High efficient alternating anaerobic/aerobic process for polyester resin wastewater treatment : performance and microbial community structure

Sun Faqian; Hu Jian; Zhou Yan; Mei Rongwu; Wang Changzhi; He Yangyang; Wu Weixiang

2018

Sun, F., Hu, J., Zhou, Y., Mei, R., Wang, C., He, Y., & Wu, W. (2018). High efficient alternating anaerobic/aerobic process for polyester resin wastewater treatment : performance and microbial community structure. *Biochemical Engineering Journal*, 138, 121-130.
doi:10.1016/j.bej.2018.07.005

<https://hdl.handle.net/10356/136911>

<https://doi.org/10.1016/j.bej.2018.07.005>

© 2018 Elsevier B.V. All rights reserved. This paper was published in *Biochemical Engineering Journal* and is made available with permission of Elsevier B.V.

Downloaded on 26 Apr 2023 18:32:49 SGT

Accepted Manuscript

Title: High efficient alternating anaerobic/aerobic process for polyester resin wastewater treatment: Performance and microbial community structure

Authors: Faqian Sun, Jian Hu, Yan Zhou, Rongwu Mei, Changzhi Wang, Yangyang He, Weixiang Wu

PII: S1369-703X(18)30223-7
DOI: <https://doi.org/10.1016/j.bej.2018.07.005>
Reference: BEJ 6991

To appear in: *Biochemical Engineering Journal*

Received date: 26-10-2017
Revised date: 6-7-2018
Accepted date: 6-7-2018

Please cite this article as: Sun F, Hu J, Zhou Y, Mei R, Wang C, He Y, Wu W, High efficient alternating anaerobic/aerobic process for polyester resin wastewater treatment: Performance and microbial community structure, *Biochemical Engineering Journal* (2018), <https://doi.org/10.1016/j.bej.2018.07.005>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

High efficient alternating anaerobic/aerobic process for polyester resin wastewater treatment: Performance and microbial community structure

Faqian Sun ^{a, b, c}, Jian Hu ^a, Yan Zhou ^{c, d}, Rongwu Mei ^e, Changzhi Wang ^e, Yangyang He ^a, Weixiang Wu ^{a, *}

^a Zhejiang Province Key Laboratory of Water Pollution Control and Environmental Safety, Department of Environmental Engineering, Zhejiang University, Hangzhou. 310058, China

^b College of Geography and Environmental Science, Zhejiang Normal University, Jinhua 321004, China

^c Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 637141, Singapore

^d School of Civil and Environmental Engineering, Nanyang Technological University, 639798, Singapore

^e Zhejiang Province Key Laboratory of Environmental Pollution Control Technology, Hangzhou 310007, China

*Corresponding author: Weixiang Wu

Address: Yuhangtang Road 866#, Hangzhou, 310058, China

E-mail: weixiang@zju.edu.cn

Tel: +86-571-88982020

Fax: +86-571-88902020

Highlights

- The two-stage A/O process treating polyester resin wastewater was evaluated
- High degradation rates of COD, phenol and PTA were achieved
- The removal for organics were monitored by GC-MS analysis
- 16S rRNA high-throughput sequencing provides new insight on bacterial community

Abstract

This study investigated organics removal and microbial community in a two-stage alternating anaerobic/aerobic (A1/O1/A2/O2) process for polyester resin wastewater treatment. It was found that chemical oxygen demand (COD) concentration could be reduced to below 500 mg L⁻¹ from 12,880 mg L⁻¹ with average COD removal rate of 2.79, 4.37, 0.50 and 0.97 kg m⁻³ d⁻¹ in four reactors, respectively. Two important chemical compounds in the wastewater, namely, purified terephthalic acid (PTA) (625 mg L⁻¹) and phenol (480 mg L⁻¹), did not display inhibitory effects on COD removal while they were almost completely degraded in the first-stage A/O process. Further, the majority of esters, alkanes, phenols, acids and alcohols could be removed in the system. However, two hazardous refractory compounds, 5,5-dimethyl-1,3-dioxane and 1,4-dioxane, were still present as major components in the final effluent. 16S rRNA

high-throughput sequencing results revealed that a distinct preference of selected bacterial groups towards anaerobic/aerobic reactors and operating conditions. High abundance of *Lewinella* genus (2.4-34.0%), which was correlated to ester-hydrolyzing ability, may play an important role in the high efficiency of organics removal.

Keywords: Polyester resin wastewater; Alternating anaerobic/aerobic; Purified terephthalic acid; Phenol; Microbial community

1. Introduction

Unsaturated polyester resins have a good balance of mechanical, electrical, thermal and chemical resistance properties at relatively low cost, hence the global industrial market demand for polyester resins continues to grow substantially in a variety of applications, ranging from automobile and electronic equipment to packaging and building materials [1, 2]. However, in polyester resin production, approximately 0.2-0.3 m³ wastewater per ton polyester resin is generated with the chemical oxygen demand (COD) concentration of 3-20 g L⁻¹. The polyester resin wastewater is very chemical-intensive in which two global important chemical compounds, purified terephthalic acid (1,4-benzenecarboxylic acid) (PTA) and phenol, are the main

pollutants. Besides, it contains other synthesis-related chemicals and their derivatives which greatly increase its organic loads during wastewater treatment [3]. These compounds have a serious environmental impact due to their high toxicity [4]. Therefore, it is important to remove these organics from the polyester resin wastewater.

Considering the complex characteristics of polyester resin wastewater, many physicochemical methods, such as air stripping, incineration, electric oxidation and Fenton oxidation, have been investigated for its wastewater treatment [5]. However, these methods are very expensive. In comparison, the biological process seems to have clear advantages due to its ability to effectively remove pollutants in a more environmentally friendly and cost-effective way [6]. However, to date, very few successful biological applications have been developed for high strength polyester resin wastewater.

