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Performance and microbial community analysis of bioaugmented activated sludge for nitrogen-containing organic pollutants removal

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ABSTRACT

Nitrogen-containing organic pollutants (quinoline, pyridine and indole) are widely distributed in coking wastewater, and bioaugmentation with specific microorganisms may enhance the removal of these recalcitrant pollutants. The bioaugmented system (group B) was constructed through inoculation of two aromatics-degrading bacteria, *Comamonas* sp. Z1 (quinoline degrader) and *Acinetobacter* sp. JW (indole degrader), into the activated sludge for treatment of quinoline, indole and pyridine, and the non-bioaugmented activated sludge was used as the control (group C). Both groups maintained high efficiencies (> 94%) for removal of nitrogen-containing organic pollutants and chemical oxygen demand (COD) during the long-term operation, and group B was highly effective at the starting period and the operation stage fed with raw wastewater. High-throughput sequencing analysis indicated that nitrogen-containing organic pollutants could shape the microbial community structure, and communities of bioaugmented group B were clearly separated from those of non-bioaugmented group C as observed in non-metric multidimensional scaling (NMDS) plot. Although the inoculants did not remain their dominance in group B, bioaugmentation could induce the formation of effective microbial community, and the indigenous microbes might play the key role in removal of nitrogen-containing organic pollutants, including *Dokdonella*, *Comamonas* and *Pseudoxanthomonas*. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis suggested that bioaugmentation could facilitate the enrichment of functional genes related to xenobiotics biodegradation and metabolism, probably leading to the improved performance in group B. This study indicated that bioaugmentation could promote the removal of nitrogen-containing organic pollutants, which should be an effective strategy for wastewater treatment.

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Introduction

Nitrogen-containing heterocyclic compounds are typical organic pollutants in coking wastewater, such as quinoline, pyridine, and indole, posing a great threat to the environment and public health (Fetzner, 1998; Joshi et al., 2017; Sun et al., 2019; Zhao and Liu, 2016). Seeking an appropriate and effective strategy for the treatment of nitrogen-containing organic pollutants is necessary. Biological treatment of wastewater has long been of interest for environmental engineering studies, and the bioaugmentation through inoculation of specific strains has gained considerable importance in recent years (Boonnorat et al., 2018; Herrero and Stuckey, 2015). The native or exogenous strains can be inoculated to activated sludges or soils improve the performance of indigenous microbial communities for waste cleanup (Cycoń et al., 2017). For example, Bai et al. (2011) found that the zeolite-biological aerated filters (Z-BAFs) bioaugmented by pyridine- and quinoline-degrading bacteria showed elevated capacities for coking wastewater treatment, and the bioaugmentation could accelerate the shift of microbial community structure. Gao et al. (2019) constructed a bioaugmentation process for quinoline degradation based on quorum sensing (QS) effects, and the quinoline degradation time could be shortened by drawing support from the addition of acylated homoserine lactones-secreting strain. Wu et al. (2018) showed that the inoculation of two triazole-degrading strains (*Shinella* sp. NJUST26 and *Sphingomonas* sp. NJUST37) could enhance the performance of bioreactor in terms of 1H-1,2,4-triazole (TZ) and tricyclazole (TC) removal, and the bioaugmentation could induce the formation of effective microbial communities. In addition, some non-biological substances, such as nutrients (e.g., nitrogen and phosphorus), coke and phenol, have also been used to stimulate activated sludge for efficient wastewater treatment (Fuentes et al., 2014; Zheng et al., 2019). In our previous study, it was found that the microbial consortia stimulated by phenol could maintain a high efficiency (> 90%) for the removal of nitrogen-containing organic pollutants with a greater stability in long-term operation (Zhang et al., 2018).

Previous studies have already shown that *Comamonas* and *Acinetobacter* are two ubiquitous bacteria in diverse ecological niches with high metabolic versatilities, which are able to degrade various xenobiotic pollutants, including phenol, indole and quinolone (Felczak et al., 2014; Ma et al., 2018). Some species of *Comamonas* can utilize quinoline as the sole carbon and energy source (Cui et al., 2004), and the enzymes catalyzing the first step of quinoline degradation from *Comamonas testosteroni* have been also identified (Schach et al., 1995). Although there is no direct evidence showing indole degradation by *Comamonas*, this genus could maintain dominant in indole-degrading activated sludge systems (Ma et al., 2015). In the meanwhile, *Acinetobacter* present a superior capacity for indole degradation, and the functional genes involved in indole biodegradation have been unveiled recently (Lin et al., 2015; Sadauskas et al., 2017). Therefore, it is possible to use *Comamonas* and *Acinetobacter* for bioaugmentation of activated sludge, which may enhance the co-metabolic degradation of nitrogen-containing organic pollutants in coking wastewater. In our previous study, an indole-degrading *Acinetobacter* sp. strain JW and a quinoline-degrading *Comamonas* sp. strain Z1 were separately isolated from activated sludge, and the genomic profiles of both strains were also determined (Zhang et al., 2019, 2020), which made them the potential candidates for the bioaugmentation treatment of nitrogen-containing organic pollutants.

