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Unraveling the Long-Term Effects of Cr(VI) on the Performance and Microbial Community of Nitrifying Activated Sludge System

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Abstract: The long-term effects of different influent Cr(VI) concentrations (0–0.5 mg L^{-1}) on the nitrification activities and microbial community structures of nitrifying activated sludge system were investigated in this study. Results showed that the performance of ammonia oxidation was significantly inhibited, and the effluent concentration of ammonia nitrogen (NH_4^+-N) increased markedly when the influent Cr(VI) loading was equal or greater than 0.2 mg L^{-1}. The specific oxygen utilization rate (SOUR), specific ammonium oxidation rate (SAOR), and specific nitrite oxidation rate (SNOR) of the system decreased from 53.24, 6.31, and 7.33 mg N g^{-1} VSS h^{-1} to 18.17, 1.68, and 2.88 mg N g^{-1} VSS h^{-1}, respectively, with an increase of Cr(VI) concentration from 0 to 0.5 mg L^{-1}. The protein/polysaccharide (PN/PS) ratio increased with the increasing Cr(VI) concentration, indicating that excessive PN secreted by microorganisms was conducive to resisting the toxicity of Cr(VI). High-throughput sequencing revealed that the relative abundance of ammonia-oxidizing bacteria (*Nitrospira*) and nitrite-oxidizing bacteria (*Nitrosomonas and Nitrosospira*) all decreased with the increasing Cr(VI) concentration, and ammonia-oxidizing bacteria were more sensitive to heavy metal toxicity than nitrite-oxidizing bacteria. The activities of nitrifying activated sludge system could not be completely recovered after a 30-d recovery process.

Keywords: nitrifying activated sludge; Cr(VI); extracellular polymeric substances; high-throughput sequencing; microbial community structures

1. Introduction

In China, overloaded operation and improper management of the treatment of industrial wastewater lead to direct discharge of raw wastewater to municipal wastewater treatment plants (WWTPs), which causes fluctuations in wastewater characteristics [1–3]. Heavy metals from industrial wastewater often resulted in performance degradation or even system collapse of wastewater treatment processes based on activated sludge systems [4,5]. Chromium (Cr) is a common heavy metal contaminant widely derived from smelting, electroplating, leather tanning, and chemical manufacturing, and is highly toxic for organisms [6]. Therefore, the presence of Cr in wastewater could negatively affect microbial activities and microbial community structures, as well as the performance of biological treatment systems [7].

Cr mainly exists in the form of Cr(III) and Cr(VI) in aqueous solution. Cr(VI) is the most toxic among chromium species, and can easily penetrate cells and react with intracellular substances to affect the activity of microorganisms [8]. The biological treatment system of WWTPs is vulnerable to wastewater containing Cr(VI), resulting in the effluent quality not meeting the discharge standard [7]. It has been

reported that nitrifying bacteria was more sensitive than heterotrophic bacteria to the toxicity of Cr(VI), and a Cr(VI) concentration of 0.5 mg L^{-1} caused significant inhibition of the nitrification process (up to 74% decrease in ammonia removal efficiency) [9]. Cheng et al. [10] found that NH_4^+-N removal decreased from 97% to 68% with the addition of Cr(VI) with 5 mg L^{-1} in an SBR. Fang et al. [11] tested the acute tolerance of an enhanced biological phosphorus removal (EBPR) system on a high of concentration Cr(VI) and revealed that P removal performance was completely inhibited by Cr(VI) with a concentration higher than 5 mg L^{-1}, but the EBPR system could recover after a short Cr(VI) shock. Subsequently, Fang et al. [2] studied the long-term effects (52-d systematic investigation) of a low concentration of Cr(VI) (0.3–0.8 mg L^{-1}) on the P removal performance of a granule-based EBPR system, and found that a high Cr(VI) concentration (0.5 mg L^{-1}) could significantly inhibit P removal; filamentous bacteria overgrew, and eventually sludge bulking occurred in long-term Cr (VI) loading. The discrepancy in results of Cr(VI) effects on microbial metabolic activities could be attributed both to metal speciation [12] and the types of assays used [10], which played an important role when comparing toxicity expressions by different studies [7]. Nevertheless, most of the above investigations studied the short-term and long-term toxicity of Cr(VI) on EBPR system, data from which cannot shed light on the likelihood of the long-term stable operation of a simple nitrifying activated sludge system. Meanwhile, the long-term effects of Cr(VI) on extracellular polymeric substances (EPS) and microbial community structures, as revealed by high-throughput sequencing of a nitrifying activated sludge system, have not been reported.

The aim of this work is to reveal the long-term effects of Cr(VI) on a continuous-flow nitrifying activated sludge system. A comparative study of the nitrification performance, EPS, and microbial community structures was conducted under different influent Cr(VI) concentrations. Results obtained in the present study will help to improve our understanding of the complicated mechanisms of Cr(VI) on nitrifying activated sludge systems for the treatment of wastewater containing Cr(VI).