In the past, the majority of studies have focused on biological treatment of wastewater containing PTA or phenol which was also identified as main components in polyester resin wastewater. Macarie et al. [7] documented that anaerobic treatment of PTA wastewater was successfully applied in fixed film reactor at the removal rate of $1.41 \text{ kg COD m}^{-3} \text{ d}^{-1}$. However, they also observed that the process was easily inhibited in the up flow anaerobic sludge blanket reactor (UASB). Kleerebezem et al. [8] further confirmed that components like benzoate and acetate in PTA wastewater inhibited the anaerobic degradation of terephthalate. Under this condition, recent studies reported encouraging results for solving bottleneck of terephthalate degradation. Kleerebezem et

al. [9] proposed a two-stage anaerobic bioreactor for high-rate treatment of PTA wastewater, which consisted of the first stage for pre-removal of acetate and benzoate, and the latter stage for terephthalate degradation. Tsuno and Kawamura [10] developed an expanded-bed anaerobic reactor with the high adsorptive function of granular activated carbon (GAC), in which more than 90% of COD could be removed at the loading rate of $4 \text{ kg COD m}^{-3} \text{ d}^{-1}$. Similarly, phenol is commonly present in various types of industrial wastewaters, including petrochemical refineries, coke production and chemical compounds production. It is also a toxic substrate for many microorganisms and has shown to severely inhibit the biological treatment at the concentration higher than 50 mg L^{-1} [11]. Compared with PTA wastewater, the inhibitory effect of phenol on the bacterial activity seems higher. Rosenkranz et al. [12] only obtained the maximum removal rate of $0.18 \text{ kg phenol m}^{-3} \text{ d}^{-1}$ with anaerobic sequencing batch reactor.

Although many anaerobic approaches have been reported to mineralize PTA or phenol, a growing body of evidence suggests that many problems (strong inhibition of anaerobic digestion in the presence of other organic matter, sensitivity to loading shocks, and long recovery periods after shocks) were still encountered in practical application [9, 13], thus requiring process improvements. In addition, the interactive effects of several kinds of toxic pollutants may further deteriorate the organics removal [14].

Polyester resin wastewater contains high concentrations of PTA, phenol, as well as many unfavorable impurities like polyester at low molecular weights. Considering the composition of polyester resin wastewater, a distinction can be made between

compounds that are readily degradable (PTA and phenol) and compounds (low-weight polyester) that can only be degraded at very low rates. In order to obtain high-efficiency in organic compounds removal, a two-stage alternating anaerobic/aerobic (A/O) process, which is composed of a first stage A/O for readily degradable compounds removal, and a second stage A/O for enhanced degradation of non-readily degradable compounds, is perhaps the most logical strategy. Compared with one-stage A/O process, the two-stage alternating A/O process could enrich various kinds of functional bacteria in the different reactors, and separately favored the degradation of readily degradable in the first A/O and non-readily degradable compounds in the second A/O, respectively, thus higher effluent quality could be obtained.

The aims of this paper are: (1) to study the removal performance of the two-stage alternating A/O process fed with high-strength polyester resin wastewater, (2) to characterize in detail organic chemical compositions in the individual reactors, (3) to assess the relationship between pollutant removal and the structure of microbial community developed in the process.

2. Materials and methods

2.1 Experimental set-up and operating conditions

The schematic diagram of the two-stage alternating A/O process is shown in **Fig. 1**. The experiments were carried out in a sequential system of anaerobic reactor (A1), an aerobic reactor (O1), a clarifier, a second anaerobic reactor (A2), followed by a second

aerobic reactor (O2), and a final clarifier. The working volume of reactors A1, O1, A2 and O2 was 8.5 L, 6.5 L, 9.5 L and 6.5 L, respectively. In the anaerobic reactors (A1 and A2), mechanical mixers were equipped in the bottom of reactors, and polyethylene carriers were packed for biofilm growth. In the aerobic reactors (O1 and O2), aeration was provided with fine bubble air diffusers, and dissolved oxygen (DO) was controlled at about 1.0 – 2.5 mg/L and 2.0 - 4.0 mg L⁻¹, respectively. Two clarifiers following the aerobic reactors had an identical working volume of 3 L. The system was operated at an external effluent flow from the clarifier to holding tank in which raw wastewater dilutions, pH adjustments and nutrients addition were carried out. Effluent from the holding tank was continuously fed into subsequent reactors at a constant rate of 5 L d⁻¹, resulting in hydraulic retention time of 1.7 d, 1.3 d, 1.9 d and 1.3 d in A1, O1, A2 and O2, respectively. Temperature in the bioreactor was about 21 ± 1 °C. The sludge retention time (SRT) of reactor O1 and O2 was maintained at about 10 d and 26 d, respectively. Mixed liquor suspended solids (MLSS) concentration in the reactor O1 and O2 was 4000 ± 1300 and 4400 ± 1100 mg L⁻¹.

As part of the start-up phase, the system was inoculated with the anaerobic sludge from polyester resin wastewater treatment plant (WWTP) (Linan, China). After a 20 day start-up in the lab, a relatively steady-state operation of the system at low COD concentrations was first assessed (Phase I). In Phase I (day 1-55), COD concentration in the holding tank was in the range of 8,180-9,480 mg L⁻¹. Afterward, the degradation capacity of the system at elevated COD concentrations was investigated in Phase II (day 56-114). COD concentration in the holding tank was gradually increased until the

effluent COD concentration was higher than 500 mg L⁻¹ (discharge limit for industrial wastewater in China).

2.2 Characteristics of polyester resin wastewater

Polyester resin wastewater was collected from Transfar Chemicals Co. Ltd, one of the largest polyester resin manufacturing factories located in the southeast of China. The wastewater was produced from a mixture of various manufacturing processes using PTA, neopentyl glycol, isophthalic acid, methylpropanediol, ethylene glycol, diethyleneglycol, trimellitic anhydride and hexanedioic acid in the decreasing order. Its physical and chemical properties are shown in **Table 1**.

2.3 Chemical analysis

Collected samples were filtered through a 0.45 µm membrane filter prior to the analysis of soluble fraction of water samples. COD were measured by the Hach COD kits TNT 822 (Hach, USA). Phenol was analyzed with 4-aminoantipyrine spectrophotometric method at the wavelength of 510 nm on a spectrophotometer (TU-1810, Persee, China) [4]. PTA was analyzed with the ultraviolet spectrophotometric method at the wavelength of 240 nm [10, 15]. The pH was measured with a pH meter (SG2, Mettler-Toledo, Greifensee, Switzerland) and DO by an oxygen probe (YSI 550A, YSI, Ohio, USA). Phosphorus (PO₄³⁻-P) and MLSS were analyzed according to the standard methods (Method 4500-P and 2540D, respectively) for the examination of water and wastewater [16].