In this study, the phenol-stimulated activated sludge of sequencing batch reactors (SBRs) was bioaugmented by both strains JW and Z1 for the treatment of wastewater containing indole, pyridine and quinoline, and the non-bioaugmented SBRs were operated under identical conditions for comparison. The SBRs were successively operated with increasing concentrations of nitrogen-containing organic pollutants in synthetic wastewater and raw wastewater, and the microbial community composition and structure were assessed by high-throughput sequencing technology. The objective of this study was to evaluate the feasibility of bioaugmentation strategy for the treatment of nitrogen-containing organic pollutants and examine the pollutants removal performance and microbial community profiles.

1. Materials and methods

1.1. Chemicals, wastewater and activated sludge

Indole was purchased from Aladdin (Shanghai, China), and phenol, pyridine and quinoline were obtained from Damao Chemical Reagent Factory (Tianjin, China). All other chemicals were of analytical grade or above.

Synthetic wastewater consisted of 180 mg/L NH_4Cl, 20 mg/L NaCl, 25 mg/L $MgSO_4 \cdot 7H_2O$, 20 mg/L KH_2PO_4, 24 mg/L $CaCl_2$, and 20 mg/L $FeSO_4 \cdot 7H_2O$. Raw wastewater after pretreatment was obtained from the collecting well of a local municipal wastewater treatment plant in Liaodong Bay New District, Panjin (China), which contained 94.1 mg/L chemical oxygen demand (COD), 5.3 mg/L total phosphorus (TP) and 12.0 mg/L total nitrogen (TN). The raw wastewater was diluted 1:4 (V/V) in deionized water prior to being filled into the SBRs, and phenol, indole, quinoline and pyridine were added with certain concentrations. Activated sludge was collected from the aeration tank of the same wastewater treatment plant.

1.2. Bacterial strains

The indole-degrading *Acinetobacter* sp. strain JW and quinoline-degrading *Comamonas* sp. strain Z1 used in this study were previously isolated from activated sludge of a local sewage farm (Dalian, China) (Zhang et al., 2019, 2020). The mineral salts medium (MSM) for the growth of bacterial strains contained 2.0 g/L $(NH_4)_2SO_4$, 2.0 g/L KH_2PO_4, 3.3 g/L $Na_2HPO_4 \cdot 12H_2O$, and 0.25 mg/L $FeCl_3$ (Zhang et al., 2019). Strains JW and Z1 were cultivated in MSM with 500 mg/L yeast extract, and 100 mg/L indole and quinoline were also added, respectively. Both strains were incubated with shaking at 30 °C for 24 hr. Then, the cells were harvested by centrifugation (10,000 × *g*, 5 min), washed with sterile MSM and resuspended in synthetic wastewater. The bacterial suspension was employed for inoculation.

1.3. Reactor configuration and operation

Six SBRs with a working volume of 1.0 L were constructed and operated under identical conditions. After seeding with activated sludge (3.97 ± 0.26 g/L, dry weight), the SBRs were domesticated with the synthetic wastewater containing 100 mg/L phenol for 30 days (stage 0). Subsequently, three SRBs (group B) were inoculated with the two strains, *Comamonas* sp. Z1 (0.06 g/L) and *Acinetobacte* sp. JW (0.06 g/L), and the other three SBRs (group C) were set as the non-augmented control. The reactors were operated in parallel over 90 days, which were divided into three stages. Stage I (Day 0–30), the synthetic wastewater was used as the influent supplemented with 200 mg/L phenol, and 100 mg/L of indole, pyridine and quinolone; stage II (Day 30–60), the concentrations of indole, pyridine and quinolone in the influent were increased to around 200 mg/L; and stage III (Day 60–90), the raw wastewater was used instead of the synthetic wastewater, which was supplemented with 200 mg/L phenol, and 200 mg/L of indole, pyridine and quinolone. Each SBR was operated with a cycle time of 48 hr, including 2 hr filling, 42 hr reacting, 2 hr settling, and 2 hr decanting. Effluent was discharged with a volumetric exchange ratio of 50%. The influent and effluent were sampled at each cycle to monitor the concentrations of COD and nitrogen-containing organic pollutants. The removal curves of the pollutants were determined at the beginning and ending cycles of each stage (Day 1, 30, 31, 60, 61 and 90). During the operation period, the concentrations of mixed liquor suspended solid (MLSS) and the sludge volume after 0.5 hr of settling (SV_{30}) were also measured. The sludge samples from Day 0, 30, 60 and 90 were collected for microbial community analysis.

