processes

MDPI

Article

Effect of Salinity on Cr(VI) Bioremediation by Algal-Bacterial Aerobic Granular Sludge Treating Synthetic Wastewater

Bach Van Nguyen [1,2,†], Xiaojing Yang [1,3,†], Shota Hirayama [1], Jixiang Wang [1], Ziwen Zhao [1], Zhongfang Lei [1,*], Kazuya Shimizu [1], Zhenya Zhang [1] and Sinh Xuan Le [2]

[1] Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8572, Japan; s1926055@s.tsukuba.ac.jp (B.V.N.); yangxj-tsukuba@outlook.com (X.Y.); s2021164@s.tsukuba.ac.jp (S.H.); s1836028@s.tsukuba.ac.jp (J.W.); tsukubazhaoziwen@outlook.com (Z.Z.); shimizu.kazuya.fn@u.tsukuba.ac.jp (K.S.); zhang.zhenya.fu@u.tsukuba.ac.jp (Z.Z.)
[2] Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology, Haiphong 180000, Vietnam; sinhlx@imer.vast.vn
[3] School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou 510275, China
* Correspondence: lei.zhongfang.gu@u.tsukuba.ac.jp
† These authors contributed equally to this work.

Abstract: Heavy metal-containing wastewater with high salinity challenges wastewater treatment plants (WWTPs) where the conventional activated sludge process is widely applied. Bioremediation has been proven to be an effective, economical, and eco-friendly technique to remove heavy metals from various wastewaters. The newly developed algal-bacterial aerobic granular sludge (AGS) has emerged as a promising biosorbent for treating wastewater containing heavy metals, especially Cr(VI). In this study, two identical cylindrical sequencing batch reactors (SBRs), i.e., R1 (Control) and R2 (with 1% additional salinity), were used to cultivate algal-bacterial AGS and then to evaluate the effect of salinity on the performance of the two SBRs. The results reflected that less filamentation and a rougher surface could be observed on algal-bacterial AGS when exposed to 1% salinity, which showed little influence on organics removal. However, the removals of total inorganic nitrogen (TIN) and total phosphorus (TP) were noticeably impacted at the 1% salinity condition, and were further decreased with the co-existence of 2 mg/L Cr(VI). The Cr(VI) removal efficiency, on the other hand, was 31–51% by R1 and 28–48% by R2, respectively, indicating that salinity exposure may slightly influence Cr(VI) bioremediation. In addition, salinity exposure stimulated more polysaccharides excretion from algal-bacterial AGS while Cr(VI) exposure promoted proteins excretion.

Keywords: algal-bacterial aerobic granular sludge; bioremediation; hexavalent chromium; salinity; wastewater treatment

Citation: Nguyen, B.V.; Yang, X.; Hirayama, S.; Wang, J.; Zhao, Z.; Lei, Z.; Shimizu, K.; Zhang, Z.; Le, S.X. Effect of Salinity on Cr(VI) Bioremediation by Algal-Bacterial Aerobic Granular Sludge Treating Synthetic Wastewater. *Processes* **2021**, *9*, 1400. https://doi.org/10.3390/pr9081400

Academic Editor: Enrico Marsili

Received: 2 July 2021
Accepted: 10 August 2021
Published: 13 August 2021

1. Introduction

The rapidly increasing global population, along with the development of industrialization, has triggered various environmental issues in many countries worldwide. Heavy metal (HM) in wastewater has emerged as a global environmental concern because of its adverse effects on human beings and ecosystems [1]. Among the HMs commonly found in wastewater, hexavalent chromium (Cr(VI)) is considered as one of the most harmful and toxic metal forms because of its high carcinogenic and mutagenic properties [2,3]. More specifically, exposure to Cr(VI) through inhalation, digestion, or direct contact can negatively affect human health, leading to liver and kidney damage, internal hemorrhage, and respiratory disorders [4]. Furthermore, Cr(VI)-contaminated wastewater is gaining more attention due to its diverse applications and higher concentrations in water contributed by various industrial processes such as metallurgy, refractory materials, textile dyeing, leather tanning, electroplating, and chemicals production [5,6].

To date, many techniques have been developed and applied to remove Cr(VI) from wastewater, such as chemical precipitation, reverse osmosis, ion exchange, and adsorp-

tion [4,7]. In general, physicochemical and biological methods are the common solutions to Cr(VI)-containing wastewater treatment [8]. However, physicochemical methods (such as electrolysis, membrane separation, and flocculation) often consume energy, with high costs in the long-term operation [9]. In addition, these techniques usually generate various secondary pollutants [10]. In contrast, biological treatment processes are cost-effective with low risk in respect of secondary pollutants. Among the biological processes, bioremediation has been extensively examined to be effective, economical, and environmentally friendly for HMs, especially Cr(VI) removal from wastewater [11,12]. The term of bioremediation commonly refers to the process of degrading contaminants from soil, water, and other environments using living organisms, such as microbes, bacteria, fungi, and plants [13].

Based on previous studies [14,15], the newly developed algal-bacterial aerobic granular sludge (AGS) is a promising biosorbent for Cr(VI) which can be applied to treat Cr(VI) contaminated wastewater. The algal-bacterial AGS has been reported to possess the merits of both algae (high surface area to volume ratio, different functional groups, and several algal cell wall components) and AGS (compact structure, superior settleability, and dense microbial structure). Nevertheless, when taking its practical application into consideration, the co-existence of Cr(VI) and salt ions is inevitable. For example, tannery wastewater may exert toxic effects on the microbial community in the wastewater treatment systems [16], thus adversely impacting the performance of wastewater treatment plants (WWTPs). Besides, the salinity in wastewater is also a key parameter for microalgae biomass production, and a higher salinity concentration may hinder the microalgae biomass production [17]. Although Yang et al. evaluated the effect of salinity on Cr(VI) adsorption efficiency by algal-bacterial AGS for the first time, the exposure time was very short (6 h) [15]. To date, the feasibility of Cr(VI) removal (with the co-existence of salt ions) by algal-bacterial AGS system has not yet been examined under a long-term operation condition. Furthermore, other parameters relating to algal-bacterial AGS system performance under the co-existence of Cr(VI) and salinity, such as nutrients removal and granule characteristics, have not been reported. Restated, little information is available regarding the efficiency of Cr(VI) removal by algal-bacterial AGS under a high salinity condition during a long-term operation. Therefore, this study aimed to investigate the effect of salinity on Cr(VI) bioremediation by algal-bacterial AGS in sequencing batch reactors (SBRs) in addition to its nutrients removal performance. Moreover, the effects of the co-existence of salinity and Cr(VI) on the characteristics of algal-bacterial AGS, such as morphological change, granule size, granule settleability, and stability, were also evaluated.

2. Materials and Methods

2.1. Experimental Design and Operation

In this study, two identical cylindrical SBRs with a working volume of 250 mL (D × H = 43 mm × 300 mm) each, namely R1 (control, no additional salinity addition) and R2 (with 1% additional salinity), were used. The reactors were operated at room temperature (25 ± 2 °C) under a 6 h cycle consisting of 7 min of feeding, 60 min of non-aeration, 282 min of aeration, 2 min of settling, 8 min of decanting, and 1 min of idling. The volume exchange rate (VER) was set at 50%, resulting in a hydraulic retention time (HRT) of 12 h. Air was introduced into each reactor from the bottom by an air pump (AK-40, KOSHIN, Japan), and the superficial uplift air velocity was controlled at 0.81 cm/s. The reactors were operated under 24 h illumination, which was provided by two artificial lights (lb055cw, RIKAKEN, Nagoya, Japan) attached to the outside wall of the reactors at an illuminance of around 6000 lux. During the experimental period, the sludge retention time (SRT) was not controlled, which varied between 30–40 days for the two SBRs according to the biomass concentration in the reactors and their effluent biomass concentrations. The whole experiment and test consisted of four stages as listed in Table 1.

