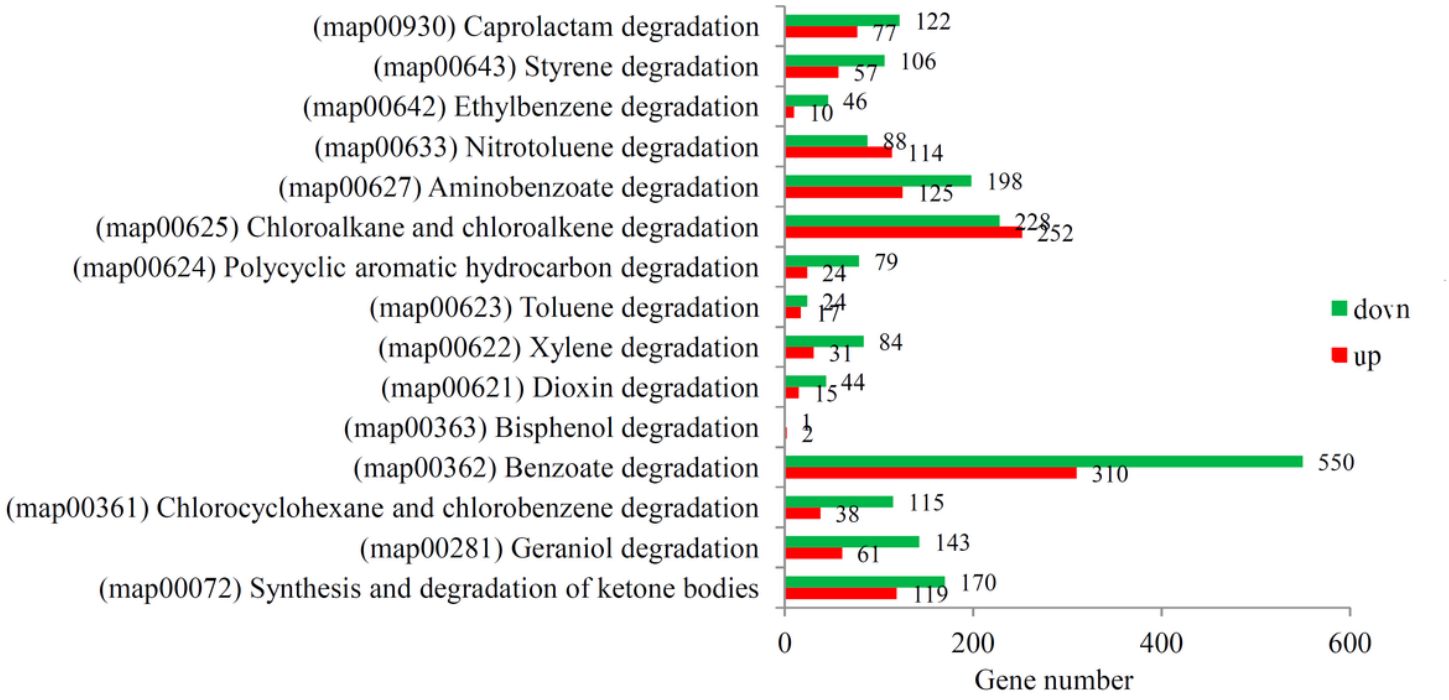

Figure 4

Analysis of xenobiotic biodegradation pathway-related KEGG terms. The number of up- and down-regulated genes related to xenobiotic biodegradation pathways after treatment.