1.4. Analytical methods

The nitrogen-containing organic pollutants were analyzed using high performance liquid chromatography (HPLC) (Agilent 1290 Infinity II, Agilent Technologies, Germany; Welch Hypersil ODS-2 column, 5 μm, 250 × 4.6 mm). The samples were eluted using linear gradient from 60% to 70% methanol/water (V/V) over 12 min at a flow rate of 0.3 mL/min, and monitored at 270, 254 and 275 nm for indole, pyridine, quinoline, respectively. COD, SV_{30} and MLSS were analyzed according to the standard methods.

1.5. DNA extraction, PCR, and high-throughput sequencing

The genomic DNA of activated sludge samples was extracted using cetyltrimethylammonium bromide (CTAB) or sodium dodecyl sulfate (SDS) methods, and the V3-V4 region of 16S rRNA gene was amplified using the primer set 515F (5′- GTG CCAG CMGC CGCG GTAA-3′) and 806R (5′-GGAC TACH VGGG TWTC TAAT-3′). The resulting PCR amplicons were used for sequencing on IonS5™ XL platform (ThermoFisher Scientific, USA) in Novogene Co., Ltd. (Beijing, China). After pretreatment of the raw sequencing data, the clean sequences were used to generate the operational taxonomic units (OTUs) by UPARSE program at a 97% similarity threshold (Edgar, 2013). For each representative sequence, the SILVA Database was used based on Mothur algorithm to annotate taxonomic information with a confidence threshold of 80% (Quast et al., 2013). Shannon index, evenness index, Chao1 and rarefaction curves were calculated with QIIME (Version1.7.0) and displayed with R software (Version 2.15.3). Non-metric multidimensional scaling (NMDS) analysis was performed by R software (Version 2.15.3). The dominant genera in the SRBs were depicted in a heatmap conducted by R software (Version 2.15.3). The metabolic functions of microbial communities were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, v1.0.0) software package by referencing the Kyoto Encyclopedia of Genes and Genome (KEGG) Orthology database. The statistical significance was examined by one-way analysis of variance (ANOVA), and the data with $P < 0.05$ were considered significant. The sequencing data have been deposited into the NCBI Sequence Read Archive (SRA) database under the BioProject number PRJNA642269.

2. Results

2.1. SBRs performance

The bioaugmented (group B) and non-bioaugmented (group C) SBRs were operated in parallel over 90 days for the treatment of indole, pyridine and quinoline. Both groups exhibited a high efficiency (> 99%) for the removal of phenol in influent (Appendix A Fig. S1), while the removal performance of nitrogen-containing organic pollutants was shown in Fig. 1. Indole was almost completely removed in each group (> 99%) during stage I. The removal of pyridine and quinoline was less effective at early days of stage I, especially in group C, and the removal efficiencies gradually increased to more than 99% in the late period. COD removal efficiency fluctuated at early days, which gradually increased from 77% to above 90%, and there was almost no difference between group B and C. The removal performance of the pollutants at the first cycle of stage I (Appendix A Fig. S2) showed that both groups presented a similar ability to remove indole, but group B was superior to group C in pyridine and quinoline degradation. Particularly, quinoline could be quickly degraded by group B within 10 hr, while only half of it was removed by group C in 40 hr. After 30 days of operation, the removal rates of the pollutants by both groups had no difference.

When the concentrations of indole, pyridine and quinoline increased to 200 mg/L in stage II, the SBRs still maintained high removal efficiencies (> 98%), though the removal of pyridine experienced a slight fluctuation in group B. The COD removal efficiency also remained stable basically (around 96%). During the stage II, Group B and C reached similar rates for the removal of nitrogen-containing organic pollutants (Appendix A Fig. S2).

In stage III, the synthetic wastewater was replaced by the raw wastewater in order to testify the stability of pollutants removal performance in the SBRs. The results showed that the removal efficiencies of indole and quinoline had no significant changes (> 99%), but the pyridine removal efficiencies slightly declined to around 94% in group C. The COD removal efficiency also decreased slightly in both groups (around 93%).