2. Materials and Methods

2.1. Experimental Apparatus and Wastewater Composition

The continuous-flow nitrifying activated sludge system consisted of an aeration tank, a sedimentation tank, and a sludge return system, as shown in Figure 1. The working volume of the aeration tank and sedimentation tank was 25 L and 10 L, respectively. The hydraulic retention time (HRT) was 8 h and the dissolved oxygen concentration was maintained at 4.5 mg L^{-1}. The sludge concentration was about 3500 mg L^{-1}, and the temperature was kept at 20 ± 2 °C using a heating rod. The inoculated activated sludge was taken from the aerobic tank of Lucun Sewage Treatment Plant (Wuxi, China). During the whole experiment, there was no sludge discharge except the sludge sampling. Detailed components of wastewater used for this experiment were as follows (per liter): chemical oxygen demand (COD) of 80 ± 6.2 mg L^{-1} (CH_3COONa), NH_4^+-N of 45 ± 3.8 mg L^{-1} (NH_4Cl), alkalinity of 260 ± 25 mg L^{-1} ($NaHCO_3$), Mg^{2+} of 1.5 ± 0.1 mg L^{-1} ($MgSO_4 \cdot 7H_2O$), and 0.3 mL trace element solution. The composition of trace element solution referred to the study of Dai et al. [13]. Potassium dichromate solution was added into the synthetic wastewater to achieve certain Cr(VI) concentrations (corresponding to Cr(VI) concentration from 0 to 0.5 mg L^{-1}).

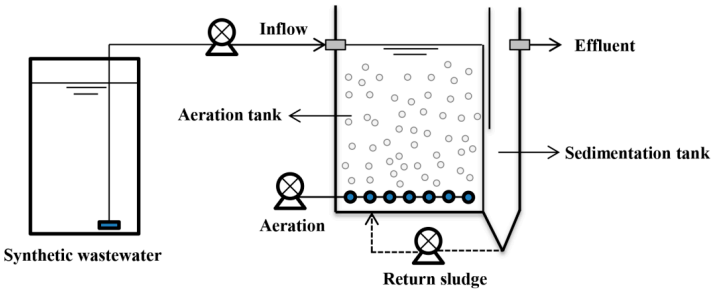

Figure 1. Schematic diagram of continuous-flow nitrifying activated sludge system.

2.2. Experimental Design

The experiment was conducted in seven stages over 180 days. The influent Cr(VI) concentration was gradually increased from 0 mg L^{-1} (days 1–50, phase I) to 0.1 mg L^{-1} (days 51–70, phase II), 0.2 mg L^{-1} (days 71–90, phase III), 0.3 mg L^{-1} (days 91–110, phase IV), 0.4 mg L^{-1} (days 111–130, phase V), and 0.5 mg L^{-1} (days 131–150, phase VI) to investigate the variations of nitrification activities, sludge characteristics, and microbial community structures at different Cr(VI) loading. Then, the influent Cr(VI) concentration was reduced to 0 mg L^{-1} (days 151–180, phase VII) to examine the recovery capability of nitrifying activated sludge system. Sampling was carried out at the end of each phase, and the sample names were NS0 (50th day), NS1 (70th day), NS2 (90th day), NS3 (110th day), NS4 (130th day), NS5 (150th day), and NSR (180th day), respectively.

2.3. Experimental Data and Analytical Methods

Relevant parameters were periodically detected to monitor the nitrification activities of the nitrifying activated sludge system during the whole experiment period. Parameters including ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), sludge volume index (SVI), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were determined following APHA (American Public Health Association) standard methods [14]. The loosely bound extracellular polymeric substances (LB-EPS) and tightly bound extracellular polymeric substances (TB-EPS) of nitrifying activated sludge were extracted using a heat extraction method [3,15]. The PN and PS in EPS were measured according to the methods reported by Fang et al. [2]. The calculation method of the specific oxygen utilization rate (SOUR), specific ammonium oxidation rate (SAOR), and specific nitrite oxidation rate (SNOR) at different influent Cr(VI) concentrations were determined according to Wang et al. [16].

2.4. Microbial Community Analysis Based on 16S rRNA High-Throughput Sequencing

The bacterial community structures of nitrifying activated sludge at different influent Cr(VI) concentrations were investigated by high-throughput sequencing. The V4 hypervariable region of the bacterial 16S rRNA genes was amplified using the primer pair 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) [17,18]. The sequence information of the obtained polymerase chain reaction (PCR) products was determined by the Illumina Miseq sequencing platform (Majorbio Bio-Pharm Co., Ltd., Shanghai, China). Subsequently, random sequencing error and low-quality sequences of the obtained sequential data were detected, trimmed, and removed to obtain the effective sequences [19]. Normalization of the sequence number in each sample was conducted to compare samples at the same sequencing depth [20]. Then, the normalized sequences were clustered into operation taxonomic units (OTUs) by setting a 0.03 distance limit (equivalent to 97% similarity) using MOTHUR program (http://www.mothur.org/wiki/MainPage). To obtain the detailed taxonomic identity of each OTU, the aligned DNA sequences were searched against the Silva database (Release 123, http://www.arb-silva.de) using the Basic Local Alignment Search Tool (BLAST). The microbial community diversity and abundance can be reflected by Alpha diversity including Chao1 richness estimator, ACE richness estimator, Shannon diversity index, Simpson diversity index, and Good's coverage, which were all calculated by MOTHUR program (version v.1.30.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity). Principal coordinate analysis (PCoA) and cluster analysis [13] were conducted based on the OTUs similarity to reveal the similarities and differences between the nitrifying activated sludge samples.