Gas chromatograph-mass spectrometer (GC-MS) was used to analyze the samples with liquid-liquid extraction pretreatment using CH_2Cl_2 as described in [Sun et al. \[17\]](#). In brief, 200 mL filtered sample was extracted using 10 mL of CH_2Cl_2 (chromatogram pure grade, Fisher, USA) three times at neutral, alkaline and acidic conditions, respectively. Then, a 1 mL extracted sample was analyzed by GC-MS system (6890N/5975B, Agilent, USA). A DB-5MS capillary column (Agilent, USA) with an inner diameter of 0.25 mm and a length of 30 m was used in the separation system. The GC column was operated in a temperature programmed mode by maintaining the temperature at 40 °C for 4 min, then increasing to 300 °C with an increment of 8 °C min^{-1} , and total run time was 43 min. The electron impact energy was set at 70 eV. Organic compounds analysis was undertaken with reference to the instrument NIST 08 library and the compound was deemed identified and reported if the match percentage was higher than 70%.

2.4 Illumina high-throughput sequencing

Microbial community structure was determined using the Illumina MiSeq. 13 activated sludge (AS) samples were periodically taken from bulk liquid in the reactors for DNA extraction. Specifically, eight AS samples were taken from reactors A1, O1, A2 and O2 at two low loading phases (L1A1, L1O1, L1A2, L1O2, and L2A1, L2O1, L2A2, L2O2) and four samples were from the elevated loading phase (HA1, HO1, HA2 and HO2), respectively, as indicated by the inverted triangle in **Fig. 2**. One AS sample was taken from the seed sludge (SS). DNA extraction of the samples was carried out in duplicate

using the Power Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's protocol.

DNA extracts of the samples were amplified in triplicate with primer pair 520F and 806R with different barcodes for the V4 region of 16S rRNA gene [18]. The PCR was performed at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. PCR products were examined on a 2% agarose gel and the band was purified by the AxyPrepDNA Gel Extraction Kit (Axygen, CA, USA). Amplicons were pooled in an equimolar mix and sent out for sequencing on the Illumina MiSeq platform at the Personal Biotechnology Co., Ltd (Shanghai, China). Sequencing raw reads were deposited in the NCBI under accession number SRP065107.

Data were processed following the procedure described previously [19]. Paired-end reads were joined by Flash, and sequences of low quality were then trimmed using Quantitative Insights into Microbial Ecology (QIIME). Chimera was detected by MOTHUR software (V 1.31.2) using UCHIME method, and the resulting high-quality sequences were clustered and assigned to operational taxonomic units (OTUs) using the QIIME implementation of UCLUST with a threshold of 97% sequence similarity. A representative sequence from each OTU was submitted to the RDP Classifier to identify bacterial taxa. The average length of all bacterial sequences without the primers was 225 bp.

The bacterial composition of the samples was compared at the family level and at the approximate genus level. Multivariate statistical analyses were performed with

Canoco 5 (Biometris–Plant Research International, Wageningen, NL) [20]. Principal Coordinates Analysis (PCoA) was used to examine the dissimilarity between samples using Bray-Curtis distance [21]. Redundancy Analysis (RDA) at the genus level was also employed to visualize the relationship between different reactor samples, microbial functional groups and main process operational variables. Rare genus, with less than 0.5% abundance, were excluded from the analysis. The significance of each operational variation on the microbial community was evaluated using the Monte Carlo permutation test (999 replicates). Pearson's correlation between two parameters under different conditions was performed using the software SPSS 18.0.

3. Results

3.1 System performance

Due to the variation of COD compositions and concentrations in the influent, a responsive mode, which was operated with adjusting influent COD concentration according to the effluent COD concentration, was developed in this study to accomplish discharge level regulations (500 COD mg/L). **Fig. 2** presented the time course of COD concentration and COD removal rate in four reactors. During Phase I, COD concentration in the feed was kept at $8,656 \pm 447 \text{ mg L}^{-1}$. Effluent COD concentrations from the reactors A1, O1, A2 and O2 were $6,585 \pm 614 \text{ mg L}^{-1}$, $1,393 \pm 657 \text{ mg L}^{-1}$, $1,319 \pm 393 \text{ mg L}^{-1}$ and $429 \pm 56 \text{ mg L}^{-1}$, respectively. The average COD removal rates in these four reactors were calculated as 0.80, 3.09, 0.03 and $0.55 \text{ kg m}^{-3} \text{ d}^{-1}$, respectively. The total COD removal efficiency could reach as high as 93%.

In the elevated phase (Phase II), COD concentration in the feed was stepwise increased. At the beginning of Phase II (day 56-69), when COD concentration in the feed was increased to 11,610 mg L⁻¹, an abrupt increase in the effluent COD concentration of reactor O2 occurred and COD concentration exceeded the discharge level of 500 mg L⁻¹. After 8 days, effluent COD concentration gradually decreased to lower than 500 mg L⁻¹. From day 70 to 90, the final effluent COD concentration remained stable. COD concentrations in four reactors averaged 6,218 ± 1,652 mg L⁻¹, 2,162 ± 777 mg L⁻¹, 1,484 ± 314 mg L⁻¹ and 388 ± 58 mg L⁻¹, respectively. Overall, the effluent COD concentrations in Phase II were more variable, especially in reactor A1. The main reason could be that with the elevated COD concentrations in the influent, the anaerobic sludge has not adapted to perform high efficiency. From day 92, COD concentration in the feed was further increased to 14,580 mg L⁻¹. On day 100, effluent COD from O2 concentration increased to 507 mg L⁻¹ ($p < 0.05$). The increase of effluent COD concentration reflected the highest capability of the process. As a result, the average COD removal rate of 2.79, 4.37, 0.50 and 0.97 kg m⁻³ d⁻¹ was achieved in reactors A1, O1, A2 and O2, respectively. To prevent deterioration of COD removal performance, COD concentration of the holding tank was again decreased to 12,880 mg L⁻¹ on day 104. As a result, the reactors again recovered good performance and effluent COD concentration from reactor O2 declined to lower than 500 mg L⁻¹.

3.2 Biodegradation of phenol and PTA

As described, phenol and PTA were two important toxic pollutants in polyester resin

wastewater. The effluent phenol and PTA concentrations of each reactor were recorded in **Fig. 3**. During the operational period, phenol concentration in the feed tank was gradually elevated to 483 mg L^{-1} with the increase of COD loading (**Fig. 3A**). Similar to COD, effluent phenol concentration in reactor A1 increased and became more variable with the elevated feeding concentration and an average concentration of $120 \text{ mg phenol L}^{-1}$ was removed in reactor A1. Phenol concentration in the reactors O1, A2 and O2 always remained low, which were about 9.9, 5.5 and 0.9 mg L^{-1} on average, respectively. During the shock loading of COD on days 70-90, low phenol concentration in the reactor O2 was consistently achieved, irrespective of an increase in COD concentration. The average phenol removal rates in reactors A1 and O1 was 0.07 and $0.18 \text{ kg m}^{-3} \text{ d}^{-1}$, respectively (**Fig. 3C**).