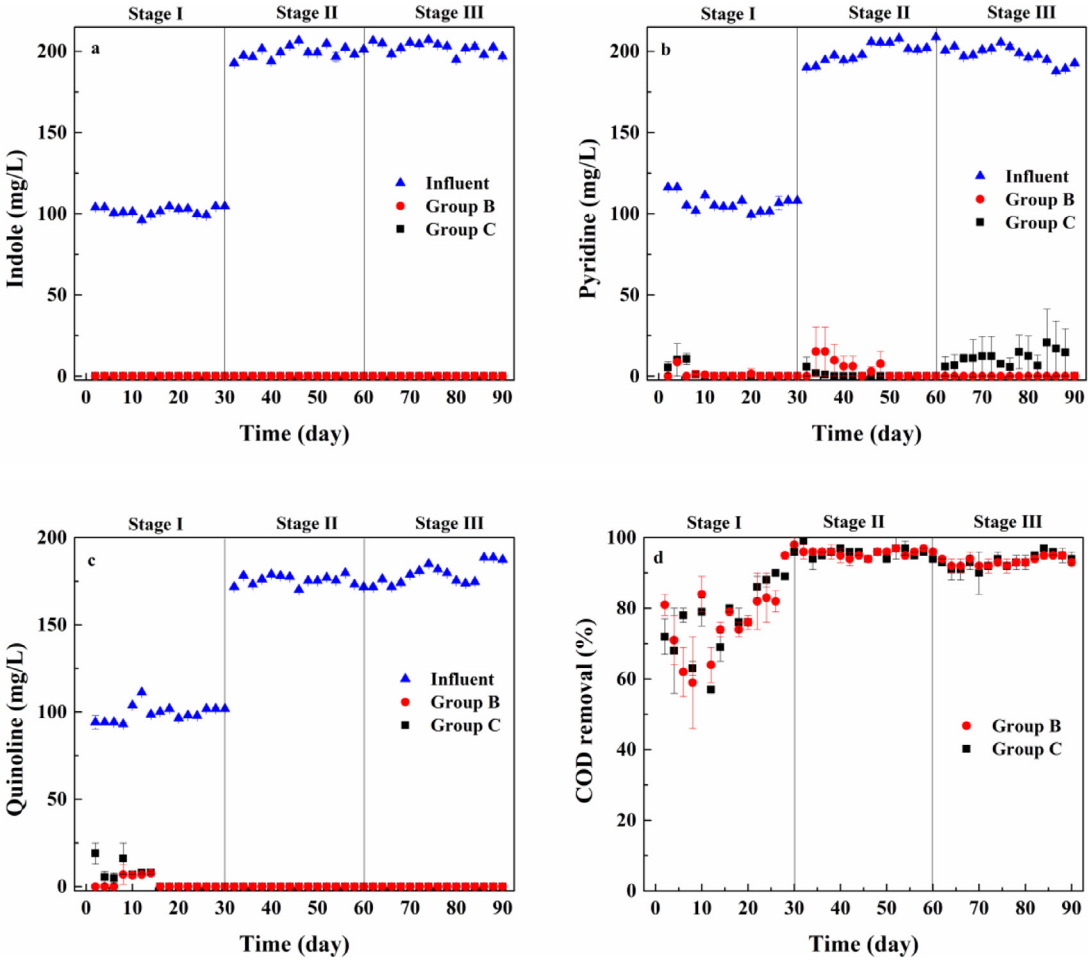

Fig. 1 – Indole (a), pyridine (b), quinoline (c) and chemical oxygen demand (COD) (d) removal performance of the sequencing batch reactors (SBRs). Group B, bioaugmented SBRs; group C, non-bioaugmented SBRs.

Furthermore, the removal rates of indole, pyridine and quinoline in group B seemed to be higher than those in group C, especially at the end of the stage III (Appendix A Fig. S2), suggesting the higher capacity for pollutants removal in group B.

To assess the sludge statue during operation, SV_{30} and MLSS were measured (Appendix A Fig. S3). SV_{30} reflected the settling ability of activated sludge, and the results showed that the SV_{30} value of both groups almost maintained steady, though some fluctuations were also observed. The MLSS measurement was used to determine the sludge concentration, which decreased in response to the high-concentration pollutants and then recovered when exposed to raw wastewater.

2.2. *Microbial community diversity and dynamics*

High-throughput sequencing of 16S rRNA gene was performed to compare the community profiles in bioaugmented and non-bioaugmented SBRs during the long-term operation, and 46,636–74,053 clean sequence reads were obtained for each sample. Rarefaction curves and alpha diversity indices indicated that the original sludges (stage 0) had the highest taxonomic richness and diversity, which decreased in both group B and C (Appendix A Fig. S4 and Table S1). In group B, Shannon index almost had no changes in response to the increasing concentration of pollutants from stage I to stage II, while evenness index was even improved, both of which decreased a little bit in stage III. On the contrary, both Shannon and evenness indices decreased from stage I to stage II in group C, but then increased in stage III. In the meantime, the microbial richness (Chao1 and OTUs) in both group B and C decreased along the operation from stage I to stage III. However, statistical analysis (One-way ANOVA) indicated that the alpha diversity indices had no significant difference between group B and C at each stage ($P > 0.05$, Appendix A Table S1).