Table 1. The 4 operation stages for the two SBRs in this study.

Reactor	Salinity and/or Cr(VI) Exposure	Operation Stage			
		I (Days 1–7)	II (Days 8–14)	III (Days 15–21)	IV (Days 22–28)
R1	NaCl (g/L)	0	0	0	0
	Cr(VI) (mg/L)	0	0	2	0
R2	NaCl (g/L)	0	10	10	10
	Cr(VI) (mg/L)	0	0	2	0

2.2. Synthetic Wastewater and Seed Mature Algal-Bacterial Granules

Synthetic wastewater was prepared and fed to the two SBRs, which consisted of the main components as follows: 300 mg chemical oxygen demand (COD)/L (with NaAc as the sole carbon source), 30 mg NH_4^+-N/L (NH_4Cl), 5 mg PO_4-P/L (KH_2PO_4), 10 mg Ca^{2+}/L ($CaCl_2 \cdot 2H_2O$), 5 mg Fe^{2+}/L ($FeSO_4 \cdot 7H_2O$), 5 mg Mg^{2+}/L ($MgSO_4 \cdot 7H_2O$), and 1 mL/L of trace elements solution. The specific composition of the trace elements solution was described in a previous study [18]. In addition, 50 mg/L $NaHCO_3$ was added to maintain the influent pH. In our previous study [15], the algal-bacterial AGS system was found to tolerate relatively low salinity <5 g/L (or 0.5%) during the 6 h Cr(VI) biosorption, which would be negatively affected when exposed to higher salinity levels. As the major purpose of this study was to examine the effect of the co-existing Cr(VI) and salinity on algal-bacterial AGS during a long-term operation, approximately 1% salinity was initially applied in the experiments. The results from the operation under higher salinity levels or real wastewaters will be included in the follow-up reports. The 1% salinity (with conductivity of about 17,000 µS/cm) in wastewater was generated by adding NaCl at a concentration of 10 g/L. The 2 mg/L Cr(VI) in the influent wastewater was obtained by diluting a 1000 mg/L stock Cr(VI) solution prepared from $K_2Cr_2O_7$. Mature algal-bacterial AGS was used in this study, which was developed from bacterial AGS. More specifically, the bacterial AGS was first sampled from the long-term bacterial AGS system operated by Wang et al. [19]. Then, the bacterial AGS was cultivated in another lab-scale SBR (D × H = 200 mm × 800 mm with a working volume of 15.7 L) under an artificial light illumination system of approximately 15k lux on the water surface and 40 k lux on the reactor wall (light on/off = 12/12 h). This lab-scale SBR was stably operated for 6 months at a mixed liquor suspended solids (MLSS) concentration of 3.4 g/L with sludge volume index in 30 min (SVI_{30}) of about 70 mL/g.

Moreover, prior to the 4-stage operation in this study, the algal-bacterial AGS was again cultivated in R1 and R2 for about 14 days by feeding the same influent wastewater without supplementation of salt and Cr(VI). Both achieved the same stable system performance before starting Stage I.

2.3. Analytical Methods

All water samples were first collected at the end of the third operation cycle (19:00) every day, then filtered through 0.22 µm membrane filters before further analysis. Ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), phosphate phosphorus (PO_4^{3-}-P), mixed liquor (volatile) suspended solids (ML(V)SS), and sludge volume index (SVI) were measured according to the standard methods [20]. In this study, total inorganic nitrogen (TIN) was calculated as the sum of NH_4^+-N, NO_2^--N, and NO_3^--N. Dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and dissolved total carbon (DTC) in the effluent samples were analyzed by a total organic carbon (TOC) analyzer (TOC-VCSN, Shimadzu, Kyoto, Japan). The concentration of Cr(VI) was measured by a UV-vis spectrophotometer (UV 1800, Shimadzu, Japan), which has been described in detail elsewhere [14]. Chlorophyll *a* (Chl-*a*) content in the granules was extracted and analyzed by the method described previously [21]. All the other assays, such as the granule size and distribution, granule strength (expressed as integrity coefficient or IC), extraction

of extracellular polymeric substances (EPS) (including loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS)), and determination of proteins (PN) and polysaccharides (PS), were the same as in a previous study [22].

2.4. Statistical Analysis

All the analyses were performed in triplicate and the results were expressed as mean or mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was conducted to compare the performance of the two SBRs and test stages using Microsoft Excel 2010. Statistically significant difference was assumed if $p < 0.05$.

3. Results and Discussion

3.1. Changes in Characteristics of Algal-Bacterial AGS in the Two Reactors

3.1.1. Morphology and Granule Size

In this study, changes in morphology and size of the granules from the two reactors were observed and recorded during the four stages' operation (Figure 1). Initially, the mature algal-bacterial AGS from both reactors was relatively regular in shape and dark green in color, demonstrating a dense and compact structure. After Stage I, there was not much change in the morphology of the granules because the salinity and Cr(VI) were not supplemented to the influent of the two reactors. Starting from Stage II, when 1% salinity was added to the influent wastewater of R2, some difference was clearly observed in the granules from the two reactors, especially the growth of filamentous bacteria outside the granules. Specifically, under the 1% salinity condition (R2), bacterial growth may have been inhibited, and further inhibition was observed under the prolonged exposure to salinity (to the end of Stage IV). In contrast, the granules in R1 (control) demonstrated the rapid growth of filamentous bacteria on their surface along with the operation. In addition, the presence of Cr(VI) in the influent to the two reactors (in Stage III) led to the appearance of specific colored precipitates (in gray and bright yellow) surrounding the surface of the granules from R1 and R2, respectively.

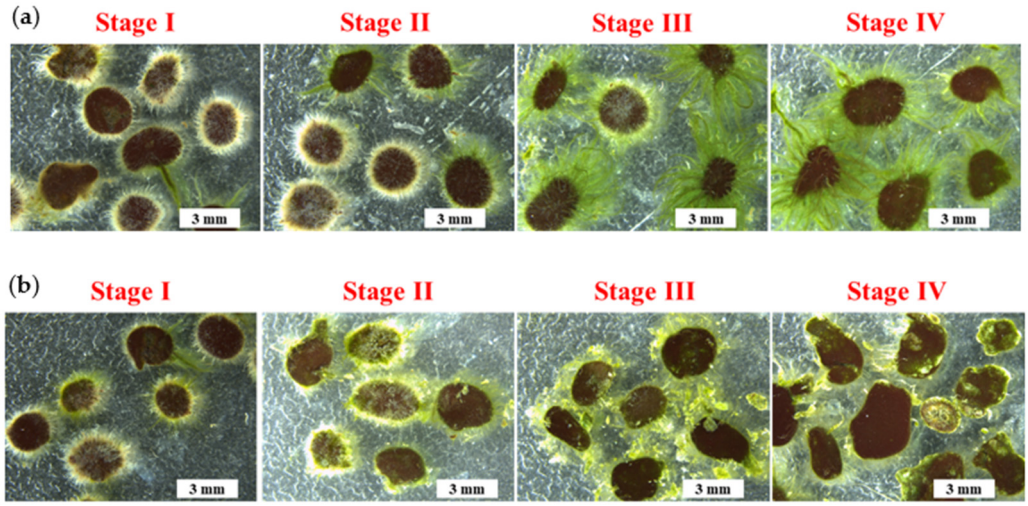

Figure 1. Microscopic images of algal-bacterial AGS from the two reactors during the four stages' operation. (**a**) R1; (**b**) R2.