3. Results and Discussion

3.1. Performance of the Nitrifying Activated Sludge System at Different Influent Cr(VI) Concentrations

The performance of nitrifying activated sludge system with different influent Cr(VI) concentrations over 180 days is shown in Figure 2. The effluent concentrations of NH_4^+-N displayed

obvious fluctuations, first rising with increasing influent Cr(VI) and then slowly decreasing during the recovery period (Figure 2a). In the Cr(VI)-free condition (0 mg L^{-1}, phase I), the effluent concentration and removal efficiency of NH_4^+-N were 2.31 mg L^{-1} and 95.05%, respectively. However, the effluent NH_4^+-N increased markedly from 2.44 to 35.31 mg L^{-1}, and the removal rate of NH_4^+-N decreased rapidly from 93.89% to 22.43% as the Cr(VI) increased from 0.1 to 0.5 mg L^{-1}. Meanwhile, the NH_4^+-N removal efficiency only returned to 29.18% during the recovery stage (phase VII). The variation trend of effluent NO_3^--N concentration was opposite to that of effluent NH_4^+-N, and the NO_2^--N was not accumulated in the effluent at different influent Cr(VI) concentrations. All of the above results indicated that a high Cr(VI) concentration negatively and permanently affected the performance of a nitrifying activated sludge system, and the presence of heavy metal ions might inhibit the growth of nitrifying bacteria and decrease the activities of ammonia and nitrite oxidoreductase, which was in agreement with the results of several previous studies [1,3,13].