NMDS analysis was performed to visualize the variation of microbial communities during the long-term operation. As shown in Fig. 2, the bacterial communities underwent a noticeable shift after receiving nitrogen-containing organic pollutants, and the communities of group B and C were clearly separated at different stages. Further increasing the concentration of nitrogen-containing organic pollutants in influent could also induce the shift in bacterial communities. When the raw wastewater was used in stage III, the communities of group B and C still clustered away.

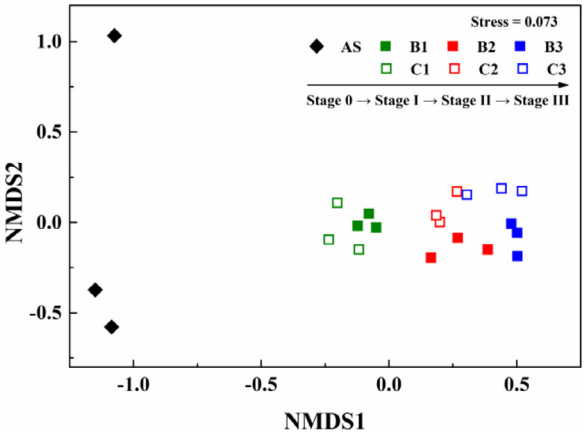

Fig. 2 – Non-metric multidimensional scaling (NMDS) plot of microbial communities. AS, original activated sludge (stage 0); B1-B3, group B at stage I-stage III; C1-C3, group C at stage I-stage III.

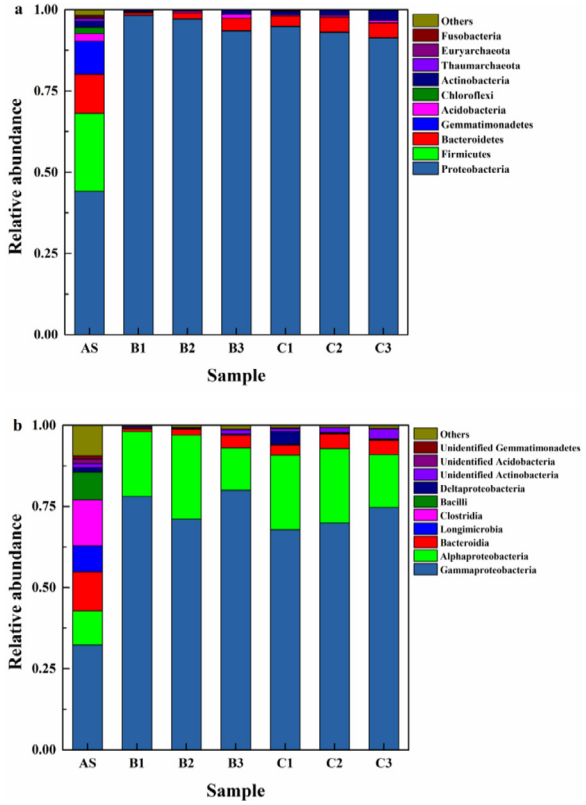

Fig. 3 – Relative abundance of dominant phyla (a) and classes (b) in the SBRs.

2.3. *Microbial community composition and structure*

Fig. 3 shows the microbial populations of activated sludge samples at the phylum and class levels. A total of 34 bacterial phyla were detected from all samples, and Proteobacteria, Firmicutes, Bacteroidetes and Gemmatimonadetes covered 90.3%–99.1% of the total sequences (Fig. 3a). Proteobacteria was the most predominate phylum in all samples (44.2%–

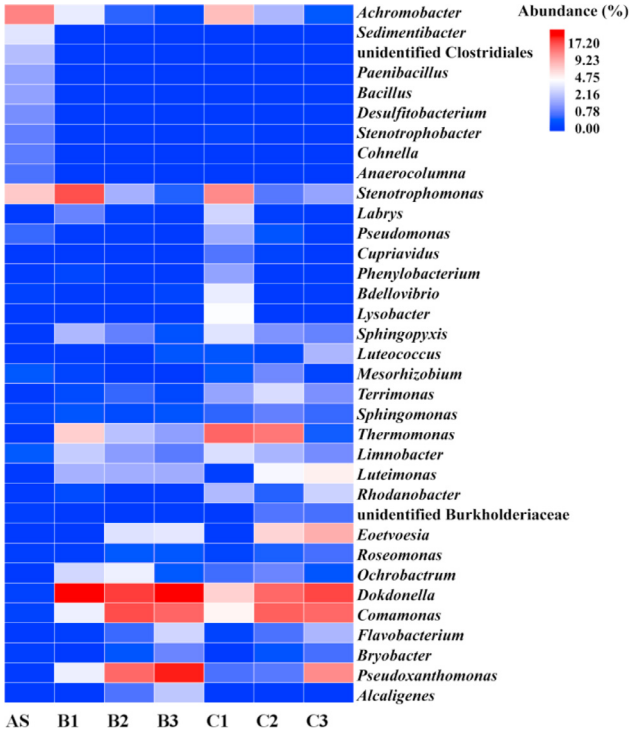

Fig. 4 – Heatmap profiles showing the top 35 genera in the SBRs.