The removal pattern of PTA was similar to that of phenol. As seen in **Fig. 3B**, the system also exhibited high efficiency for PTA removal. PTA concentration of reactor A1 fluctuated with feeding concentration, and an average of $140 \text{ mg PTA L}^{-1}$ was removed in reactor A1 and the majority of PTA was decomposed in reactor O1. During the whole experimental period, effluent PTA from reactor O2 remained at a constant concentration of $22 \pm 9 \text{ mg L}^{-1}$, and the average removal rate of PTA in reactors A1, O1, A2 and O2 was 0.06 , 0.20 , 0.02 and $0.01 \text{ kg m}^{-3} \text{ d}^{-1}$, respectively (**Fig. 3D**).

During the study, both PTA and phenol concentrations in the effluent were very low, implying that the process showed good performance for PTA and phenol removal.

When PTA and phenol concentrations in the feed were elevated to 625 and 483 mg L^{-1} ,

PTA and phenol concentrations in the reactor O1 increased coinstantaneously, reaching its peak on day 111.

3.3 Biodegradation of other organic compounds

In order to gain more insight into the composition of organic compounds in the polyester resin wastewater and explore the remaining component of organic chemicals in each reactor, GC–MS analysis was carried out with samples collected on days 3, 55 and 114. The details of organic compounds are summarized in **Table 2**. It appears that certain compounds, such as the alkanes (5,5-dimethyl-1,3-dioxane, 4-ethyl-4-methyl-2-pentadecyl-1,3-Dioxolane), esters (butanoic acid, hexyl ester, propanoic acid, 2-methyl-butyl ester, propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester), 3-thiol-5-methyl-1H-1,2,4-Triazole, and alcohols (2,2-dimethyl-1,3-propanediol, 3-chloro-2,2-dimethyl-1-propanol), which each covered more than 5% of integration area, dominated in the raw wastewater, whereas other compounds were the minor species. These organic compounds in the influent are generally composed of polyester resin synthesis-related chemicals and their derivatives. After the treatment by reactor A1, many compounds were degraded or converted, such as 2,2-dimethyl-1,3-propanediol, 3-buten-1-ol. It is interesting to note that the abundance of many esters, such as butanoic acid, hexyl ester, propanoic acid, 2-methyl-butyl ester, propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester, declined greatly. Meanwhile, it was observed that some new intermediates, benzoic acid, 2-methyl-propanoic acid, butanoic acid, were produced, indicating that several esters

were probably converted due to the hydrolysis/acidification in reactor A1.

The residual compounds from the A1 effluent were removed at the aerobic stage of reactor O1. It can be seen from **Table 2** that a considerable amount of benzene series organics and alkanes, which were hardly biodegraded at the anaerobic stage, were degraded in aerobic tank. Similar to previous phenol removal analysis, phenol was removed completely in reactor O1. Furthermore, the majority of new intermediates produced from reactor A1 were removed at reactor O1. However, it could also be observed that the relative abundance of two compounds, 5,5-dimethyl-1,3-dioxane and 1,4-dioxane, increased to up to 32.4 and 20.3%, respectively.

In the second stage of A/O process, some new intermediates, such as para-methoxybenzenethiol, 4-methyl-phenol, benzeneacetic acid, and n-Hexadecanoic acid, were produced in reactor A2. The majority of the residual compounds in the A2 effluents were further degraded in the reactor O2. However, two compounds, 5,5-dimethyl-1,3-dioxane and 1,4-dioxane, corresponding to 14.1-37.1% and 21.2-26.2% of the total integration area, respectively, were quite stable in the system.

3.4 Taxonomic complexity of the bacterial community

Microbial community analysis demonstrated that the communities in the reactor differed substantially from the original inoculum. The selected effective bacterial and archaeal sequences yielded for 13 samples were assigned to different taxa levels using the RDP Classifier at 50% threshold. **Fig. 4** summarizes the relative bacterial

community abundances on the phylum level for each sample. In the sample SS, *Firmicutes* was the most abundant phylum, accounting for 33.6% of all detected OTUs. *Bacteroidetes*, *Chloroflexi*, *Thermotogae* and *Proteobacteria*, were more abundant groups, comprising 13.8%, 9.9%, 9.1% and 6.7% of the reads, respectively. After the operation in the two-stage alternating A/O process, AS samples exhibited a similar pattern in which marked change in community structure was observed. In reactor samples A1 (L1A1, L2A1 and HA1), *Bacteroidetes*, *Firmicutes* and *Proteobacteria* were three abundant phyla comprising 13.2-35.2%, 18.0-34.0% and 19.5-22.7% of the reads, respectively. In contrast, in the other 9 reactor samples, *Bacteroidetes* and *Proteobacteria* were still highly represented groups (38.1-54.1% and 13.0-47.5%). However, *Firmicutes* and *Chloroflexi* were comprised of only 0.6 to 5.1% and 0.9 to 7.4% of the reads, respectively. Overall, these four phyla represented approximately 61.0 to 93.6% of bacteria detected within the 12 reactor samples.

At the class level (**Table S1**), the class *Clostridia* within the phylum *Firmicutes*, was most dominant in reactor A1 (L1A1, L2A1 and HA1, 14.1, 23.3 and 18.0%) and samples SS (30.2%), less abundant in reactor A2 (2.0, 4.0 and 3.4%), but was only a minor component of AS samples from reactor O2 (L1O2, L2O2 and HO2, 0.6, 0.3 and 0.6%). Within the phylum *Bacteroidetes*, two classes, *Sphingobacteria* (4.3-36.3%) and *Bacteroidia* (0.5-23.7%) was highly represented in all reactor samples. Within the phylum *Proteobacteria*, the classes *Betaproteobacteria* (4.7-34.8%), *Alphaproteobacteria* (3.6-14.2%) and *Deltaproteobacteria* (1.0-6.9%), fluctuated in reactor samples and exhibited higher abundance in aerobic reactors.