Figure 2 demonstrates the changes in granular size and its distribution in the two reactors (R1 and R2). Generally, granules ranging between 1.5–2 mm and 2–3 mm were predominant in both R1 and R2 during the test period. In addition, an opposite trend was observed in the occupying ratio of the smallest (≤0.5 mm, decreased) and largest (>3 mm, increased) granules in both reactors. This trend was more obvious in R2, possibly due to the salinity exposure. Regarding the average diameter of granules, there was a gradual

increase over time in both reactors, from the initial 1.92 ± 0.87 (R1) and 1.90 ± 0.87 (R2) mm to 2.58 ± 1.39 (R1) and 2.62 ± 1.11 (R2) mm at the end of test, respectively.

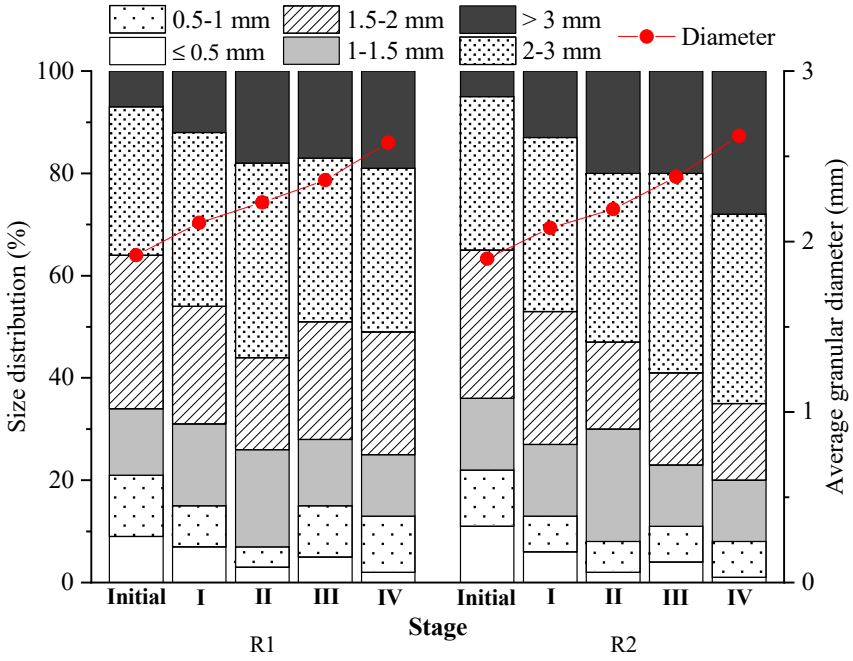

Figure 2. Changes in the diameter and its size distribution of algal-bacterial AGS in R1 and R2.

3.1.2. Granular Biomass Growth and Settleability

Figure 3a shows the changes of the MLSS and MLVSS/MLSS ratio in the two reactors during the four operation stages. Obviously, the MLSS concentration in the two reactors increased gradually over time, from the initial 3.40 to 6.67 g/L in R1 and 9.08 g/L in R2, respectively. A larger increase in MLSS was detected in R2, which is probably attributable to the effect of salinity exposure. The larger increase in biomass under the salinity exposure condition (R2 in this study) is consistent with previous studies [23,24], possibly due to the increased accumulation of inorganic materials into the granules. In addition, the presence of Cr(VI) in wastewater (Stage III) also triggered the increase in biomass growth in R1. In terms of MLVSS/MLSS, this ratio in R1 fluctuated between 80–92% during the experiment, while that in R2 decreased from 86% (Stage I) to 52% after exposure to 1% NaCl (Stage II), and then stabilized around 89% during the rest period of test.

On the other hand, salinity exposure also enhanced the granule settleability (Figure 3b), as the SVI_5 of granules from R2 decreased from 62.0 mL/g (Stage I) to 26.4 mL/g (Stage IV). This finding agreed well with other previous works [23,25]. In addition, Dong et al. [25] attributed the excellent settleability of algal-bacterial AGS under salinity conditions to its inhibitory effect on the growth of filamentous bacteria.

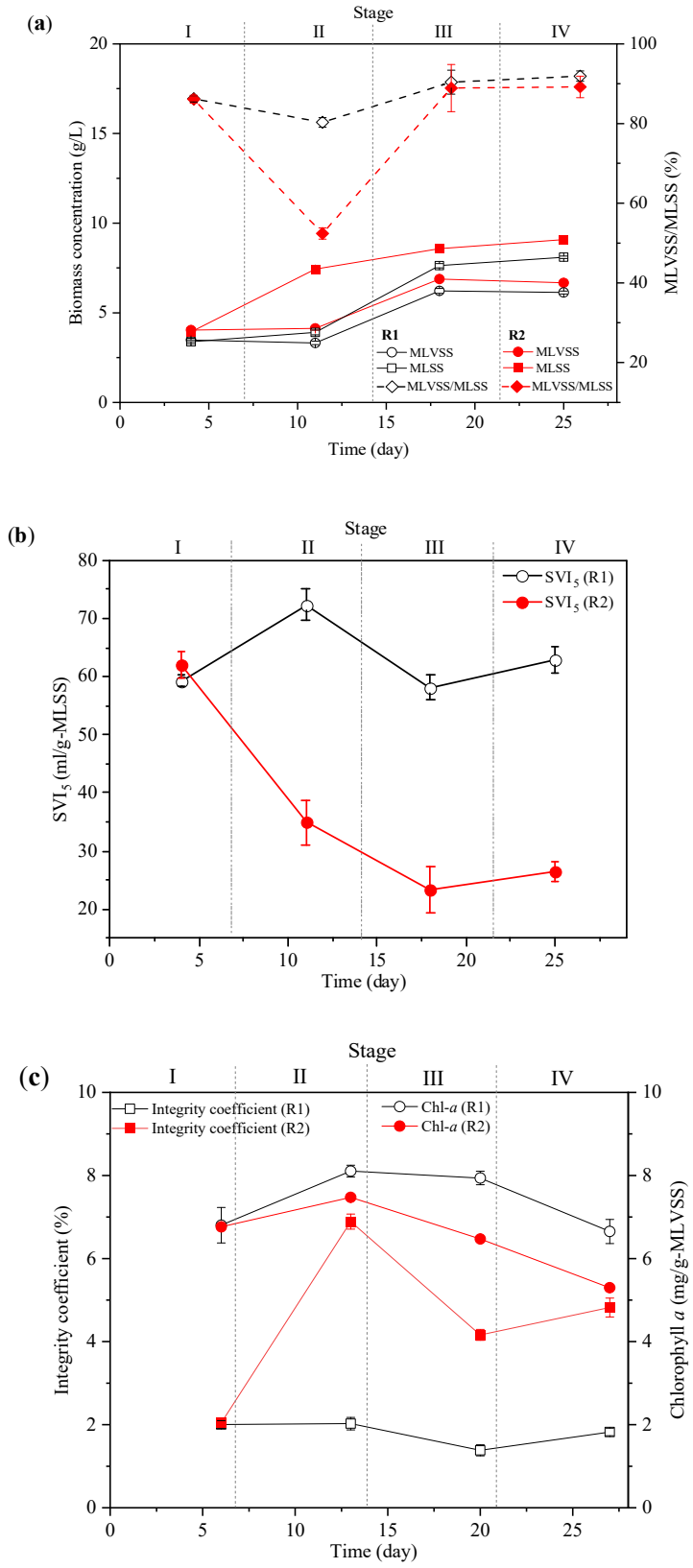

Figure 3. Characteristic changes of algal-bacterial AGS in R1 and R2 during the 28 days' operation. (**a**) ML(V)SS and MLVSS/MLSS ratio; (**b**) SVI_5; (**c**) integrity coefficient and chlorophyll *a* content.