98.2%), which increased significantly in group B and C from original sludges (stage 0) to stage I (One-way ANOVA, $P < 0.05$), while the other three dominant phyla showed an opposite trend, decreasing in relative abundance in stage I. Thereafter, the abundance of Proteobacteria slightly decreased in stage II and III, and the abundance of Bacteroidetes increased on the contrary. Gammaproteobacteria (32.3%–80.0%) and Alphaproteobacteria (10.5%–25.9%) were the most dominant subdivisions of Proteobacteria in all samples (Fig. 3b). Gammaproteobacteria was more abundant in group B, which increased in abundance from original sludges (stage 0) to stage I and maintained relatively high abundance afterward. Alphaproteobacteria was also more enriched in group B during the stage I and II. But in stage III, the abundance of Alphaproteobacteria was higher in group C.

A total of 249 genera were detected and the top 35 genera were shown in Fig. 4. *Achromobacter, Stenotrophobacter, Sedimentibacter*, unidentified Clostridiales, *Paenibacillus, Bacillus* and *Desulfitobacterium* were the major components (> 1%) in original sludges (stage 0), but they decreased dramatically when nitrogen-containing organic pollutants (indole, pyridine and quinoline) were added in stage I. *Dokdonella* was negligible in original sludges (< 0.5%), but the relative abundance significantly increased in group B (31.5%) at stage I (One-way ANOVA, $P < 0.05$), becoming the predominant genus, which still maintained its dominance in stage II and III (> 20%). In group C, *Dokdonella* also increased to be the foremost genus at stage III, accounting for 20.1% of the total sequences. Furthermore, *Pseudoxanthomonas, Flavobacterium* and *Alcaligenes* increased during the operation in both groups, especially in group B at stage III, while *Thermomonas, Limnobacter, Luteimonas* and *Rho-*

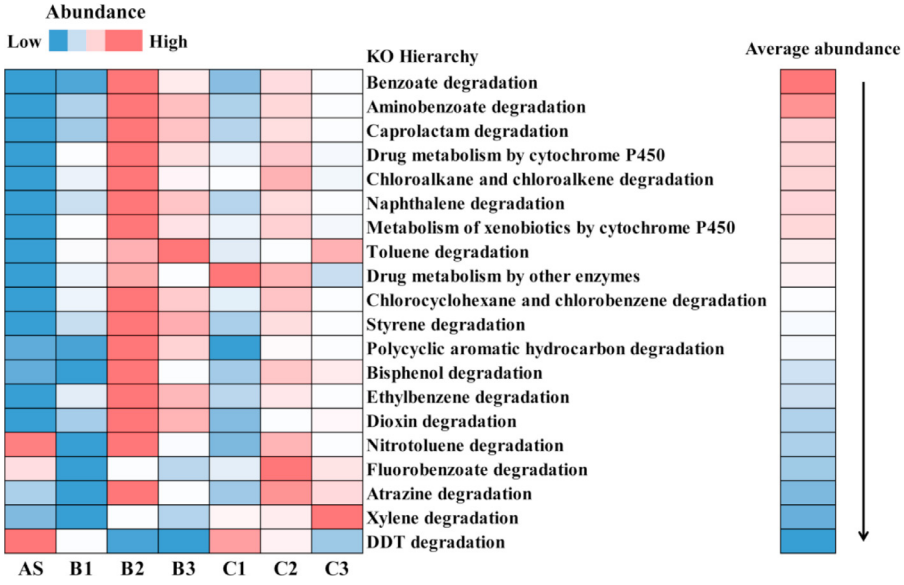

Fig. 5 – Comparison of the functional categories of bacterial communities relevant to xenobiotics biodegradation and metabolism. In each row, the color intensity shows the relative proportion of a functional category among different samples. The color bar on the right side represents the average abundances of the functional categories. The redder the color, the higher the abundances, and the bluer the color, the lower the abundances.

danobacter were more abundant in group C. *Comamonas* also increased significantly from stage 0 to stage I (One-way ANOVA, $P < 0.05$), and maintained high relative abundance (> 15%) in stage II and III. But almost no difference was observed in relative abundance of *Comamonas* between group B and C. Moreover, *Acinetobacter* presented a low relative abundance (< 0.5%) in both groups.