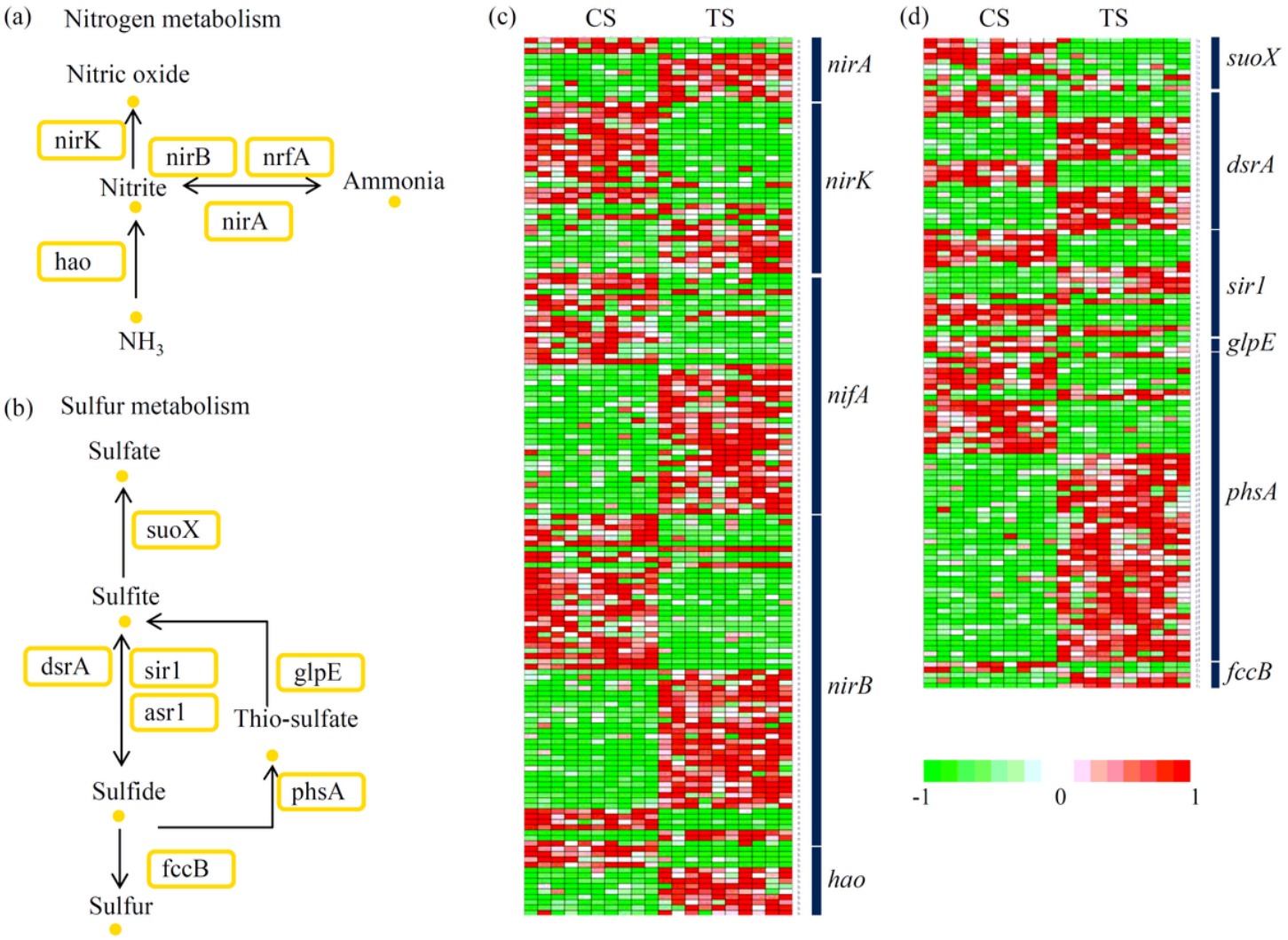

Figure 5

Analysis of the genes involved in the nitrogen metabolic and sulfur metabolic pathways. (a) Overview of the nitrogen metabolic pathway. (b) Overview of the sulfur metabolic pathway. (c) The relative abundances of the genes involved in the nitrogen metabolic pathway. (d) The relative abundances of the genes involved in the sulfur metabolic pathway. Red indicated low expression level and green indicated high expression level of each gene. The heatmap scale ranges from −1 to +1 on a log2 scale.