3.1.3. Granular Stability and Chl-*a* Content

In this study, the integrity coefficient (IC) and Chl-*a* represent the granular stability (or granular strength) and algal content in granules, respectively. From Figure 3c, during Stage I with no salinity exposure and Cr(VI) addition, the IC values of granules from R1 and R2 were similar at 2.01% and 2.04%, respectively. However, in the presence of 10 g/L of NaCl (during Stage II), the IC of algal-bacterial AGS in R2 was consistently higher than that in R1 (no salinity exposure), indicating the decrease in granular strength in R2. The negative effect of salinity on granular strength in this study is consistent with previous studies [25,26]. Interestingly, when 2 mg/L Cr(VI) (Stage III) was co-existing, the IC values of granules in both reactors slightly decreased in comparison to their previous stage, and then slightly increased to 1.82% (R1) and 4.82% (R2), respectively, when Cr(VI) exposure ceased (Stage IV), implying a positive effect of Cr(VI) on granule stability. As a whole, a very slight change in IC value was observed in the granules from both reactors during the test period, probably due to the very compact structure of algal-bacterial AGS and the low concentrations of NaCl and/or Cr(VI) tested.

As seen from Figure 3c, after the exposure to 1% salinity (Stage II), the Chl-*a* content in the granules from R2 was consistently lower than that from R1, implying the negative influence of salinity on the growth of microalgae. On the other hand, in the presence of Cr(VI), the Chl-*a* contents tended to decrease in the granules from both reactors, which continued to decline even after Cr(VI) exposure ceased (Stage IV), to 6.65 mg/g-VSS and 5.30 mg/g-VSS in the granules from R1 and R2, respectively.

3.2. Performance of the Two Reactors

3.2.1. Organics Removal

The changes in effluent DIC, DOC concentrations, and DOC removal efficiency are shown in Figure 4. As seen, little impact was observed on organics removal under the salinity exposure and/or Cr(VI) presence, achieving, on average, >93% of DOC removal by the two reactors ($p = 0.152 >> 0.05$). Specifically, the effluent DOC concentrations from the two reactors were consistently lower than 18 mg/L, with an average DOC removal efficiency of 96.7 $\pm$ 1.1% by R1 and 96.5 $\pm$ 0.8% by R2 during Stage I, respectively. After the 1% salinity exposure (Stage II), the effluent DOC concentration from R2 began to increase and exceeded that from R1 (no salinity exposure) by 10.52 mg/L and 6.70 mg/L, respectively. Then, both DOC concentrations tended to increase after the exposure to 2 mg/L Cr(VI) (Stage III). When the exposure to Cr(VI) ceased during Stage IV, a slight decrease in DOC was detected in the effluents from both reactors, suggesting that both reactors could recover their DOC removal capacity. The above results imply the limited negative effect of salinity and Cr(VI) on algal-bacterial AGS with respect to DOC removal performance. The relatively low salinity level (1%) may lead to a result of "no significant difference" in DOC removal between the two reactors.

Compared with the effluent DIC concentration from R1, that from R2 was slightly higher. This phenomenon may be associated with the less algae growth in R2, which can be clearly reflected from the lower Chl-*a* content in the granules from R2 after salinity exposure (from Stage II to the end of test, Figure 3c). Thus, salinity exposure may partially contribute to the increase of effluent DIC concentration. For instance, according to Dong et al. [25], high salinity (1% and 3%) conditions can negatively affect the algal growth in algal-bacterial AGS system, resulting in high DIC concentrations in the effluent of SBR. Interestingly, after exposure to 2 mg/L Cr(VI) (Stage III), both reactors produced a lower DIC concentration effluent, and then became relatively stable when the Cr(VI) exposure ceased.

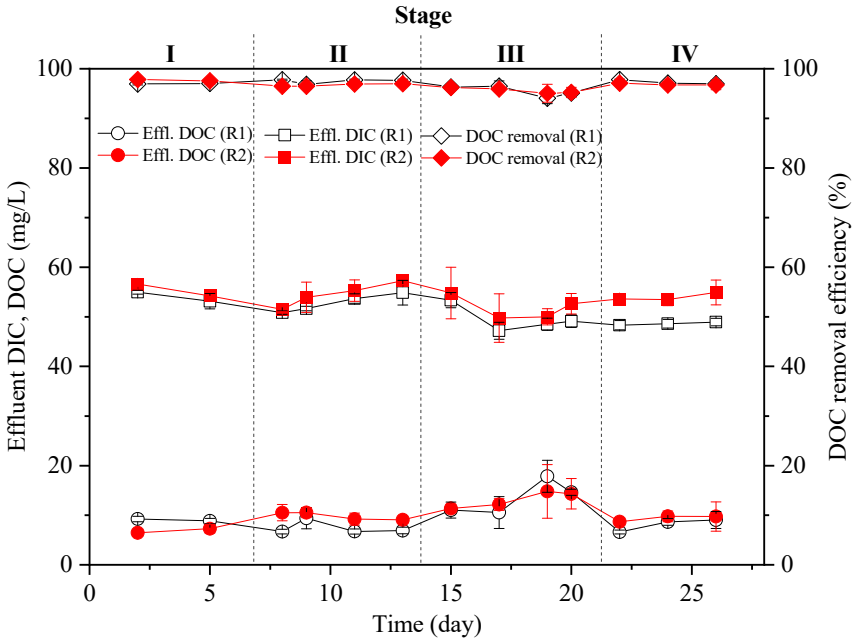

Figure 4. Variations of the effluent DOC and DIC concentrations from R1 and R2, and their DOC removals during the 28 days' operation.

3.2.2. Nitrogen Removal

The changes in effluent N species (including NH_4^+-N, NO_2^--N, and NO_3^--N) and total inorganic nitrogen (TIN) removal are shown in Figure 5. Among the variations of the three N species, the concentrations of effluent NH_4^+-N from the two reactors showed the most apparent fluctuations. More specifically, the effluent NH_4^+-N concentration from R2 gradually increased after the exposure to 1% salinity began (Stage II). This increasing trend continued when the granules were exposed to 2 mg/L Cr(VI) (Stage III), while the NH_4^+-N concentration steadily decreased when the exposure ceased from Stage IV. In contrast, the effluent NH_4^+-N concentration from R1 (no salinity exposure) gradually increased from the beginning of exposure to Cr(VI) (Stage III), but decreased during the later period of Stage III. Being different from the apparent fluctuation in effluent NH_4^+-N concentrations, the effluent NO_2^--N concentrations remained at a relatively constant low level, averagely 0.61 mg/L and 1.22 mg/L from R1 and R2, respectively. As for NO_3^--N, Cr(VI) exposure did not exert a noticeable effect on the granules in the two reactors. In addition, as can be seen from Figure 5, the effluent NO_3^--N concentrations from R2 were generally lower than those from R1 during most of the test period, to some extent reflecting the negative effect of salinity exposure on nitrification.

Based on the changes of TIN removal during the 28 days' operation of R1 and R2, the main findings can be summarized as follows: (i) In the test SBR (R2), nitrification process was inhibited when exposed to 1% salinity, which became worse after the granules were exposed to 2 mg/L Cr(VI) together. The granules could be recovered when the exposure to Cr(VI) ceased. (ii) In the control SBR (R1), Cr(VI) exposure also exhibited an inhibitory effect on nitrification, and the granules could be acclimated and recovered along with the operation. Once the Cr(VI) exposure ceased, the granules could quickly be recovered to the initial level.