2.4. *Predicted functional profiling of microbial communities*

PICRUSt was used to assess the functional capacities of the microbial communities, and the inferred genes were annotated against KEGG orthology groups. The top 10 functional categories were observed at KEGG level 2 (Appendix A Fig. S5). Membrane transport, amino acid metabolism, carbohydrate metabolism, and replication and repair were the ubiquitous functions in all samples. The functional genes relevant to xenobiotics biodegradation and metabolism were also abundant, especially in group B at stage II with high concentrations of nitrogen-containing organic pollutants. Within the category of xenobiotics biodegradation and metabolism (Fig. 5), benzoate degradation and aminobenzoate degradation were the two dominant functions, followed by caprolactam degradation, drug metabolism by cytochrome P450, chloroalkane and chloroalkene degradation, naphthalene degradation, metabolism of xenobiotics by cytochrome P450, and toluene degradation (> 0.2% of total functional genes). It was worth noting that most of these dominant functional genes increased in abundances after receiving nitrogen-containing organic pollutants and peaked at stage II in both groups, which were more enriched in group B than in group C. In addition, the contribution of OTUs to the functional categories within xenobiotics biodegradation and metabolism (> 0.2% of total functional genes) was further analyzed at the genus level (Appendix A Fig. S6). The results implied that the high abundances of these functional categories were mainly contributed by the dominant bacterial community compositions, such *Dokdonella, Comamonas, Pseudoxanthomonas, Achromobacter* and *Thermomonas*, which further confirmed the critical role of these taxa in nitrogen-containing organic pollutants removal.

3. Discussion

Nitrogen-containing organic pollutants (e.g., indole, pyridine and quinoline) and phenols are the common constituents of coking wastewater (Fetzner, 1998; Zhao and Liu, 2016). Our previous study indicated that nitrogen-containing organic pollutants could be effectively removed by phenol-stimulated activated sludge (Zhang et al., 2018). In this study, it was further proven that the SBRs could be biologically enhanced by inoculation with specific functional bacteria, and the performance of nitrogen-containing organic pollutants removal was improved. Compared with the non-bioaugmented control, the bioaugmented SBRs could quickly adapt to the nitrogen-containing organic pollutants, leading to the faster removal rates at early operation stage (Appendix A Fig. S2). Both groups maintained high efficiencies (> 94%) for the removal of COD and pollutants during the long-term operation (Fig. 1). When raw wastewater was used, both groups exhibited robust capacity and stability for pollutants removal, and group B presented accelerated removal rates of nitrogen-containing pollutants in response to the changes of influent (Appendix A Fig. S2). In terms of reactor operation, no deterioration was observed in group B after bioaugmentation (Appendix A Fig. S3). These results suggested that the bioaugmentation could pro-

mote the performance of SBRs for the treatment of nitrogen-containing organic pollutants.

Indole, pyridine and quinoline are toxic to microbes. Bai et al. (2011) reported that the high loadings of pyridine and quinoline led to a decrease of bacterial richness and diversity, and Ma et al. (2015) found that indole could significantly reduce the diversity of microbial communities in activated sludge. The nitrogen-containing pollutants also reduced the richness (Chao1 and OTU) and diversity (Shannon and evenness) of microbial communities in group B and C compared with original sludges (Appendix A Table S1). In the face of high concentrations of nitrogen-containing organic pollutants (indole, pyridine and quinoline), the microbial diversity in bioaugmented SBRs (group B) exhibited a higher stability than that in non-bioaugmented control (group C), suggesting that the bioaugmentation could improve the resistance of microbial communities to the shock loading of pollutants. Meanwhile, the differences of alpha diversity indices between group B and C were insignificant (One-way ANOVA, $P > 0.05$, Appendix A Table S1), indicating that the bioaugmentation had little influence on microbial richness and diversity. NMDS analysis indicated that the characteristics of influent (e.g., pollutant concentration, raw wastewater) could change the overall microbial community structure of activated sludge (Fig. 2). Moreover, the inoculation of specific functional bacteria could clearly induce the alteration of bacterial community structure, leading to the separation of clusters between group B and C (Fig. 2).