The relative bacterial community abundances on the genus level for each sample were shown in supplementary **Table 3**. At the genus level, it was found that *Lewinella*, a genus within the family *Saprospiraceae* and the phylum *Bacteroidetes*, was highly represented (2.4-34.4%) in all AS samples, particular in reactor O1, A2, O2. In addition, the members of *Hydrogenophaga*, *Thauera* and *Terrimonas* were also common genera (abundance > 0.5%) in all samples. Except these four shared genera, other abundant populations in each sample were further analyzed. For reactor A1 samples, the abundance of top genera was in the range of 2.5-7.5% and no core genus was observed. However, the abundant genera under different COD loadings showed a different pattern. Three genera extensively reported in sewage AS, that is *Trichococcus*, and *Acinetobacter*, were more abundant in LA1, whereas in HA1, *Sedimentibacter* and *Azotobacter* were more abundant genera. With regard to other reactors, the more abundant genera in LO1 were *Sediminibacterium* and *Zoogloea*, while members of *Brevundimonas* and *Rhodococcus* were positively enriched in HO1. In the reactor O2, several genera such as *Bosea*, *Plesiocystis*, *Thauera* and *Ohtaekwangia*, greatly enriched.

3.5 The relationships between bacterial community and operating process

To visualize the similarities of bacterial communities among four successive reactors and examine whether bacterial communities correlate with critical operational parameters under three operating conditions, PCoA together with RDA analysis at the genus levels were conducted. The PCoA analysis (**Fig. 5A**) showed that the two first ordination axes explained 29.2 and 17.1% of the variability observed in community

structure based on bacterial genus relative abundances. It was observed that the community structure of AS samples from reactor A1 is different from those from other 3 reactors. To further explore the possible association of high-rank genus ($> 0.5\%$ in any AS sample) in response to critical operational parameters (COD concentration, pH and DO concentration), an RDA analysis was performed, as indicated in **Fig. 5B**. According to the permutation test, COD concentration and pH were two significant factors ($p < 0.05$) explaining the variations of bacterial structure. From **Fig. 5B** and **Table 3**, it is also possible to confirm that several abundant genera, such as *Lewinella*, *Thauera*, *Azoarcus* and *Acetobacterium* was associated to different operational parameters.

4. Discussion

This work shows that the two-stage alternating A/O process appears to be an attractive technological option for polyester resin wastewater treatment. There are no other comparative studies for polyester resin wastewater treatment, but removal rate values observed can be compared with values reported for similar industrial wastewater systems in the literature. The COD removal rate in this study was comparable to the value reported by Tsuno and Kawamura [10] with anaerobic expanded-bed GAC reactor for treating PTA wastewater. The maximum influent COD concentration in the present study could reach up to 12880 mg L^{-1} , which was much higher than previous studies [10, 22]. Under this condition, the final biological effluent could meet the national discharge standard of water pollutants for industrial wastewater in China ($\text{COD} < 500 \text{ mg L}^{-1}$), and was discharged to municipal WWTP for further treatment. However, the influent

COD concentration of 14800 mg L⁻¹ deteriorated the final effluent quality probably due to the accumulation of more recalcitrant organics.

Phenol is a common toxic contaminant in polyester resin wastewater that may have significant effects on reactor performance. Fang et al. [23] reported that over 99% of phenol was effectively degraded in UASB at 55 °C with HRT of 40 h for a raw wastewater containing 630 mg phenol L⁻¹. Rosenkranz et al. [12] found that influent phenol concentration lower than 800 mg L⁻¹ had little effect on the phenol removal efficiency with the removal rate up to 0.18 kg m⁻³ d⁻¹. Our study is similar to these studies in terms of adaptation to increased phenol concentration, indicating that the influent phenol concentration of 500 mg L⁻¹ had little effect on reactor performance.

PTA is another important toxic substance in polyester resin wastewater. In previous studies, the presence of readily degradable compounds in wastewater, such as benzoic acid, had strongly rate limiting effects on terephthalate degradation [9]. Only in the absence of readily degradable compounds in the influent, substantial terephthalate degradation can be achieved. In the present study, such inhibitory effect may be negligible, since the removal of COD, phenol and PTA occur simultaneously.

The majority of esters, alkanes, phenols, acids and alcohols could be removed in the system (Table 2). However, there are many refractory contaminants present in the final effluent which may reduce the water quality. Although the relative abundances of 5,5-dimethyl-1,3-dioxane and 1,4-dioxane changed due to COD concentration variations, in fact, these two compounds were not effectively degraded or converted in

the system, which in turn led to higher COD concentration in the final effluent with step-wise increased loading. In addition, 1,4-dioxane was frequently detected in industrial effluents including polyester wastewater [24-26]. It is a well-known hazardous waste and falls under the priority pollutant regulations of the Environmental Protection Agency (EPA), which has set a discharge limit of $30 \mu\text{g L}^{-1}$. In addition, this compound was among the most refractory compounds due to its high resistance to biotransformation. Therefore, advanced oxidation processes such as electrochemical oxidation and $\text{O}_3/\text{H}_2\text{O}_2$ are recommended to further polish the effluent.

Illumina high-throughput sequencing indicated that after operated with alternating A/O process, microbial community structure changed greatly. In the AS samples from SS and reactor A1, *Firmicutes*, *Bacteroidetes*, *Chloroflexi*, *Thermotogae* and *Proteobacteria* were top 5 abundant phyla, which were frequently found in soil and wastewater communities [27, 28]. Many members of these phyla were the main groups in anaerobic environment related to hydrolysis and fermentation and could utilize a wide range of carbon sources including phenol and phenolic derivatives [21, 29]. In other reactors, *Bacteroidetes* and *Proteobacteria* become more enriched phyla, which showed no distinct different pattern from AS samples treating municipal wastewater [30, 31]. At the class level, *Sphingobacteria* within the phylum *Bacteroidetes* was more abundant in all reactor samples which is consistent with a few previous results from pet food, petroleum refinery and whey processing industry (35-50%) [21].

Specific taxonomic comparison down to the genus level revealed that the genus

Lewinella within the class *Sphingobacteria* was highly presented in all reactors and was relatively associated to the WWTP that treated wastewater from the polyester resin synthesis plant. According to previous report by Ibarbalz et al. [21], this genus was present in particular systems such as the whey processing industry WWTP, in which *Lewinella* displayed high abundance of 23.6% of the total sequences, while it showed a rather low abundance in municipal WWTPs [30]. The reason could be that *Lewinella*, is a hydrolyser-related genus that played an important role in the breakdown of complex organic compounds, such as starch, CM-cellulose, casein, gelatin, as well as the polysorbate Tween detergents [32, 33]. According to GC-MS analysis results (Table 2), many different kinds of esters were highly presented in polyester resin wastewater, and were removed in the system. Thus, the high abundance of *Lewinella* supported that these genera might be crucial for high efficiency of organics removal in this study. The observation of *Thauera*, *Azoarcus* group suggests that these genera are also important in the aromatics removal of the alternating A/O system [34]. Further isolation and enrichment of these understudied groups of bacteria may be helpful for enhancing process performance with potential biotechnological application.