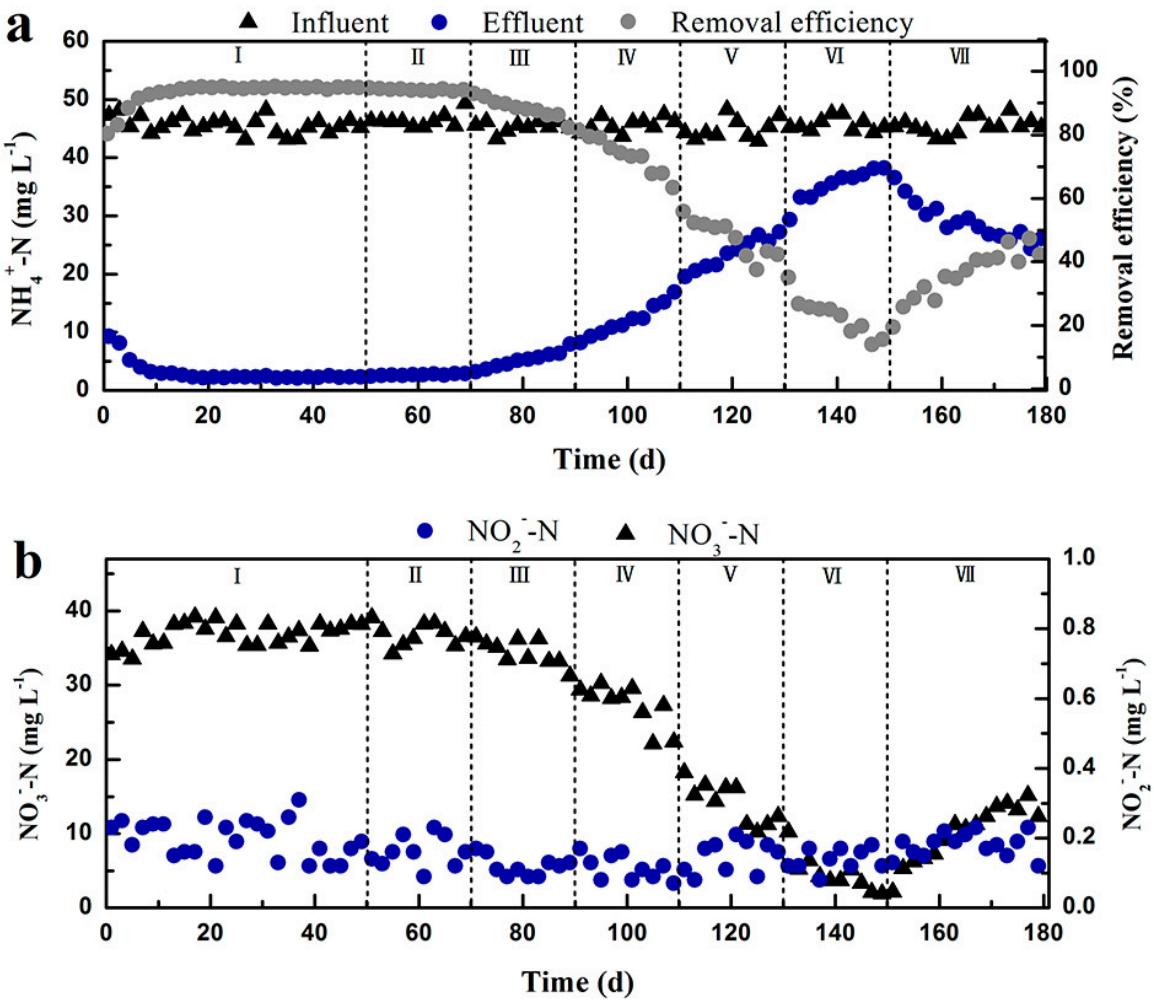

Figure 2. The variations of NH_4^+-N (**a**); NO_3^--N and NO_2^--N (**b**) concentrations in the influent and effluent at different Cr(VI) concentrations. The Cr(VI) concentrations were 0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0 mg L^{-1}, corresponding to the phases I, II, III, IV, V, VI, and VII, respectively.

3.2. Characteristics of Nitrifying Activated Sludge at Different Influent Cr(VI) Concentrations

The variations in SVI, MLVSS/MLSS, SOUR, SAOR, and SNOR at different influent Cr(VI) concentrations are shown in Table 1. An obvious increase in SVI value was observed from 72.35 to 187.36 mL g^{-1} MLSS with increasing Cr(VI) concentrations from 0 to 0.5 mg L^{-1}, indicating that the sludge bulking occurred in the nitrifying activated sludge system. From stage I to stage VI, the MLVSS/MLSS ratio significantly increased approximately 33%. The high MLVSS/MLSS ratio might be attributed to the disintegration of zoogloea under high Cr(VI) loading, which was confirmed by the SVI values. Similar results were found in studies on the effects of Cr(VI), nitrite, and Ca^{2+} on enhanced biological phosphorus removal systems [2,13,21]. As the Cr(VI) concentration increased from 0 to 0.5 mg L^{-1}, the SOUR, SAOR, and SNOR of nitrifying activated sludge system decreased from 53.24 to 18.17 mg N g^{-1} VSS h^{-1}, 6.31 to 1.68 mg N g^{-1} VSS h^{-1}, and 7.33 to 0.88 mg N g^{-1} VSS h^{-1}, respectively. Heavy metal ions could interact with thiol groups and destroy the protein structure and function of autotrophic microorganisms, resulting in a decrease of SOUR, SAOR, and SNOR [3]. The decrease in SNOR values was faster than that of SAOR with increasing Cr(VI) concentration because nitrite oxidation was more sensitive to the biological toxicity of Cr(VI) than ammonia oxidation. Hu et al. [22] found a similar phenomenon in their study of the effects of nickel and cadmium on an activated sludge system.

Table 1. Characteristics of nitrifying activated sludge at different influent Cr(VI) concentrations.

Cr(VI) (mg L^{-1})	Sampling Time (d)	SVI (mL g^{-1} MLSS)	MLVSS/MLSS (mg mg^{-1})	SOUR (mg N g^{-1} VSS h^{-1})	SAOR (mg N g^{-1} VSS h^{-1})	SNOR (mg N g^{-1} VSS h^{-1})
0	50	72.35 ± 2.78	0.72 ± 0.05	53.24 ± 3.29	6.31 ± 0.56	7.33 ± 0.45
0.1	70	83.25 ± 3.69	0.75 ± 0.07	45.36 ± 3.36	5.28 ± 0.22	4.21 ± 0.29
0.2	90	92.77 ± 3.25	0.81 ± 0.02	32.55 ± 2.98	3.47 ± 0.18	3.54 ± 0.23
0.3	110	123.55 ± 5.21	0.85 ± 0.03	28.79 ± 2.15	2.25 ± 0.21	1.56 ± 0.18
0.4	130	156.78 ± 5.79	0.92 ± 0.03	22.39 ± 3.12	1.79 ± 0.17	1.12 ± 0.16
0.5	150	187.36 ± 7.12	0.96 ± 0.04	18.17 ± 2.36	1.68 ± 0.09	0.88 ± 0.11
0	180	136.77 ± 4.65	0.83 ± 0.02	30.15 ± 1.02	2.97 ± 0.13	1.13 ± 0.19

