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RESEARCH ARTICLE

Bioremediation Effects and Functional Microorganisms Associated with Cr (VI) Pollution in Groundwater, Using a Mixed Nutrition Model Involving Siderite and Hydrochar

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Abstract The global industrialization process has led to the introduction of heavy metal Cr (VI) into groundwater, posing a significant threat to human health and survival. To address this issue, bio-experimental columns were constructed utilizing siderite and hydrochar as mixed nutrient sources to investigate the effects of microorganisms on Cr (VI) removal. Results indicated that both the hydrochar group and the siderite-hydrochar group achieved complete Cr (VI) removal at an influent Cr (VI) concentration of 0.6 mmol/L. At a higher influent Cr (VI) concentration of 1 mmol/L, the siderite-hydrochar group still exhibited a superior Cr (VI) removal efficiency compared to the individual use of siderite or hydrochar. The X-ray diffraction (XRD) spectra confirmed the reduction of Cr (VI) to insoluble Cr (III) through both microbiological and chemical pathways. Notably, the highest proportion of *Acidovorax* was observed in the experimental columns of the hydrochar group and siderite-hydrochar group, which may account for the notable removal of Cr (VI) observed in these two experimental columns, Additionally, other bacteria, such as *Pseudoxanthomonas*, *Ruminococcus*, and *Ruminiclostridium* were present in the system, potentially playing crucial roles in Cr (VI) removal. This study underscores the potential of using mixed nutrient sources and microbial communities for effective Cr (VI) remediation in groundwater, providing valuable insights for future research and practical applications in environmental protection.

Keywords: Mixed nutrition, Cr (VI) pollution, microorganism.

Introduction

Chromium, a hard and corrosion-resistant metal, is widely used in many industrial processes, such as electroplating, steel production, and leather tanning [1]. It exists in different valence states, with Cr (III) being an essential trace element for human health. However, Cr (VI) is classified as toxic due to its carcinogenic, teratogenic, and mutagenic properties [1]. The primary sources of chromium pollution are anthropogenic, including industrial wastewater and chromium-containing sludge [2]. Inadequate treatment and uncontrolled discharge of Cr (VI)-containing wastewater have positioned Cr (VI) as one of the most critical heavy metal pollutants in both surface and groundwater, posing a significant threat to human health and ecosystems [3].

To address this issue, one main approach has been adopted to controlling and remediating Cr (VI) pollution in water: converting soluble Cr (VI) into insoluble Cr (III) [4]. Traditional chemical remediation methods are costly and easy to produce secondary pollutants, such as toxic byproducts from incomplete chemical reactions or sludge from precipitation processes [4]. In contrast, biological remediation technology offers a safe and economic alternative that is both efficient and environmentally friendly [5].

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However, the natural reduction of Cr (VI) is slow, necessitating the acceleration of microbial reduction processes. Organic and inorganic substances can be added as electron donors and energy sources to enhance the metabolism and reproduction of Cr-reducing bacteria. Heterotrophic and autotrophic microbial reduction of Cr (VI) has been widely reported, with siderite and hydrochar being potential electron donors and sorbents for Cr (VI) removal [6,7]. Fe (II) in siderite can act as an electron donor for iron-oxidising microorganisms under aerobic and anaerobic conditions [8], and siderite has a strong capacity to remove and reduce Cr (VI) in anoxic aqueous environments such as wastewater or soil pore water [9]. Hydrochar is produced by mixing biomass and water in a proportion into a closed reactor with high-pressure resistance and heating carbonization under certain temperature and pressure conditions [10]. Due to its unique properties, such as rich functional group content, strong cation exchange capacity, and stable chemical properties, hydrochar is often used to remove heavy metals and organic pollutants from water bodies [11].

While low levels of natural organic matter in groundwater are insufficient to support heterotrophic reduction [12], adding organic carbon sources may lead to secondary contamination and aquifer plugging [13]. Purely heterotrophic (solely organic carbon-dependent) or autotrophic processes (solely inorganic carbon-dependent) rarely exist in natural or artificial biological processes. Therefore, mixotrophic techniques combining autotrophic and heterotrophic reduction processes have received increasing attention for their ability to overcome the drawbacks of both autotrophy and heterotrophy. Studies on the removal of Cr (VI) from groundwater using siderite-based mixotrophic techniques have not been reported yet, and hydrochar can form iron-carbon microelectrodes with siderite to facilitate electron transfer [14]. Therefore, this study aims to investigate the effect of microorganisms on the removal of Cr (VI) under a mixed mode of siderite and hydrochar. Soil containing mixed bacteria was collected from a Cr (VI) contaminated site, and the dominant bacterial groups were analyzed to provide a theoretical basis for managing Cr (VI) pollution.