Proteobacteria was the primary component in both groups, and Alpha- and Gamma-proteobacteria were the predominant subgroups (Fig. 3), which were in accordance with previous studies on bioremediation of nitrogen-containing organic pollutants (Bai et al., 2011; Ma et al., 2015; Zhang et al., 2018). However, the inoculated bacteria, *Comamonas* and *Acinetobacter*, did not remain dominant in the bioaugmented SBRs. Although *Comamonas* was still abundant in both groups at stage II and III, it might be derived from the indigenous microbes of activated sludges. In contrast, the abundance of *Acinetobacter* was relatively low. The results suggested that the indigenous degrading bacteria played the most significant role in the treatment of nitrogen-containing organic pollutants. Similarly, Wu et al. (2018) found that the inoculated bacterial strains could not maintain dominant in the bioreactor after a long-term operation, but the removal of target pollutants (1H-1,2,4-triazole and tricyclazole) was still in high efficiency. Bai et al. (2011) also showed that the treatment of quinoline and pyridine was highly efficient by bioaugmentation, but the introduced bacteria lost their dominance in the bioaugmented biofilm. Here, the bioaugmentation with quinoline-degrading and indole-degrading strains could promote the formation of effective microbial communities in SBRs, leading to the enhanced removal of indole, pyridine and quinoline in group B.

Among the dominant genera (Fig. 4), *Dokdonella*, *Comamonas* and *Pseudoxanthomonas* could be the core taxa for the removal of nitrogen-containing organic pollutants. In previous studies, *Dokdonella* showed potential capability in paracetamol biodegradation and nitrite transformation (Du et al., 2017; Palma et al., 2018), while *Pseudoxanthomonas* exhibited a good ability for degradation of BTEX monoaromatic hydrocarbons (benzene, toluene, ethylbenzene and xylene) (Choi et al., 2013; Nayak et al., 2009). *Comamonas* possessed an extraordinary metabolic versatility, which enabled it to degrade a variety of xenobiotic pollutants, including quinolone (Cui et al., 2004; Ma et al., 2015; Zhang et al., 2020). These genera were abundant in both groups (average abundances > 8% of the total sequences), and group B contained higher relative abundances. The enrichment of these functional species in bioaugmented SBRs could be beneficial for the treatment of wastewater containing indole, pyridine and quinoline. Furthermore, *Flavobacterium* and *Alcaligenes* were also reported to be the versatile aromatics-degrading bacteria (Chaudhary et al., 2019; Claus and Kutzner, 1983; Palma et al., 2018; Qiu et al., 2018), and the higher abundances of both genera in group B could contribute to the efficient removal of pollutants. Besides, *Thermomonas* (denitrifying species) (Wang et al., 2019), *Limnobacter* (phenol degrader) (Vedler et al., 2013), *Luteimonas* (hydrocarbon degrader) (Ke et al., 2018) and *Rhodanobacter* (xenobiotic degrader) (Pugazhendi et al., 2015) were more abundant in group C, which might also help to maintain the stable performance of SBRs.

According to PICRUSt prediction, a high abundance of functional genes relevant to xenobiotics biodegradation and metabolism were detected in group B and C, and these metabolic activities should be important for degradation of nitrogen-containing organic pollutants (Ye et al., 2004; Zhang et al., 2018). More importantly, most of the subclass pathways within xenobiotics biodegradation and metabolism category were notably enriched in group B (Fig. 5), which suggested that bioaugmentation could boost the capacity of activated sludge for xenobiotics removal. In previous study, Ma et al. (2020) also found that the bioaugmentation could improve the abilities of xenobiotics transportation and metabolism in activated sludge. Thus, the enhanced metabolic functions could lead to the improved removal performance of nitrogen-containing organic pollutants in bioaugmented group, which further confirmed the feasibility of bioaugmentation strategy for wastewater treatment.

Nevertheless, the loss of the dominance of the inoculated strains was probably due to their poor competitiveness compared to the indigenous microbes, which might result in the failure of bioaugmentation in some cases (Cycoń et al., 2017; Wu et al., 2018). To overcome this problem, repeated inoculation of the introduced microorganisms was an easy and simple way in practical wastewater treatment, and the use of immobilized microorganisms on different carriers could also achieve the purpose (Cycoń et al., 2017; Herrero and Stuckey, 2015). Furthermore, bioaugmentation with mobile genetic elements was a potential alternative, which involved the horizontal transfer of degradative genes from exogenous strains to indigenous microbes (Mapelli et al., 2017; Top et al., 2002). A proper strategy for using bioaugmentation in wastewater treatment should be paid more attention in further investigations.

4. Conclusions

In this study, the bioaugmented activated sludge constructed by inoculation of quinoline-degrading strain *Comamonas* sp. Z1 and indole-degrading strain *Acinetobacte* sp. JW presented

an excellent capability for removal of indole, pyridine and quinolone, especially at the starting period and exposed to raw wastewater. Bioaugmentation had little influence on microbial diversity, but indeed induced the alteration of bacterial community structure. *Dokdonella*, *Comamonas* and *Pseudoxanthomonas* originated from the indigenous microbes of activated sludges could be responsible for the efficient removal of pollutants. Furthermore, bioaugmentation could also facilitate the bacterial communities with elevated capacities for xenobiotics removal. This study should highlight the potential application of bioaugmentation strategy in wastewater treatment.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.09.002.

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