EPS consists of a mixture of macromolecules, and has a significant influence on the maintenance of the bioaggregate structure and stability of an activated sludge system [23,24]. PS and PN, as the two main components of EPS, contain negatively charged functional groups that could act as a permeability barrier to effectively bind metal cations and reduce the toxicity of heavy metals to cells [3,25]. The variations of PN and PS contents in the LB-EPS and TB-EPS of nitrifying activated sludge system with different influent Cr(VI) concentrations are shown in Figure 3. As the Cr(VI) concentration increased from 0 to 0.5 mg L^{-1}, LB-EPS and TB-EPS increased from 25.34 to 72.35 mg g^{-1} VSS and 60.33 to 121.03 mg g^{-1} VSS, respectively. PN content in the LB-EPS and TB-EPS increased from 17.45 to 62.79 mg g^{-1} VSS and 34.01 to 89.80 mg g^{-1} VSS, respectively, and the PS content in the LB-EPS and TB-EPS increased from 7.89 to 16.01 mg g^{-1} VSS and 26.32 to 32.23 mg g^{-1} VSS, respectively. The above results indicated that, with the addition of Cr(VI), bacteria could secrete more PN and PS in the LB-EPS and TB-EPS to decrease the heavy metal toxicity to bacteria. The ratio of PN/PS in the two types of EPS presented a rising trend, increasing from 2.21 to 6.56 and 1.29 to 2.83, respectively. The amount of PN secreted was more than that of PS because EPS contained some exoenzymes that could reduce heavy metal ions [3,26], and an increase in Cr(VI) concentration could induce bacteria to produce more proteins. Thus, the increase of PN production was regarded as a protective response of bacteria to the increase of Cr(VI) concentration. During the recovery stage (phase VII, 30 days recovery), the values of all parameters were not comparable to the initial phase (phase I), indicating that the nitrifying activated sludge system was not revived after long-term Cr (VI) loading ($\leq$0.5 mg L^{-1}).

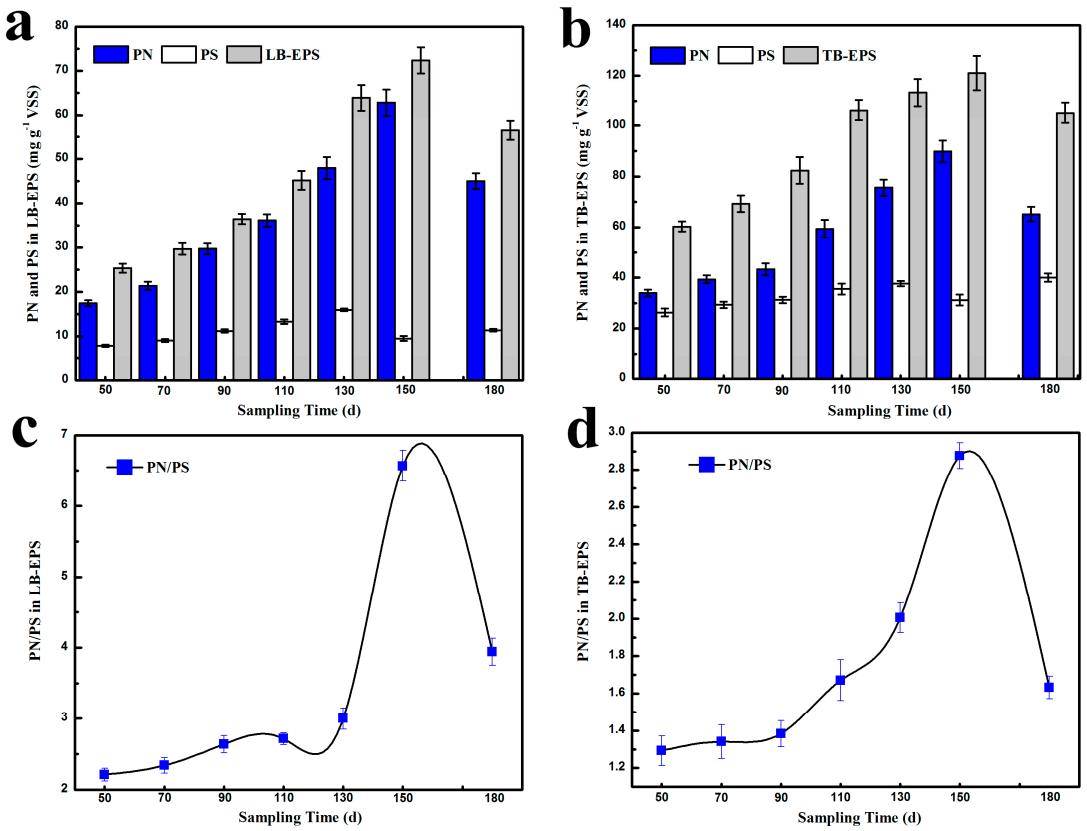

Figure 3. Variation of EPS at different Cr(VI) concentrations: PN and PS in LB-EPS (**a**) and TB-EPS (**b**) EPS content; PN/PS in LB-EPS (**c**), and TB-EPS (**d**).