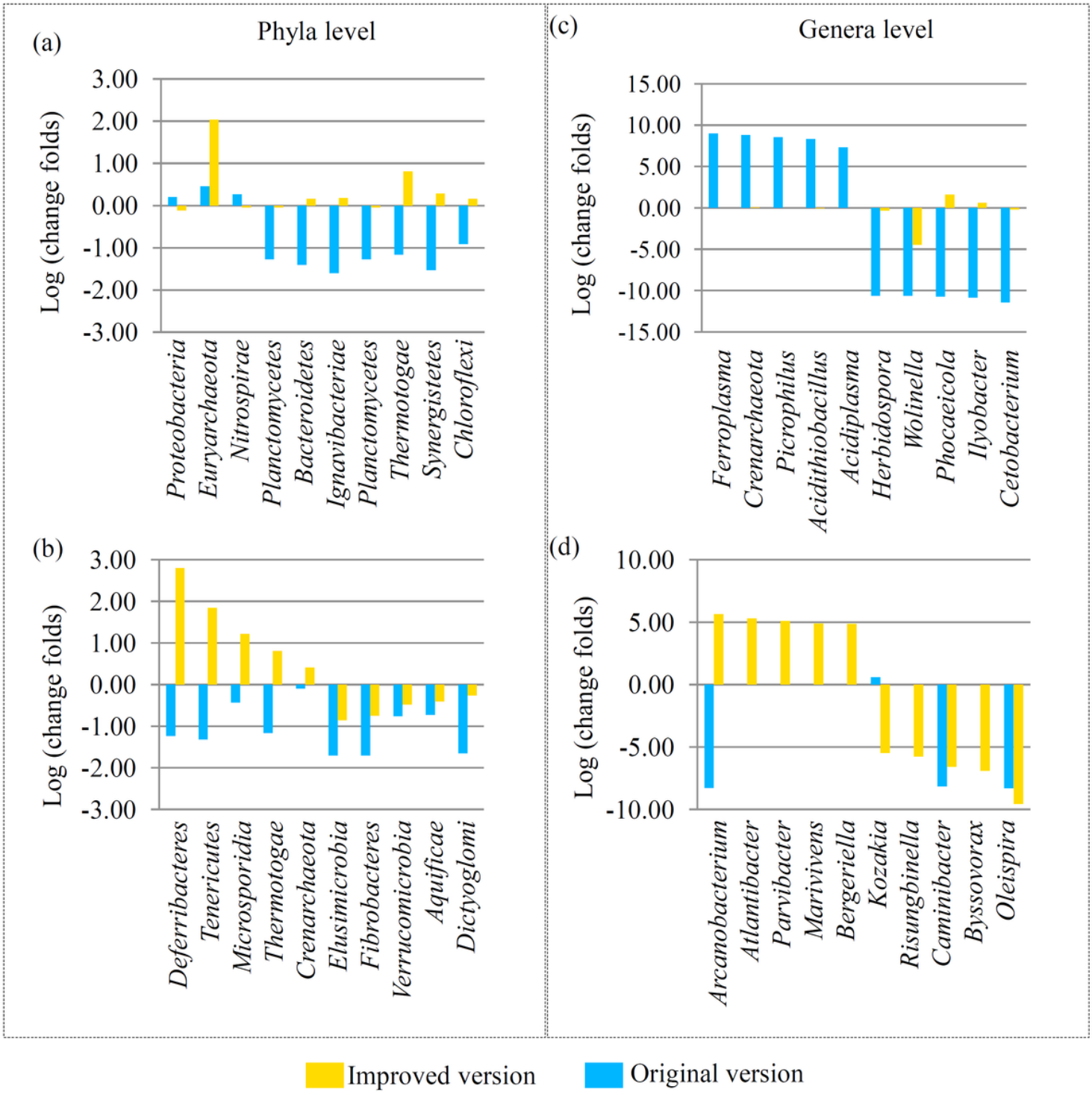

Figure 6

Comparison of the microbial community between original and improved biofilters. (a) Changes in microbial communities of the top 10 phyla that were significantly changed in the original biofilter. (b) Changes in microbial communities of the top 10 phyla that were significantly changed in the improved biofilter. (c) Changes in microbial communities of the top 10 genera that were significantly changed in the original biofilter. (d) Changes in microbial communities of the top 10 phyla that were significantly changed in the improved biofilter.