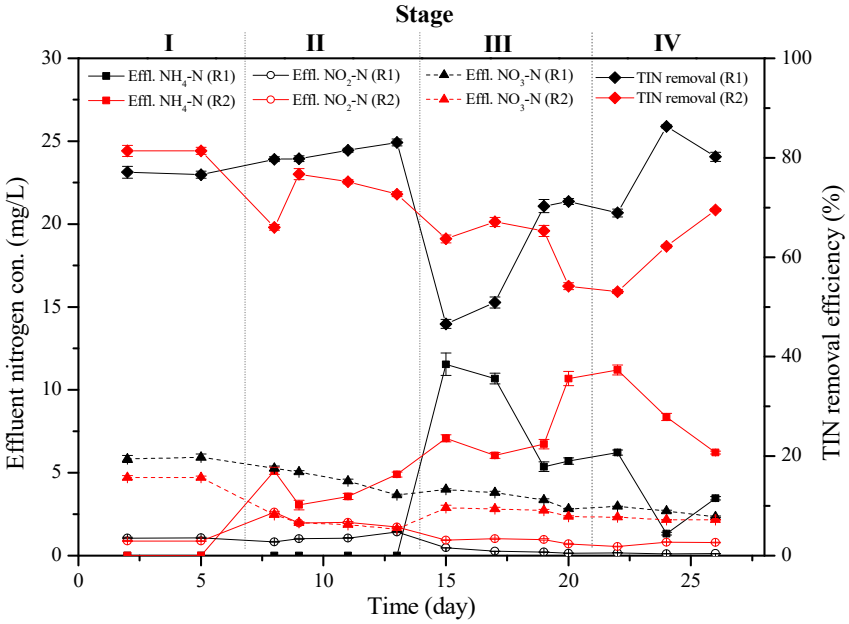

Figure 5. Profiles of the effluent nitrogen species from R1 and R2, and their TIN removals during the 28 days of operation.

The above results indicate that exposure to 1% salinity and/or 2 mg/L Cr(VI) may negatively affect nitrogen removal performance to different extents. This observation is probably associated with the inhibitory effects of the above harsh environment on the growth of algae or bacteria that are responsible for nitrogen uptake and conversion, in agreement with previous works [25,27].

3.2.3. Phosphorus Removal

Figure 6 shows the effluent TP concentration and TP removal efficiency of the two SBRs during the 28 days of operation. At Stage I with no exposure to 1% salinity and 2 mg/L Cr(VI), the TP removal efficiency was averagely kept at about 68.8% by R1 and 80.8% by R2, respectively, and this difference was mainly due to their different SRTs (no SRT control). After that, an obvious increase in the effluent TP concentration from R2 was detected, ranging from 0.97 mg/L to 3.59 mg/L when the granules were exposed to 1% salinity (Stage II), which led to a dramatic reduction in TP removal to the lowest level of 28.1%. However, this P removal performance was quickly recovered over the operation time. When the granules were exposed 2 mg/L Cr(VI) (Stage III), the effluent TP concentrations from R1 and R2 increased from 0.73 mg/L and 1.26 mg/L to 2.34 mg/L and 1.99 mg/L, corresponding to the reductions in TP removal efficiency to 53.3% and 60.3%, respectively. Even so, their TP removal efficiencies could be rapidly recovered, reaching 97.2% and 78.1% by R1 and R2 when the exposure to Cr(VI) ceased (Stage IV). This means that the algal-bacterial granules had a good TP removal capability and recoverability under fluctuated wastewater quality and variable environmental conditions, which could quickly recover from the short-term inhibition of 1% salinity and/or 2 mg/L Cr(VI) exposure. This observation is different from a previous study [25], i.e., the salt-stressed algal-bacterial AGS (under sudden salinity disturbance conditions) could not recover its original TP removal rate. The different response of algal-bacterial AGS in these two works are most probably attributable to the differences in SBR operating conditions (dimension and size, cycle time, aeration time, operating duration, etc.), light condition, salinity exposure procedure, and the different characteristics of the initial granules used.

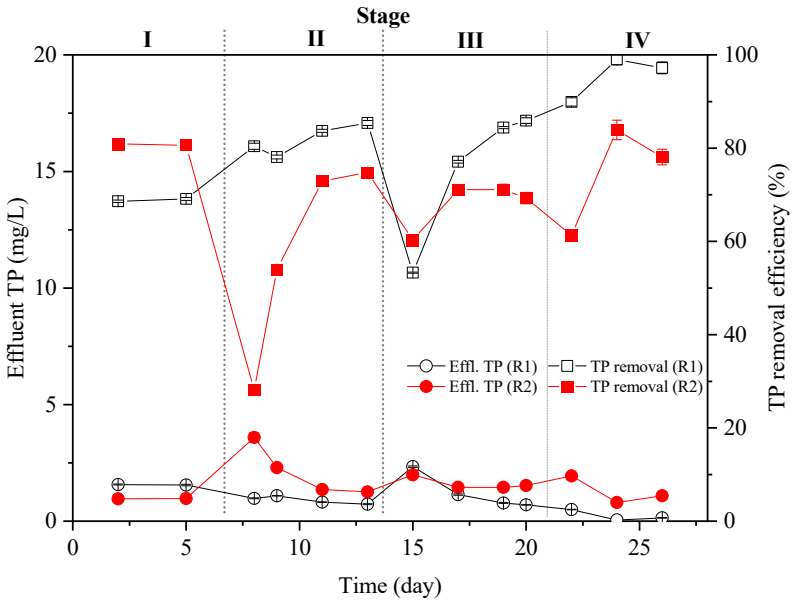

Figure 6. Profiles of the effluent P concentrations from R1 and R2, and their TP removals during the 28 days of operation.

Further studies are still necessary on the effect of salinity and/or Cr(VI) exposure on the anaerobic P release rate and aerobic P uptake rate, thereby shedding light on the mechanism regarding the varied P removal performance of the algal-bacterial AGS system.

3.2.4. Chromium Removal

In order to assess the effect of salinity on Cr(VI) removal efficiency by the algal-bacterial AGS system, the effluent Cr(VI) and Cr(III) concentrations, as well as the Cr(VI) removal efficiency, were monitored during Stage III (Figure 7). It is noteworthy that the Cr(VI) removal rate of R2 was generally lower than that of R1 in Stage III, possibly due to the slightly negative effect of salinity exposure. Specifically, the Cr(VI) removal efficiencies of R1 and R2 fluctuated between 31–51% (37.3% on average) and 28–48% (34.8% on average), respectively ($p = 0.005 < 0.05$). This observation might be attributable to the inhibitory impact of salinity on microbial activity in the AGS system [28], leading to the decline in Cr(VI) removal efficiency. In addition, the increase in ionic strength under the 1% salinity condition (R2) might have some shielding effect on the surface electrostatic field, thus suppressing the adsorption of chromium species on the active sites of algal-bacterial AGS [29].

The effluent Cr(III) concentrations from the two reactors could be obtained by subtracting the effluent Cr(VI) concentration from the effluent total Cr concentration. During Stage III, the effluent Cr(III) concentrations from R1 and R2 were very low, fluctuating between 0.01–0.04 mg/L and 0.01–0.06 mg/L, respectively. Still, it is necessary to further analyze the total Cr (Cr(III) + Cr(VI)) content in the granules, as well as in the EPS samples, to evaluate the effect of salinity on the Cr accumulation capability by the algal-bacterial granules.