Besides, the results also show close similarity between the bacterial compositions of samples from two low loading rates. RDA results revealed that COD concentration was correlated most strongly to the variance of bacterial communities and the bacterial compositions. It is possible to infer that appropriate COD concentration and loading should be maintained for an efficient and stable system performance.

5. Conclusions

Results demonstrate that the two-stage alternating A/O process appears to be an effective technological option for polyester resin wastewater treatment. At the influent COD concentration up to 12880 mg L⁻¹, the effluent COD concentration lower than 500 mg L⁻¹ was achieved in the system. Two important chemical compounds presented in the wastewater, PTA and phenol, were mainly degraded in the first-stage A/O. The majority of esters, alkanes, phenols, acids, alcohols could be removed in the system. However, two hazardous compounds, 5,5-dimethyl-1,3-dioxane and 1,4-dioxane, total corresponding to 42.2-59.3% of relative mass percentage, were the major components in the effluent. Specific comparison down to the genus level revealed that the relative high abundance of *Lewinella* related to ester-hydrolyzing ability may play an important role in highly efficient organic removal.

Acknowledgements

This work was financially supported by Open Project of Zhejiang Province Key Laboratory of Environmental Pollution Control Technology, and by Zhejiang Provincial Natural Science Foundation of China under Grant No. LQ18E080008. We also acknowledge the funding support from the Project of Jinhua Science and Technology Department. We thank Dr. Yuan Mengdong for her support in the molecular analysis.

References

- [1] M. Alae, P. Arias, A. Sjödin, Å. Bergman, An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release, *Environ. Int.*, 29 (2003) 683-689.
- [2] B.M. Gorelov, O.I. Polovina, A.M. Gorb, S. Dacko, M. Kostrzewa, A. Ingram,

Fumed silica concentration effect on structure and dielectric properties of a styrene-cross-linked unsaturated polyester resin, *J. Appl. Phys.*, 112 (2012) 094321.

[3] R.A. Caffaro-Filho, R. Wagner, G.A. Umbuzeiro, M.J. Grossman, L.R. Durrant, Identification of alpha-beta unsaturated aldehydes as sources of toxicity to activated sludge biomass in polyester manufacturing wastewater, *Water Sci. Technol.*, 61 (2010) 2317-2324.

[4] R.A. Caffaro-Filho, D.M. Morita, R. Wagner, L.R. Durrant, Toxicity-directed approach of polyester manufacturing industry wastewater provides useful information for conducting treatability studies, *J. Hazard. Mater.*, 163 (2009) 92-97.

[5] X. Yang, Interior microelectrolysis oxidation of polyester wastewater and its treatment technology, *J. Hazard. Mater.*, 169 (2009) 480-485.

[6] G. Moussavi, M. Mahmoudi, B. Barikbin, Biological removal of phenol from strong wastewaters using a novel MSBR, *Water Res.*, 43 (2009) 1295-1302.

[7] H. Macarie, A. Noyola, J.P. Guyot, Anaerobic treatment of a petrochemical wastewater from a terephthalic acid plant, *Water Sci. Technol.*, 25 (1992) 223-235.

[8] R. Kleerebezem, L.W.H. Pol, G. Lettinga, The role of benzoate in anaerobic degradation of terephthalate, *Appl. Environ. Microbiol.*, 65 (1999) 1161-1167.

[9] R. Kleerebezem, J. Beckers, L.W. Hulshoff Pol, G. Lettinga, High rate treatment of terephthalic acid production wastewater in a two-stage anaerobic bioreactor, *Biotechnol. Bioeng.*, 91 (2005) 169-179.

[10] H. Tsuno, M. Kawamura, Development of an expanded-bed GAC reactor for anaerobic treatment of terephthalate-containing wastewater, *Water Res.*, 43 (2009) 417-422.

[11] A. Krastanov, Z. Alexieva, H. Yemendzhiev, Microbial degradation of phenol and phenolic derivatives, *Engineering in Life Sciences*, 13 (2013) 76-87.

[12] F. Rosenkranz, L. Cabrol, M. Carballa, A. Donoso-Bravo, L. Cruz, G. Ruiz-Filippi, R. Chamy, J.M. Lema, Relationship between phenol degradation efficiency and microbial community structure in an anaerobic SBR, *Water Res.*, 47 (2013) 6739-6749.

[13] J.Y. Joung, H.W. Lee, H. Choi, M.W. Lee, J.M. Park, Influences of organic loading disturbances on the performance of anaerobic filter process to treat purified terephthalic acid wastewater, *Bioresour. Technol.*, 100 (2009) 2457-2461.

[14] C. Fajardo, J.P. Guyot, H. Macarie, O. Monroy, Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products, *Water Sci. Technol.*, 36 (1997) 83.

[15] Z. Yang, Determination of TA in discharged sewage of PTA purified equipment,

Industrial Water Treatment, 22 (2002) 38-39.

[16] APHA, Standard Methods for Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, USA, 1998.

[17] F. Sun, B. Sun, J. Hu, Y. He, W. Wu, Organics and nitrogen removal from textile auxiliaries wastewater with A²O-MBR in a pilot-scale, J. Hazard. Mater., 286 (2015) 416-424.

[18] R.F. Vasconcellos, T. Zucchi, R. Taketani, F. Andreote, E.B.N. Cardoso, Bacterial community characterization in the soils of native and restored rainforest fragments, A. Van Leeuw., 106 (2014) 947-957.

[19] Q. Ma, Y. Qu, W. Shen, Z. Zhang, J. Wang, Z. Liu, D. Li, H. Li, J. Zhou, Bacterial community compositions of coking wastewater treatment plants in steel industry revealed by Illumina high-throughput sequencing, Bioresour. Technol., 179 (2015) 436-443.

[20] C.J.F. ter Braak, P. Šmilauer, Canoco reference manual and user's guide: software for ordination, version 5.0., Microcomputer Power, Ithaca, USA, 2012.