3.3. Richness, Diversity, and Similarity of Microbial Community at Different Cr(VI) Concentrations

Seven sludge samples (NS0, NS1, NS2, NS3, NS4, NS5, and NSR) were collected at the end of each stage for studying the variations in microbial community under different influent Cr(VI) loading. A total of 244.08 K effective sequences of 16S rRNA gene's V4 region were obtained from the sludge samples (Table 2). The first 30,265 sequences in each sample were extracted to compare the samples at the same sequencing depth. The statistical analysis index, including Good's coverage, Ace, Chao 1, Shannon, and Simpson for evaluating the richness and diversity of microbial community, is also summarized in Table 2. The coverage index in all samples exceeded 0.99, indicating that all sequencing data could sufficiently reflect the structure of microbial communities [18]. The NS0 sample had the richest effective reads and OTUs number because it was collected from the culture without Cr(VI) loading. With the increase of influent Cr(VI) concentration, the OTUs number, richness indexes (Ace and Chao 1), and diversity indexes (Shannon) all showed a decreased trend. The above results indicated that high influent Cr(VI) ($\geq$0.2 mg L^{-1}) had a negative effect on the abundance and diversity of microbial community in a nitrifying activated sludge system.

Table 2. Alpha diversity estimators for the abundance and diversity of microbial communities of a nitrification sludge system at different Cr(VI) concentrations.

Sample ID	Effective Reads	Normalization No.	OTUs No.	Good's Coverage	Richness		Diversity	
					Ace	Chao 1	Shannon	Simpson
NS0	39,527	30,265	893	0.9974	941.87	925.57	4.37	0.029
NS1	37,564	30,265	882	0.9947	823.60	811.61	4.34	0.032
NS2	36,117	30,265	856	0.9968	724.52	699.23	4.24	0.047
NS3	36,129	30,265	821	0.9902	494.56	525.07	3.83	0.041
NS4	31,580	30,265	815	0.9968	418.51	422.32	3.35	0.091
NS5	30,265	30,265	802	0.9946	377.26	378.77	3.23	0.135
NSR	32,896	30,265	833	0.9956	526.39	465.38	3.97	0.105

PCoA and cluster analysis were conducted to compare the similarity of the microbial communities among the nitrifying activated sludge samples on the basis of OTUs numbers with 97% similarity (Figure 4). PCoA analysis showed that NS0 and NS5 had the longest relative distance from other samples, and shorter relative distances emerged between the samples of NS1 and NSR, NS2, and NS3, respectively (Figure 4a). The above results indicated that the microbial community structure was changed markedly by the imported Cr(VI), and a 30-d recovery process could revive the bacterial population of nitrifying activated sludge system to a certain extent. This view was also confirmed by the results of Cluster analysis (Figure 4b), which showed that the sludge samples of NS1, NSR, NS5, and NS4 were clustered together as one group, and the sample of DS0 formed another group.

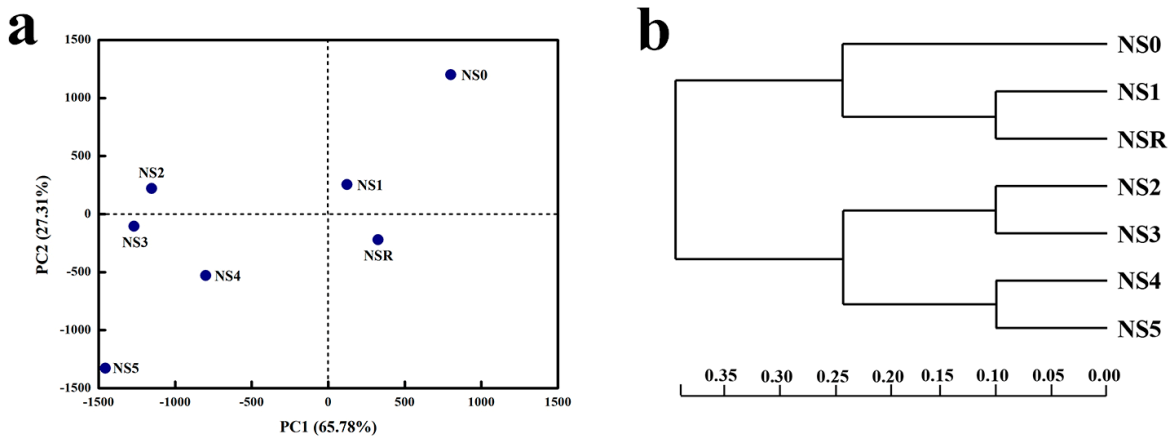

Figure 4. Principal coordinate analysis (PCoA) and cluster analysis for the similarity analysis of nitrification sludge samples at different Cr (VI) concentrations: (**a**) PCoA; (**b**) cluster analysis.