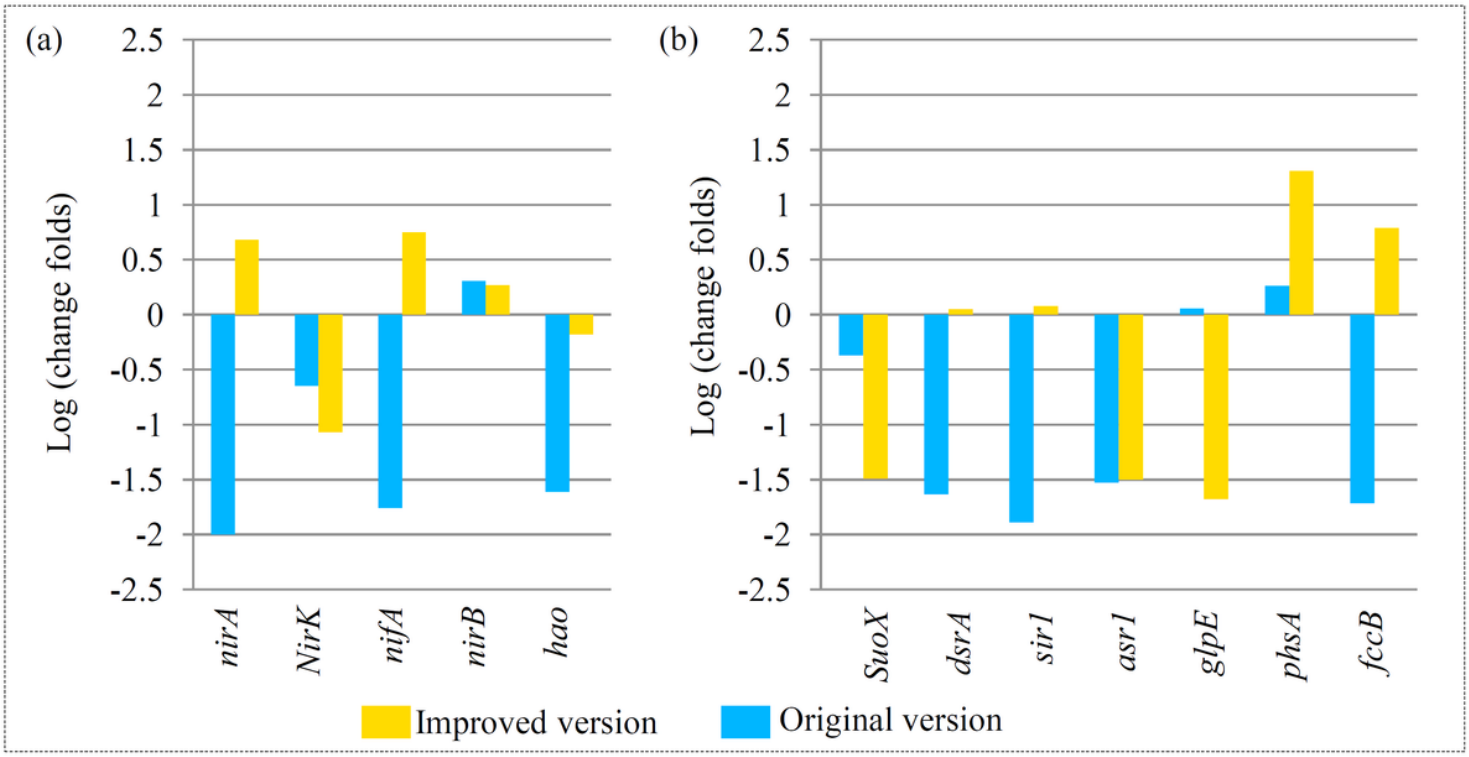

Figure 7

Comparison of the functional genes between original and improved biofilters. (a) The average expression levels of nitrogen metabolic pathway-related genes in both of the original and improved biofilters. (b) The average expression levels of sulfur metabolic pathway-related genes in both of the original and improved biofilters.

Supplementary Files

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