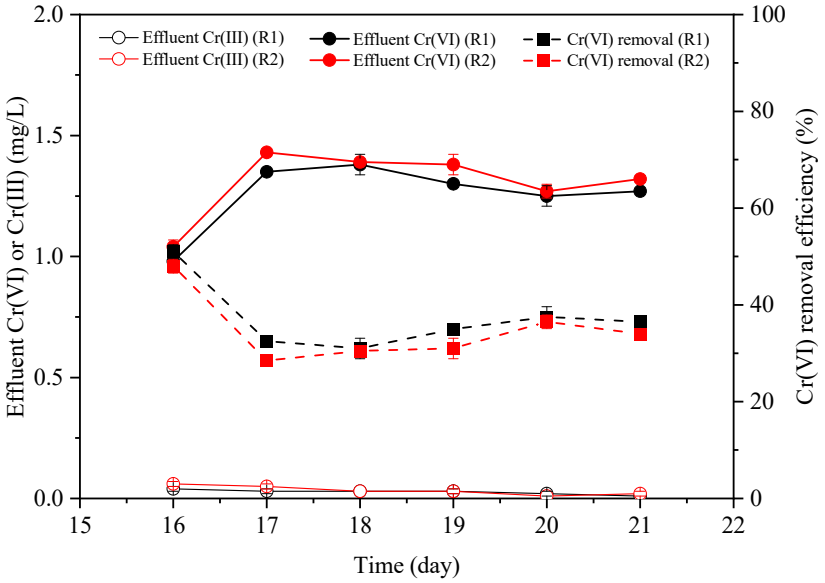

Figure 7. Profiles of the effluent chromium species from R1 and R2, and their Cr(VI) removals during Stage III.

Considering the Cr bioremediation mechanisms, the chemical fractionation of Cr in the algal-bacterial AGS (shown in Supplementary Figure S2), as well as surface functional groups (from FTIR spectra in Supplementary Figure S3), were also examined. For the granules in R1 (control, no salinity exposure), organic bound Cr contributed to the largest fraction, about 51.6%, indicating that metal complexation might be the main mechanism involved in Cr(VI) removal by algal-bacterial AGS in this study. This observation is consistent with previous studies [30,31], in which bacterial AGS and algal-bacterial AGS were used to adsorb Cr(III) and Cr(VI), respectively.

As for the granules in R2 (1% salinity exposure), the Fe/Mn oxides bound Cr fraction accounted for the highest percentage, about 52.0%, implying that Fe/Mn oxides in granules play a more important role in binding and adsorption of Cr than other fractions. Compared with the fractionation results of the granules from R1, under 1% salinity, some influence on Cr removal could be discerned, as the granules in R2 exhibited a higher chromium removal and immobilization by Fe/Mn oxides, and lower organic bound and residual fractions. It is possible that under some harsh condition (such as the 1% salinity condition in this study), the two most stable fractions (residual and organic matter bound parts) could be converted to less stable fractions, resulting in the increase in Cr bioavailability. Further studies are necessary on the leaching risk of Cr accumulated in algal-bacterial AGS with prolonged salinity exposure to better understand the effects of Cr-loaded granules on the environment. Moreover, the results from FTIR analysis (Supplementary Figure S3) and the morphological changes on granule surfaces (Supplementary Figure S1) partly confirmed that the functional groups such as carboxyl and phosphate played an important role in the metal complexation and/or chemical precipitation.

Based on the above results, the algal-bacterial AGS cultivated in the SBR system can potentially treat wastewaters containing both Cr(VI) and high salinity during the long operation period. Still, future works are necessary for a clear and insightful explanation of the mechanisms involved in the effects of co-existing Cr(VI) and salinity, which are also included in our follow-up research. For instance, further work on the changes of microbial composition and algae species during the long-term operation of algal-bacterial AGS system could help to clearly elucidate their roles in Cr(VI) removal.

3.3. EPS Secretion from Algal-Bacterial Granules

Algal-bacterial granule samples used for EPS extraction and EPS content determination were obtained from the two SBRs at the end of each operation stage. EPS are mainly composed of PN and PS that are secreted by microorganisms, which play an essential role in granular structure and stability [32,33]. The changes in PN and PS contents under different test stages are illustrated in Figure 8.

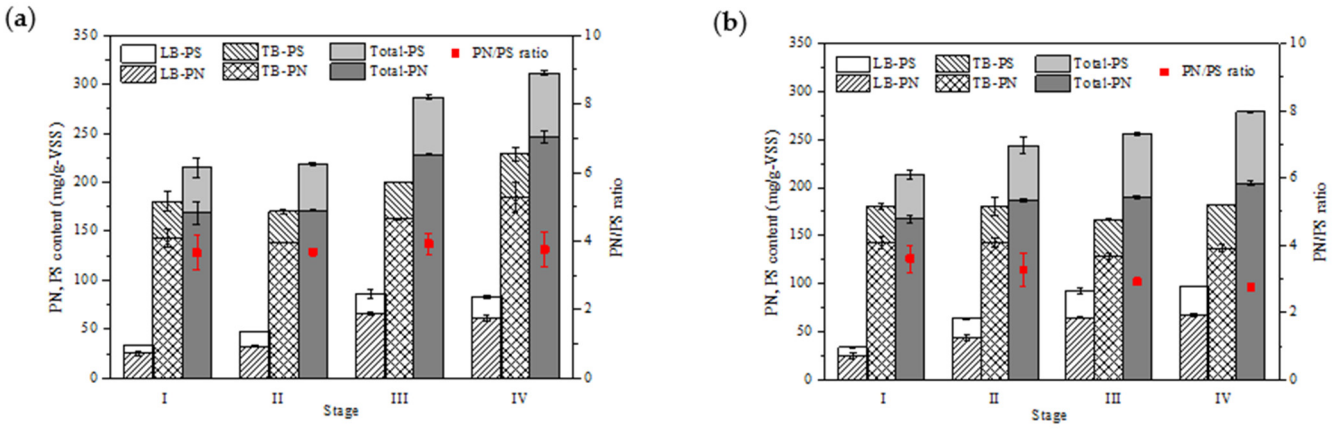

Figure 8. Variation of the EPS content (LB-EPS, TB-EPS, and total EPS), composition (PN and PS), and the PN/PS ratios of algal-bacterial AGS at the end of each test stage during the 28 days of operation. (**a**) R1; (**b**) R2.

As shown in Figure 8a, the total EPS content in the granules from R1 remained stable during Stages I and II. Meanwhile, for the granules in R2, after exposure to 1% salinity (Stage II), a slight increase in the EPS content, from 213.82 90 mg/g-MLVSS to 243.90 mg/g-MLVSS, was detected (Figure 8b). These results indicate that algal-bacterial AGS can produce more EPS to protect themselves from the increasing osmotic pressure due to the increase of influent salinity, in agreement with previous studies using granular sludge to treat saline wastewater [24,25,34,35]. For instance, Corsino and coworkers [35] found that the osmotic pressure generated by the increasing salinity can stimulate the production of exopolymers by microorganisms. EPS might mitigate the osmotic pressure on the cell wall of bacteria because of their gelatinous matrix.

As the influent Cr(VI) concentration increased from 0 mg/L to 2 mg/L (Stage III), the total EPS content in granules from both reactors increased. However, the extent of their increase was different. Specifically, after exposure to 2 mg/L Cr(VI), the granules from R1 excreted more EPS, which was remarkably increased to 286.64 mg/g-MLVSS. This increase trend continued, reaching 311.53 mg/g-MLVSS even after Cr(VI) exposure ceased (Figure 8a). On the other hand, in R2, the granular EPS content rose slightly to 259.39 mg/g-MLVSS and 278.73 mg/g-MLVSS during Stages III and IV, respectively. The significant increase of EPS in granules from R1 during Stage III might help protect microorganisms from harsh external condition (e.g., Cr(VI) presence in the influent) [27,32,36]. The granules from R2 may have better tolerance of Cr(VI) under the co-existence of 1% salinity.

From Figure 8a, the TB-EPS content showed a similar trend to the total EPS content during the four operation stages, while the LB-EPS content displayed a somewhat different variation trend. This finding implies that TB-EPS is an essential part affecting the algal-bacterial granule structure and stability under Cr(VI) exposure condition. On the contrary, the variation of LB-EPS content better matched with the total EPS change than the TB-EPS content in the granules from R2. Therefore, the LB-EPS played a more important role in algal-bacterial granule structure and stability in the presence of salinity, which is in line with a recent study [25].