Materials and Methods

Materials and Reagents

K_2CrO_7 (99.8%) and siderite powder were obtained from Chengdu Colon Chemical Co., Ltd. (Chengdu, China), and Changsha Jingkang New Material Technology Co., Ltd. (Hunan, China) respectively. The microbial inoculum was collected as chromium contaminated soil from Doumen Town, Shaoxing City, Zhejiang Province (30°13'31.238" N, 120°43'34.817" E). Corn straw was sourced from Mashan Town, Shaoxing City, Zhejiang Province, China. All chemical reagents used were of analytical grade.

Preparation of Hydrochar

An amount of 10 g of corn straw was weighed and added to a 500 mL hydrothermal reactor, along with 250 mL of ultrapure water. Subsequently, the reactor was placed in an oven at 200°C for 2 hours. After allowing it to cool completely, the product was centrifuged at 8000rpm for 5 minutes, and the supernatant was discarded. The precipitate was collected, washed three times with ultrapure water, and dried at 105 °C for 24 hours to obtain the hydrochar.

Experimental Column System Construction

Each experimental column (5 cm in diameter, 40 cm in height) was filled with 30 g of quartz sand with a particle size of 1-2 mm and 10 g of soil containing the microbial inoculum. Subsequently, three different experimental columns were filled with 10 g of siderite powder, 10 g of hydrochar, and a mixture of 10 g of siderite powder and hydrochar (ratio 1:1), respectively. The remaining space in each experimental column was filled with larger particles of quartz sand, and all three columns were wrapped in tinfoil to provide a dark environment. Synthetic groundwater containing Cr (VI) was introduced into the experimental columns at a 0.5mL/min flow rate. The concentration of Cr (VI) in the synthetic groundwater was maintained at 0.6 mmol/L (31.2 mg/L) from weeks 0 to 11 and adjusted to 1 mmol/L (52 mg/L) in the 12th week by the addition of K_2CrO_7. The composition of synthetic groundwater is as follow (per litre): NH_4Cl (0.1557 g), $CaCl_2$ (0.2464 g), $MgCl_2$ (1.05721 g), NaCl (0.4459 g), KCl (0.0283 g), $NaHCO_3$ (0.882 g) and KH_2PO_4 (0.0299 g) and was prepared using ultrapure water [15].

Analysis of Effluent Water

Samples were taken from each column every seven days for subsequent analyses. A sample of 20 ml of the initial effluent water sample was collected after 30 hours. Then, 20 mL samples were collected every seven days and 15 ml of each sample was filtered through a 0.45 μm nylon disposable filter and stored at -4°C. The remaining 5 mL were stored in a centrifugal tube and refrigerated at -4°C. The Cr (VI) content was determined using the diphenylcarbamide dihydrazine spectrophotometry method [16].

The total organic carbon (TOC) and total nitrogen (TN) contents were analyzed with an AR-element analyzer (Analytical Instruments Jena GMBH, Ulti N/C 3100), samples were pretreated with acidification (2 M HCl), blank correction used ultrapure water, calibration employed KHP (0–50 mg C/L) and KNO_3 (0–20 mg N/L) standards (5-point curve, $R^2 > 0.995$). The Cr (VI) removal efficiency was calculated using the formula:

$$\text{Removal efficiency (\%)} = \left(\frac{\mathrm{C1} - \mathrm{C0}}{\mathrm{C1}}\right) * 100$$

the amount of Cr (VI) was determined using the formula:

$$\text{Removal amount} = \frac{(\mathrm{C1} - \mathrm{C0}) * \mathrm{Q}}{\mathrm{V}}$$

Where C1 is the influent Cr (VI) concentration; C0 is the is the effluent Cr (VI) concentration, Q is the quantity of flow, V is the effective volume of the experimental column. Statistical analysis was performed using SPSS (Version 27) with ANOVA.

Solid Sample Analysis

After 18 weeks of operation, solid phase samples were collected from the experimental column. The samples were prepared for analysis by oven-drying at 60 °C for 24 hours to remove moisture, followed by homogenization using an agate mortar and pestle. The dried solids were sieved through a 63 μm mesh to ensure uniformity. The composition and structure of these solid samples were characterized using an X-ray diffractometer (Empyrean, Malvern Panaco).

Microbial Community Evolution

The solid-phase samples were extracted from the experimental columns and collected via low-temperature centrifugation (8,000 × g, 15 min, 4 °C). These samples were stored in cryotubes, flash-frozen using dry ice, and transported to Qingdao Bemac Biotechnology Co., Ltd. for shotgun metagenomic sequencing (Illumina NovaSeq PE150 platform). This sequencing approach was employed to analyze microbial community dynamics and identify functional taxa and genes associated with Cr (VI) reduction.