[21] F.M. Ibarbalz, E.L.M. Figuerola, L. Erijman, Industrial activated sludge exhibit unique bacterial community composition at high taxonomic ranks, Water Res., 47 (2013) 3854-3864.

[22] X.-k. Li, K.-l. Ma, L.-w. Meng, J. Zhang, K. Wang, Performance and microbial community profiles in an anaerobic reactor treating with simulated PTA wastewater: From mesophilic to thermophilic temperature, Water Res., 61 (2014) 57-66.

[23] H.H.P. Fang, D.W. Liang, T. Zhang, Y. Liu, Anaerobic treatment of phenol in wastewater under thermophilic condition, Water Res., 40 (2006) 427-434.

[24] J.S. Han, M.H. So, C.G. Kim, Optimization of biological wastewater treatment conditions for 1,4-dioxane decomposition in polyester manufacturing processes, Water Sci. Technol., 59 (2009) 995-1002.

[25] J.H. Suh, M. Mohseni, A study on the relationship between biodegradability enhancement and oxidation of 1,4-dioxane using ozone and hydrogen peroxide, Water Res., 38 (2004) 2596-2604.

[26] P.F. Greenwood, L.J. Berwick, J.P. Croue, Molecular characterisation of the dissolved organic matter of wastewater effluents by MSSV pyrolysis GC-MS and search for source markers, Chemosphere, 87 (2012) 504-512.

[27] A. Geng, A. Soh, C. Lim, L. Loke, Isolation and characterization of a phenol-degrading bacterium from an industrial activated sludge, Appl. Microbiol. Biot., 71 (2006) 728-735.

- [28] J.L. VandeWalle, G.W. Goetz, S.M. Huse, H.G. Morrison, M.L. Sogin, R.G. Hoffmann, K. Yan, S.L. McLellan, *Acinetobacter*, *Aeromonas* and *Trichococcus* populations dominate the microbial community within urban sewer infrastructure, *Environ. Microbiol.*, 14 (2012) 2538-2552.
- [29] L. Ye, M.F. Shao, T. Zhang, A.H.Y. Tong, S. Lok, Analysis of the bacterial community in a laboratory-scale nitrification reactor and a wastewater treatment plant by 454-pyrosequencing, *Water Res.*, 45 (2011) 4390-4398.
- [30] T. Zhang, M.F. Shao, L. Ye, 454 Pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants, *ISME J*, 6 (2012) 1137-1147.
- [31] X. Wang, M. Hu, Y. Xia, X. Wen, K. Ding, Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China, *Appl. Environ. Microbiol.*, 78 (2012) 7042-7047.
- [32] S. McIlroy, P. Nielsen, The family *Saprospiraceae*, in: E. Rosenberg, E. DeLong, S. Lory, E. Stackebrandt, F. Thompson (Eds.) *The Prokaryotes*, Springer Berlin Heidelberg 2014, pp. 863-889.
- [33] F. Ju, F. Guo, L. Ye, Y. Xia, T. Zhang, Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years, *Environ. Microbiol. Rep.*, 6 (2014) 80-89.
- [34] F. Sun, S. Wu, J. Liu, B. Li, Y. Chen, W. Wu, Denitrification capacity of a landfilled refuse in response to the variations of COD/NO₃⁻-N in the injected leachate, *Bioresour. Technol.*, 103 (2012) 109-115.

Fig. 1 Schematic diagram of the two-stage alternating A/O process

Fig. 2 Dynamic profiles of COD concentration as well as COD removal rate in each reactor. I indicates Phase I, II indicates Phase II, dashed line indicates the boundary between Phase I and II, inverted triangle indicates the time collected for DNA analysis, arrows indicate changing feedstock concentrations.

Fig. 3 Dynamic profiles of phenol (A) and PTA (B) concentration as well as their removal rates (C, D) in each reactor. I indicates Phase I, II indicates Phase II, dashed line indicates the boundary between Phase I and II.

Fig. 4 Patterns of bacterial phyla distribution in each activated sludge sample. Taxa represented occurred at $> 1\%$ abundance in at least one sample. Minor phyla refer to the taxa with their maximum abundance $< 1\%$ in any sample.

Fig. 5 (A) Principal Coordinates Analysis (PCoA) using Bray-Curtis distance based on the bacterial genus relative abundances (> 0.5%). (B) Redundancy Analysis (RDA) of bacterial genus relative abundances and measured operational parameters: pH, DO and COD. For clarity, operational parameters were labeled in B, whereas only both high-abundant and high-score genus was labeled in B. The correlation between bacterial groups and operational parameters variable was indicated by a perpendicular projection of the group arrow-tips onto the line overlaying the operational parameters arrow. The further a projection point falls in the direction indicated by the arrow, the higher the correlation. The percentage numbers labeling in the ordination axis indicate the explained variation.

Table 1

Characteristics of polyester resin wastewater observed during the experiment study

Item	Range
pH	3.40-4.35
COD (mg L ⁻¹)	31,400-36,000
Phenol (mg L ⁻¹)	714-887
PTA (mg L ⁻¹)	861-1,114
PO ₄ ³⁻ -P (mg L ⁻¹)	0.26-1.00

Table 2

Main organic compounds detected by GC-MS in the influent and the effluent in each sample.