As shown in Figure 8b, the PN/PS ratio gradually decreased over time under the presence or absence of Cr(VI), suggesting that 1% salinity exposure may trigger more

excretion of PS than PN, somewhat helping the granules maintain a more stable structure to resist saline conditions. This observation is consistent with the finding from a previous work [25]. One possible explanation for this phenomenon is that PS contains abundant polar functional groups that are often strongly water-binding and can thicken the hydration layer around the cells, mitigating the adverse effect of osmotic pressure generated by salinity [37]. Meanwhile, for the granules from R1, the PN/PS ratio remained stable at 3.66 and 3.68 during Stages I and II, respectively (Figure 8a). However, after exposure to 2 mg/L Cr(VI) (Stage III), their PN/PS ratio increased to 3.92, which fell to 3.76 when Cr(VI) exposure ceased (Stage IV). More excretion of PN than PS was detected from the granules in R1 during Stage III when compared to Stage II, which is probably associated with some exoenzymes contained in EPS that could reduce heavy metal ions [38] as enzymes are mainly composed of proteins. Interestingly, during the four test stages, the granules from both R1 and R2 contained consistently higher PN content than PS content. To summarize, salinity exposure promoted algal-bacterial AGS to secrete more PS, while Cr(VI) exposure stimulated more PN excretion.

4. Conclusions

The present study examined the effects of salinity on Cr(VI) bioremediation and nutrients removal performance by algal-bacterial AGS. The results from this work showed that algal-bacterial AGS reflected less filamentous and a rougher surface under salinity exposure, with little influence on organics removal being observed. However, the removals of total inorganic nitrogen and total phosphorus were noticeably impacted at the 1% salinity condition, and were further decreased with the co-existence of 2 mg/L Cr(VI). The Cr(VI) removal efficiency, on the other hand, was 31–51% in the control SBR and 28–48% in the test SBR, respectively, indicating that salinity exposure may slightly influence Cr(VI) bioremediation. Furthermore, in this study, it was found that 1% salinity exposure stimulated more PS excretion from algal-bacterial AGS, while PN excretion was enhanced with the introduction of 2 mg/L Cr(VI).

Supplementary Materials: The followings are available online at https://www.mdpi.com/article/10.3390/pr9081400/s1. Figure S1: Morphological changes of algal-bacterial AGS from R1 and R2 at the beginning and the end of the test, respectively. Figure S2: Chemical fractionation of different metals extracted from the Cr-loaded granules. Figure S3: FTIR spectrum of algal-bacterial AGS at the beginning (a) and (b) the end of test from R1 (without salinity addition), and (c) the end of the test from R2 (with 1% salinity addition).

Author Contributions: Conceptualization, B.V.N., X.Y., and Z.L.; Methodology, B.V.N., X.Y., J.W., Z.Z. (Ziwen Zhao), Z.L., and S.H.; Software, B.V.N. and X.Y.; Validation, B.V.N. and X.Y.; Formal analysis, B.V.N. and X.Y.; Investigation, B.V.N., X.Y., J.W., Z.Z. (Ziwen Zhao), and S.H.; Data curation, B.V.N., X.Y., J.W., Z.Z. (Ziwen Zhao), Z.L., S.H., and S.X.L.; Writing–original draft preparation, B.V.N. and X.Y.; Writing–review and editing, B.V.N., X.Y., Z.L., K.S., Z.Z. (Zhenya Zhang), and S.H.; Supervision, Z.L., K.S., and Z.Z. (Zhenya Zhang); Project administration, Z.L.; Funding acquisition, Z.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by JSPS KAKENHI Grant Number JP18H03403.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The first author, Bach Van Nguyen, would like to thank the financial and technical support by the Project for Human Resource Development Scholarship by Japanese Grant Aid (JDS), Japan International Cooperation Agency (JICA), and the Institute of Marine Environment and Resources (IMER), Vietnam Academy of Science and Technology (VAST), Vietnam for supporting his study in University of Tsukuba, Japan.

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

References

1. Sartore, L.; Dey, K. Preparation and heavy metal ions chelating properties of multifunctional polymer-grafted silica hybrid materials. *Adv. Mater. Sci. Eng.* **2019**, *2019*, e7260851. [CrossRef]
2. Gibb, H.J.; Lees, P.S.; Pinsky, P.F.; Rooney, B.C. Lung cancer among workers in chromium chemical production. *Am. J. Ind. Med.* **2000**, *38*, 115–126. [CrossRef]
3. Zhitkovich, A. Chromium in drinking water: Sources, metabolism, and cancer risks. *Chem. Res. Toxicol.* **2011**, *24*, 1617–1629. [CrossRef]
4. Tumolo, M.; Ancona, V.; De Paola, D.; Losacco, D.; Campanale, C.; Massarelli, C.; Uricchio, V.F. Chromium pollution in european water, sources, health risk, and remediation strategies: An overview. *Int. J. Environ. Res. Public. Health* **2020**, *17*, 5438. [CrossRef]
5. Mitra, S.; Sarkar, A.; Sen, S. Removal of chromium from industrial effluents using nanotechnology: A review. *Nanotechnol. Environ. Eng.* **2017**, *2*, 11. [CrossRef]
6. Brasili, E.; Bavasso, I.; Petruccelli, V.; Vilardi, G.; Valletta, A.; Bosco, C.D.; Gentili, A.; Pasqua, G.; Di Palma, L. Remediation of hexavalent chromium contaminated water through zero-valent iron nanoparticles and effects on tomato plant growth performance. *Sci. Rep.* **2020**, *10*, 1920. [CrossRef] [PubMed]
7. Fu, F.; Wang, Q. Removal of heavy metal ions from wastewaters: A review. *J. Environ. Manag.* **2011**, *92*, 407–418. [CrossRef] [PubMed]
8. Pushkar, B.; Sevak, P.; Parab, S.; Nilkanth, N. Chromium pollution and its bioremediation mechanisms in bacteria: A review. *J. Environ. Manag.* **2021**, *287*, 112279. [CrossRef] [PubMed]
9. Joseph, L.; Jun, B.-M.; Flora, J.R.V.; Park, C.M.; Yoon, Y. Removal of heavy metals from water sources in the developing world using low-cost materials: A review. *Chemosphere* **2019**, *229*, 142–159. [CrossRef]
10. Zabochnicka-Świątek, M.; Krzywonos, M. Potentials of biosorption and bioaccumulation processes for heavy metal removal. *Pol. J. Environ. Stud.* **2014**, *23*, 551–561.
11. Ashraf, S.; Naveed, M.; Afzal, M.; Ashraf, S.; Rehman, K.; Hussain, A.; Zahir, Z.A. Bioremediation of tannery effluent by cr- and salt-tolerant bacterial strains. *Environ. Monit. Assess.* **2018**, *190*, 716. [CrossRef]
12. Zeng, Q.; Hu, Y.; Yang, Y.; Hu, L.; Zhong, H.; He, Z. Cell envelop is the key site for Cr(VI) reduction by oceanobacillus oncorhynchi w4, a newly isolated Cr(VI) reducing bacterium. *J. Hazard. Mater.* **2019**, *368*, 149–155. [CrossRef]
13. U.S. Environmental Protection Agency. *Engineered Approaches to in Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications*; U.S. Environmental Protection Agency: Washington, DC, USA, 2000.
14. Yang, X.; Zhao, Z.; Yu, Y.; Shimizu, K.; Zhang, Z.; Lei, Z.; Lee, D.-J. Enhanced biosorption of Cr(VI) from synthetic wastewater using algal-bacterial aerobic granular sludge: Batch experiments, kinetics and mechanisms. *Sep. Purif. Technol.* **2020**, *251*, 117323. [CrossRef]
15. Yang, X.; Zhao, Z.; Zhang, G.; Hirayama, S.; Nguyen, B.V.; Lei, Z.; Shimizu, K.; Zhang, Z. Insight into Cr(VI) biosorption onto algal-bacterial granular sludge: Cr(VI) bioreduction and its intracellular accumulation in addition to the effects of environmental factors. *J. Hazard. Mater.* **2021**, *414*, 125479. [CrossRef]
16. Stasinakis, A.S.; Thomaidis, N.S.; Mamais, D.; Papanikolaou, E.C.; Tsakon, A.; Lekkas, T.D. Effects of chromium (VI) addition on the activated sludge process. *Water Res.* **2003**, *37*, 2140–2148. [CrossRef]
17. Minhas, A.K.; Hodgson, P.; Barrow, C.J.; Adholeya, A. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. *Front. Microbiol.* **2016**, *7*, 546. [CrossRef] [PubMed]
18. Huang, W.; Cai, W.; Huang, H.; Lei, Z.; Zhang, Z.; Tay, J.H.; Lee, D.-J. Identification of inorganic and organic species of phosphorus and its bio-availability in nitrifying aerobic granular sludge. *Water Res.* **2015**, *68*, 423–431. [CrossRef]
19. Wang, J.; Lei, Z.; Tian, C.; Liu, S.; Wang, Q.; Shimizu, K.; Zhang, Z.; Adachi, Y.; Lee, D.-J. Ionic response of algal-bacterial granular sludge system during biological phosphorus removal from wastewater. *Chemosphere* **2021**, *264*, 128534. [CrossRef] [PubMed]
20. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association, American Water Work Association and Water Environment Federation: Washington, DC, USA, 2012.
21. Wang, Q.; Shen, Q.; Wang, J.; Zhang, Y.; Zhang, Z.; Lei, Z.; Shimizu, K.; Lee, D.-J. Fast cultivation and harvesting of oil-producing microalgae ankistrodesmus falcatus var. Acicularis fed with anaerobic digestion liquor via biogranulation in addition to nutrients removal. *Sci. Total Environ.* **2020**, *741*, 140183. [CrossRef]
22. Zhao, Z.; Yang, X.; Cai, W.; Lei, Z.; Shimizu, K.; Zhang, Z.; Utsumi, M.; Lee, D.-J. Response of algal-bacterial granular system to low carbon wastewater: Focus on granular stability, nutrients removal and accumulation. *Bioresour. Technol.* **2018**, *268*, 221–229. [CrossRef] [PubMed]
23. Li, X.; Luo, J.; Guo, G.; Mackey, H.R.; Hao, T.; Chen, G. Seawater-based wastewater accelerates development of aerobic granular sludge: A laboratory proof-of-concept. *Water Res.* **2017**, *115*, 210–219. [CrossRef]
24. Meng, F.; Liu, D.; Huang, W.; Lei, Z.; Zhang, Z. Effect of salinity on granulation, performance and lipid accumulation of algal-bacterial granular sludge. *Bioresour. Technol. Rep.* **2019**, *7*, 100228. [CrossRef]
25. Dong, X.; Zhao, Z.; Yang, X.; Lei, Z.; Shimizu, K.; Zhang, Z.; Lee, D.-J. Response and recovery of mature algal-bacterial aerobic granular sludge to sudden salinity disturbance in influent wastewater: Granule characteristics and nutrients removal/accumulation. *Bioresour. Technol.* **2021**, *321*, 124492. [CrossRef]