Quality control of raw sequences was performed using Fastp (v0.23.3) to remove splice and low-quality sequences. Megahit (v1.2.9) was then used to assemble the clean reads for each sample in a single sample to obtain the overlapping group sequences and to filter out the overlapping group sequences shorter than 300 bp [15]. The assembly results were evaluated using QUAST software. Alpha diversity indices (Shannon, Simpson, Chao1, ACE) were calculated using QIIME2 (version 2024.2) [17]. Statistical differences in alpha diversity were assessed using ANOVA.

Results and Discussion

Removal Efficiency of Cr (VI)

Figure 1 illustrates the removal rate and removal amount of Cr (VI) of experimental columns of the siderite group, hydrochar group, and siderite-hydrochar group. Initially, the influent Cr (VI) concentration was 0.6 mmol/L, the siderite group achieved a Cr (VI) removal rate of 53.32±1.06%, which increased to a maximum of 65±1.95% by week 1, corresponding to a Cr (VI) removal amount of 20.51 g/m³ (Figure 1a). However, this efficiency declined over time, reaching 4±0.12% with a corresponding Cr (VI) removal amount of 1.24 g/m³ by week 11. The poor Cr (VI) removal performance of siderite alone stems from its stable crystal structure, which tightly encapsulates Fe (II) ions within the mineral lattice. This structural stability significantly hinders the release of Fe (II) from the solid phase into the aqueous solution, particularly under neutral or alkaline conditions where siderite's solubility is further reduced. Consequently, the limited Fe (II) availability diminishes its effectiveness in Cr (VI) reduction [18]. It has also been shown that the reduction of Cr (VI) is accompanied by the oxidation of Fe (II) to Fe (III), leading to the formation of Fe (III)-Cr (III) hydroxide passivation layer on the surface of siderite, this layer can reduce the life and long-term effectiveness of siderite, preventing further Cr (VI) reduction reaction, and thus reducing its Cr (VI) removal ability [9].