Match %	Compounds	Relative mass percentage on 3 d					Relative mass percentage on 55 d					Relative mass percentage on 114 d				
		(%)					(%)					(%)				
		Raw	A1	O1	A2	O2	Raw	A1	O1	A2	O2	Raw	A1	O1	A2	O2
83-91	1,4-Dioxane	2.9	5.7	15.6	25.0	26.2	2.0	4.1	20.3	17.3	21.2	4.8	5.0	14.3	14.0	22.2
68-91	1,3,6-Trioxane	-	-	0.9	0.9	1.9	0.1	0.2	1.0	0.9	1.0	0.3	0.2	0.9	0.5	2.3
80-91	5,5-Dimethyl-1,3-dioxane	15.0	18.7	32.4	31.5	14.1	12.5	16.8	30.4	24.7	37.1	11.9	12.3	13.2	10.7	19.4
72-96	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.5	0.3	1.6	0.3	1.7	0.4	0.3	0.3	0.5	0.4	0.6	0.2	0.9	1.7	0.4
45-64	1H-1,2,4-Triazole, 3-thiol-5-methyl-	28.8	21.7	6.9	2.2	0.5	9.1	11.1	1.3	5.7	2.2	15.6	10.9	0.7	0.3	3.8
50-60	4-ethyl-4-methyl-2-pentadecyl-1,3-Dioxolane	16.5	20.4	0.2	-	0.7	9.4	12.8	-	-	-	13.7	16.9	-	-	0.4
76-91	Phenol	2.0	-	-	-	-	2.0	2.9	-	-	-	2.1	9.4	-	-	-
50-90	3-Chloro-2,2-dimethyl-1-propanol	0.7	1.7	1.3	-	-	6.5	3.3	-	-	-	0.9	5.5	-	8.5	-
78-86	Butanoic acid, hexyl ester	9.6	-	-	-	-	6.5	4.2	-	-	-	17.7	-	-	-	-
56-72	Propanoic acid, 2-methyl-, butyl ester	-	-	-	-	-	7.3	8.4	-	-	-	-	4.5	-	-	-
72-78	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	-	-	-	-	-	6.2	-	-	-	-	-	7.0	-	-	-
50-64	Adipic acid, di(but-2-en-1-yl) ester	-	1.4	-	-	-	2.9	1.8	-	-	-	4.4	2.3	-	-	-
91	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	-	-	-	-	0.2	0.1	0.5	-	-	0.7	0.1	0.1	-	1.5	0.6
53-64	Adipic acid, di(but-2-en-1-yl) ester	-	1.4	-	-	-	2.9	1.8	-	-	-	4.4	2.3	-	-	-
83-93	Cyclohexasiloxane, dodecamethyl-	0.2	0.3	0.5	0.8	0.9	-	-	-	-	-	-	-	-	-	-
90-94	Cyclopentasiloxane, decamethyl-	0.2	0.3	-	-	0.7	-	-	-	-	-	-	-	-	-	-

[illegible]

Note: Relative mass percentage was calculated based on the area of the peaks of the detected compounds. “-” = not detected.

Table 3

Abundances of the most abundant genus in each AS sample.

	SS	L1A1	L1A2	L2A1	L2A2	HA1	HA2	L1O1	L1O2	L2O1	L2O2	HO1	HO2
<i>Lewinella</i>	0.45	2.68	34.35	4.92	16.57	3.46	21.88	23.68	31.63	15.53	2.37	13.49	33.99
<i>Coprothermobacter</i>	2.88	0.05	0.01	0.01	0.01	0.01	0	0	0.02	0	0.01	0	0
<i>Soehngenia</i>	2.87	0.21	0.27	0.02	0.1	0.09	0.05	0.01	0.07	0	0.01	0.13	0.01
<i>Methanothermobacter</i>	2.82	0.08	0.02	0.03	0.01	0.01	0.01	0.01	0.02	0	0	0	0.01
<i>Sedimentibacter</i>	2.66	0.35	0.03	3.2	0.02	3.79	0.05	0.01	0.02	0.03	0.01	0.09	0.01
<i>Syntrophomonas</i>	2.62	0.24	0.02	0.6	0.13	0.11	0.11	0	0.02	0	0	0.02	0
<i>Thauera</i>	0.77	1.48	7.96	3.53	4.59	0.36	1.51	3.7	7.15	0.93	2.92	0.41	4.37
<i>Acholeplasma</i>	0.73	2.29	0.08	0.01	0.03	0.05	1.21	0.01	0.04	0.02	0.01	0.71	0.01
<i>Azoarcus</i>	0.21	0.66	3.31	0.52	0.73	0.06	0.2	1.14	1.96	1.34	0.45	0.21	0.42
<i>Hydrogenophaga</i>	0.17	0.55	3.08	0.55	2.44	4.24	0.6	3.85	1.39	4.54	5.16	1.99	1
<i>Ohtaekwangia</i>	0.11	0.2	0.03	0.25	0.34	0.13	2.45	0.05	0.12	0.67	1.46	0.11	8.6
<i>Ignavibacterium</i>	0.1	0.09	0.23	0.27	1.38	0.07	0.53	0.09	0.26	0.15	0.94	0.07	0.13
<i>Acidovorax</i>	0.09	0.39	2.59	0.18	1.36	0.44	0.42	4.46	1.85	12.65	0.98	0.83	0.29
<i>Brachymonas</i>	0.07	0.29	1.37	0.15	0.8	0.09	0.09	3.2	0.39	0.14	0.6	0.21	0.08
<i>Bosea</i>	0.06	0.3	0.36	0.27	0.9	0.33	0.56	0.36	0.41	1.6	5.18	0.9	1.04
<i>Terrimonas</i>	0.04	0.13	0.23	0.87	2.77	2.38	1.07	1.2	1.32	1.1	2.5	1.67	0.35
<i>Rhodococcus</i>	0.03	0.38	0.1	0.32	0.27	1.66	1.11	0.08	0.31	0.5	0.31	2.31	0.82
<i>Acetobacterium</i>	0.03	2.9	0.03	6.87	0.02	0.64	0.02	0.03	0.02	0.08	0.01	0.07	0.01
<i>Gp2</i>	0.03	1.17	0.03	0.58	0.01	0.02	0.03	0.05	0.02	0.04	0.01	0.03	0.1
<i>Zoogloea</i>	0.02	0.15	1.11	1.73	0.62	0.13	0.09	0.45	0.41	3.58	0.31	0.38	0.1
<i>Sediminibacterium</i>	0.01	0.08	0.02	1.04	0.28	0.25	0.17	0.14	0.02	3.09	0.16	1.27	0.26
<i>Trichococcus</i>	0.01	0.02	0.01	7.51	0.01	0.1	0	0.01	0	0.03	0.01	0.01	0.01
<i>Nannocystis</i>	0.01	0.22	1.29	0.02	0.02	0	0.01	1.94	1.96	0.01	0.01	0.01	0.06
<i>Byssovorax</i>	0.01	0.02	0	0.01	0.03	0.01	0.48	0	0	0.01	0.29	0.01	2.92
<i>Azotobacter</i>	0.01	0.64	0	0.31	0.01	2.5	0	0	0.01	0	0	0.04	0.01

Note: The abundance is presented in terms of percentages of the total sequences in a sample. Green color intensity, key provided underneath, indicates the particular genus relative abundance in each sample. TOP 5 genera in each sample is shown. Abbreviation: SS, seed sludge; L1A1, L1O1, L1A2, L1O2 indicate the samples from reactor A1, O1, A2 and O2 at low loading rate 1; L2A1, L2O1, L2A2, L2O2 indicate the samples from four reactors at low loading rate 2; HA1, HO1, HA2, HO2 indicate the samples from four reactors at high loading rate.