26. Meng, F.; Huang, W.; Liu, D.; Zhao, Y.; Huang, W.; Lei, Z.; Zhang, Z. Application of aerobic granules-continuous flow reactor for saline wastewater treatment: Granular stability, lipid production and symbiotic relationship between bacteria and algae. *Bioresour. Technol.* **2020**, *295*, 122291. [CrossRef]
27. Wang, X.; Dai, H.; Zhang, J.; Yang, T.; Chen, F. Unraveling the long-term effects of Cr(VI) on the performance and microbial community of nitrifying activated sludge system. *Water* **2017**, *9*, 909. [CrossRef]
28. Bassin, J.P.; Pronk, M.; Muyzer, G.; Kleerebezem, R.; Dezotti, M.; van Loosdrecht, M.C.M. Effect of elevated salt concentrations on the aerobic granular sludge process: Linking microbial activity with microbial community structure. *Appl. Environ. Microbiol.* **2011**, *77*, 7942–7953. [CrossRef] [PubMed]
29. Shukla, A.; Zhang, Y.-H.; Dubey, P.; Margrave, J.L.; Shukla, S.S. The role of sawdust in the removal of unwanted materials from water. *J. Hazard. Mater.* **2002**, *95*, 137–152. [CrossRef]
30. Yao, L.; Ye, Z.; Tong, M.; Lai, P.; Ni, J. Removal of Cr3+ from aqueous solution by biosorption with aerobic granules. *J. Hazard. Mater.* **2009**, *165*, 250–255. [CrossRef] [PubMed]
31. Yang, X.; Zhao, Z.; Nguyen, B.V.; Hirayama, S.; Tian, C.; Lei, Z.; Shimizu, K.; Zhang, Z. Cr(VI) bioremediation by active algal-bacterial aerobic granular sludge: Importance of microbial viability, contribution of microalgae and fractionation of loaded Cr. *J. Hazard. Mater.* **2021**, *418*, 126342. [CrossRef]
32. Fang, J.; Su, B.; Sun, P.; Lou, J.; Han, J. Long-term effect of low concentration Cr(VI) on p removal in granule-based enhanced biological phosphorus removal (EBPR) system. *Chemosphere* **2015**, *121*, 76–83. [CrossRef]
33. Lin, H.; Ma, R.; Hu, Y.; Lin, J.; Sun, S.; Jiang, J.; Li, T.; Liao, Q.; Luo, J. Reviewing bottlenecks in aerobic granular sludge technology: Slow granulation and low granular stability. *Environ. Pollut.* **2020**, *263*, 114638. [CrossRef]
34. Wan, C.; Yang, X.; Lee, D.-J.; Liu, X.; Sun, S.; Chen, C. Partial nitrification of wastewaters with high nacl concentrations by aerobic granules in continuous-flow reactor. *Bioresour. Technol.* **2014**, *152*, 1–6. [CrossRef]
35. Corsino, S.F.; Capodici, M.; Torregrossa, M.; Viviani, G. Physical properties and extracellular polymeric substances pattern of aerobic granular sludge treating hypersaline wastewater. *Bioresour. Technol.* **2017**, *229*, 152–159. [CrossRef] [PubMed]
36. Wang, Z.; Gao, M.; Wang, S.; Xin, Y.; Ma, D.; She, Z.; Wang, Z.; Chang, Q.; Ren, Y. Effect of hexavalent chromium on extracellular polymeric substances of granular sludge from an aerobic granular sequencing batch reactor. *Chem. Eng. J.* **2014**, *251*, 165–174. [CrossRef]
37. Li, J.; Ye, W.; Wei, D.; Ngo, H.H.; Guo, W.; Qiao, Y.; Xu, W.; Du, B.; Wei, Q. System performance and microbial community succession in a partial nitrification biofilm reactor in response to salinity stress. *Bioresour. Technol.* **2018**, *270*, 512–518. [CrossRef] [PubMed]
38. Wang, Z.; Gao, M.; Wei, J.; Ma, K.; Zhang, J.; Yang, Y.; Yu, S. Extracellular polymeric substances, microbial activity and microbial community of biofilm and suspended sludge at different divalent cadmium concentrations. *Bioresour. Technol.* **2016**, *205*, 213–221. [CrossRef] [PubMed]