3.4. Variations of Microbial Communities and Functional Bacterial Populations at Different Influent Cr(VI) Concentrations

The normalized effective reads were blasted to the bacterial SILVA database (version SSU123) with a set confidence threshold of 80% to obtain the detailed taxa levels (from phylum to genus) [17]. The abundance of functional microorganisms in nitrifying activated sludge system at the level of phyla and the classes in *Proteobacteria* was investigated to reveal the variation in bacterial populations of nitrifying activated sludge at different influent Cr(VI) concentrations (Figure 5). *Proteobacteria* was the most abundant phylum in all samples (accounting for 39.23–53.28%), which was lower than the microbial communities in the conventional activated sludge system [27–29]. This might be because of the different cultivation and domestication conditions of the nitrifying sludge system. The other dominant phyla were *Nitrospirae* (accounting for 10.75–13.28%), *Bacteroidetes* (accounting for 9.25–12.32%), *Planctomycetes* (accounting for 7.79–11.23%), *Chloroflexi* (accounting for 6.32–18.14%), *Actinobacteria* (accounting for 1.52–9.13%), and *Chlorobi* (accounting for 2.24–4.51%), followed by several other major phyla (abundance >1% in any sample) including *Cyanobacteria*, *Bacteria_unclassified*, and *Firmicutes*. It was apparent that the microbial community structure at the phylum level was changed significantly under different influent Cr(VI) concentrations. Among them, *Nitrospira*, related to nitrifying bacteria, dramatically decreased from 13.28% to 0.75% with the increase of Cr(VI) concentration from 0 to 0.5 mg L^{-1}, indicating that high Cr(VI) loading severely inhibited the growth of nitrifying bacteria, resulting in the performance deterioration of the nitrifying activated sludge system (Figure 2, Table 1). *Chloroflexi*, the main filamentous related bacteria causing sludge bulking and foaming [30], increased from 7.25% to 18.14% with the increasing influent Cr(VI) concentration, indicating that high Cr(VI) loading could result in sludge bulking, which was in agreement with the variations of SVI (Table 1).

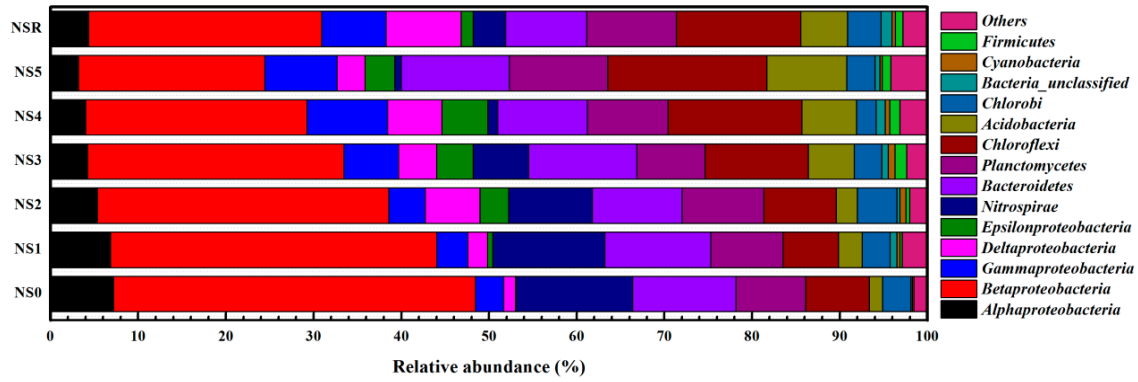

Figure 5. The relative abundances of different classes of *Proteobacteria* and phyla (>1% in any sample) in sludge samples (NS0, NS1, NS2, NS3, NS4, NS5, and NSR).

Within *Proteobacteria*, *β-proteobacteria* (21.23–41.25%) was the dominant class in all samples, followed by *γ-proteobacteria* (3.24–9.23%), *α-proteobacteria* (3.21–7.18%), *δ-proteobacteria* (0.31–8.55%), and *ε-proteobacteria* (0.11–5.22%). The relative abundance of *β-proteobacteria* and *α-proteobacteria* decreased from 41.25% to 21.23% and 7.18% to 3.21%, respectively, with the increase of influent Cr(VI) concentration from 0 to 0.5 mg L^{-1}, while the relative abundance of *δ-proteobacteria* and *ε-proteobacteria* increased with the increasing influent Cr(VI) concentration. This is because ammonia-oxidizing bacteria (*Nitrosomonas* and *Nitrosococcus*) and nitrite-oxidizing bacteria (*Nitrobacter*, *Nitrococcus*, and *Nitrospira*) mainly belong to *α-proteobacteria* and *β-proteobacteria* [31–33].

Further comparison of the functional bacterial populations at genus level was conducted to reveal more detailed information about the variation of microbial community structure under different influent Cr (VI) concentrations. The relative abundances of the dominant functional bacteria (>1% in any sample) are presented in Figure 6. The relative abundance of nitrification-related bacteria (*Nitrospira, Nitrosomonas*, and *Nitrosospira*) accounted for 16.52% without Cr(VI) loading. As the Cr(VI) concentration increased from 0.1 to 0.5 mg L^{-1}, nitrification-related bacteria decreased from 13.12% to 0.63%. Meanwhile, *Nitrosomonas and Nitrosospira* (nitrite-oxidizing bacteria) only accounted for 0.08% and 0.001%, respectively, under the Cr(VI) loading of 0.5 mg L^{-1}. The results indicated that nitrite-oxidizing bacteria were more sensitive to the toxicity of heavy metal ions than ammonia-oxidizing bacteria. *Pseudomonas*, a common pathogenic bacterium, increased from 5.10% to 10.23% with the increase of influent Cr(VI) from 0 to 0.5 mg L^{-1}, indicating that deteriorated nitrifying activated sludge under high Cr(VI) loading was a potential public health hazard. After a 30-d recovery operation, the relative abundance of nitrification-related bacteria returned to 4.83%, which only accounted for 29.23% of the initial microbial community (NS0), indicating that the activities of the nitrifying activated sludge system could not be completely recovered after long-term Cr(VI) loading.

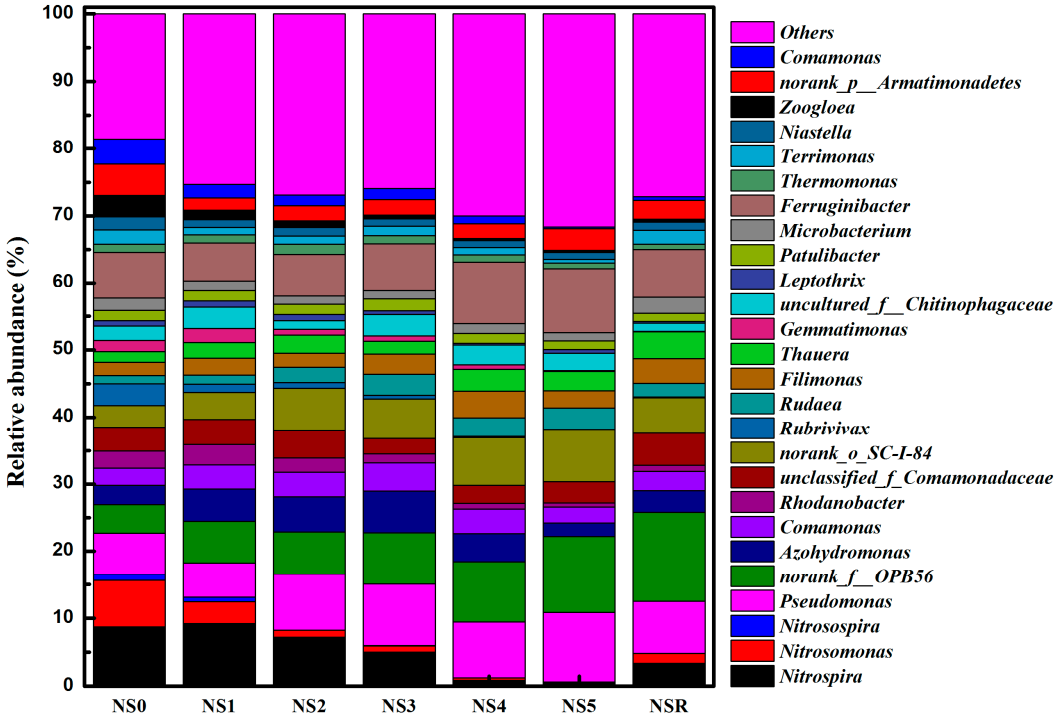

Figure 6. The relative abundance of the predominant microbial (>1% in any sample) in sludge samples (NS0, NS1, NS2, NS3, NS4, NS5, and NSR) at the genus level.

4. Conclusions

The present work revealed the long-term effects of Cr(VI) loading on nitrifying activated sludge system using the performance, EPS, and bacterial population analysis. It was proved that high influent Cr(VI) concentrations ($\geq$0.2 mg L^{-1}) not only seriously inhibited the nitrification performance but also markedly changed the microbial community structure of nitrifying activated sludge system. Meanwhile, an obvious sludge bulking was observed in the high Cr(VI) loading. The decrease in SNOR values was faster than that of SAOR with the increasing Cr(VI) concentration because nitrite oxidation was more sensitive to biological toxicity of Cr(VI) than ammonia oxidation. The variations of PN and PS contents in EPS showed that bacteria could secrete more PN and PS in the LB-EPS and TB-EPS to decrease the heavy metal toxicity to bacteria, and the amount of PN secreted was more than that of PS because EPS contained some exoenzymes that could reduce heavy metal ions. Microbial community structure analysis revealed that high Cr(VI) loading severely inhibited the growth of nitrifying bacteria, resulting in performance deterioration for the nitrifying activated sludge system, which poses a potential public health hazard. After a 30-d recovery operation, the activities of the nitrifying activated sludge system could not be completely recovered after long-term Cr(VI) loading.

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Conflicts of Interest: The authors declare no conflict of interest.

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