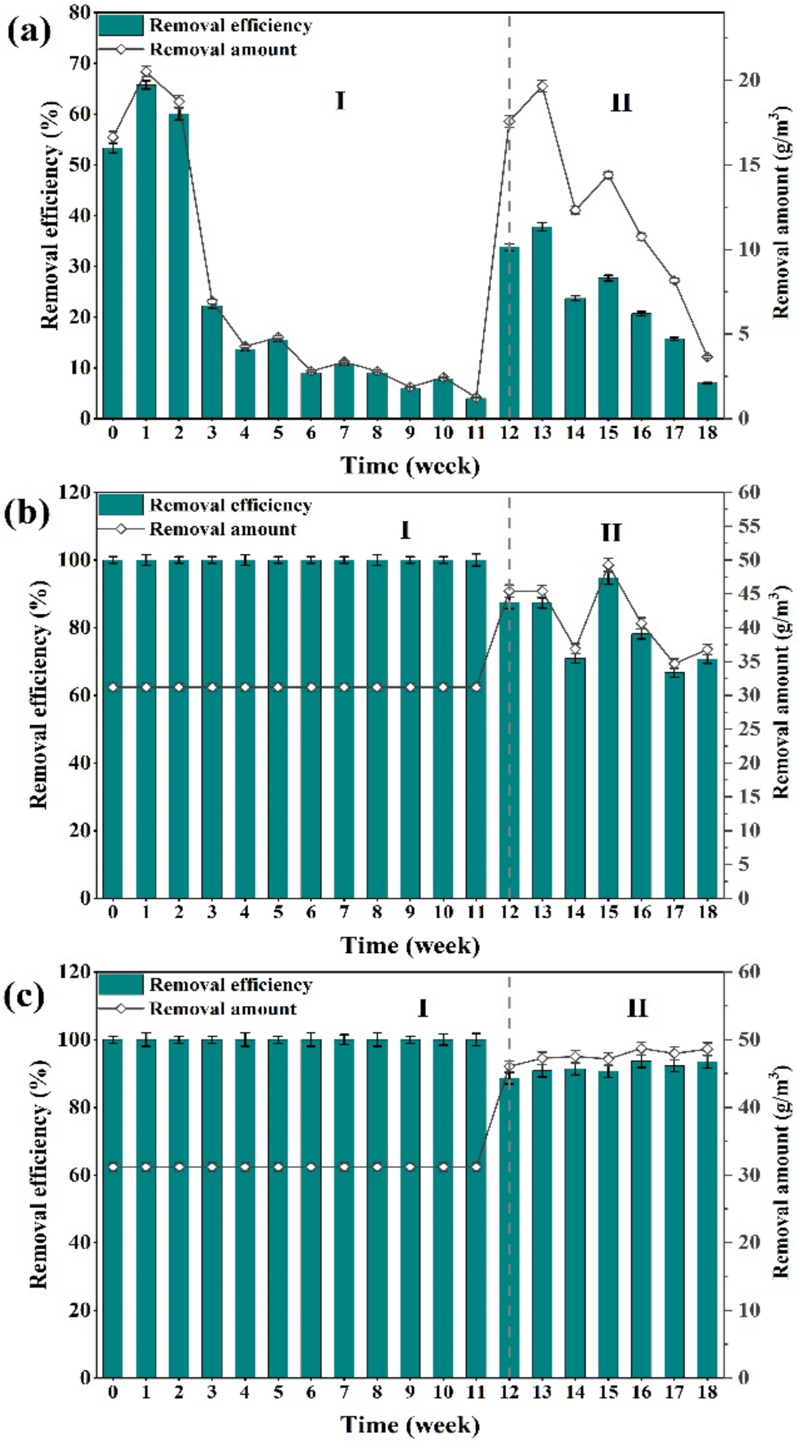

Figure 1. shows the changes in Cr (VI) removal efficiency and removal amount from the experimental columns of the siderite (a), hydrochar (b), siderite - hydrochar (c). The bar graph denotes the removal efficiency, the line graph denotes the removal amount, the phase Ⅰ with an influent Cr (VI) concentration of 0.6 mmol/L, phase Ⅱ with an influent Cr (VI) concentration of 1 mmol/L

The hydrochar group (Figure 1b) demonstrated a remarkable ability to remove Cr (VI). During weeks 0-11, the Cr (VI) removal rate reached 100%, with a total removal amount of 31.20 g/m^3. In a previous study, the hydrochar prepared by hydrothermal co-carbonization of sewage sludge and rice straw exhibits significant adsorption potential for Cr (VI) [19]. In the study, the hydrochar can remove Cr (VI) through adsorption and reduction, and Cr (VI) was adsorbed to the surface and interior of the hydrochar through the pore-filling effect [20]. Additionally, the hydroxyl group (-OH) present on the surface of the hydrochar can undergo oxidation to form C=O, releasing electrons that are subsequently transferred to Cr (VI), reducing it to Cr (III). Furthermore, the furan structure within the hydrochar also serves as an electron donor, providing π electrons that aid in the reduction of Cr (VI) [21].

The siderite-hydrochar group (Figure 1c) also demonstrated a high capacity for Cr (VI) removal, achieving complete removal from the system during weeks 0-11. This resulted in a Cr (VI) removal rate of 100% and a total removal amount of 31.20 g/ m^3. Qu *et al*. (2024) prepared Fe/N co-doped porous hydrochar (MP1HCMW) through microwave pyrolysis. MP1HCMW effectively removed Cr (VI) via pore filling, electrostatic attraction, ion exchange, reduction, complexation, and precipitation [22].

From the 12th week, the influent Cr (VI) concentration increased to 1 mmol/L, and the Cr (VI) removal rate of the three groups of experimental columns showed a turning point of decline. The removal efficiency of Cr (VI) in the siderite group increased from 4±0.12% to 33.8±0.60%, while the removal rate of Cr (VI) fluctuated and showed a trend decline. At the 18th week, the removal rate of Cr (VI) was 7± 0.14%, and the removal amount of Cr (VI) was 3.64g /m^3. Cr (VI) removal firstly increased and then decreased, which may be related to the growth of chromium-reducing bacteria. At the beginning of the reaction, the growth of some chromium-reducing autotrophic microorganisms was first promoted because of the increase in chromium concentration [23]. With prolonged reaction time, the reduction of Cr (VI) was accompanied by oxidation of Fe (II) to Fe (III), leading to the formation of a Fe (III)-Cr (III) hydroxide precipitate on the siderite surface. This precipitation was minimally soluble and highly stable, ultimately inhibiting the release of iron ions and consequently limiting microbial growth [9].

The Cr (VI) removal efficiency of the hydrochar group decreased from 100% to 87.31±0.75% upon the addition of higher levels of Cr (VI), followed by a slow decreasing trend in Cr (VI) removal efficiency, to 70.75±1.42% in the 18th week. Such changes may be related to the saturation of adsorption sites on the hydrochar surface, competition for adsorption, and changes in surface chemistry and structural properties [24]. The hydrochar-microbial system appears to be more robust compared to the siderite-microbial system, and was able to recover partially when challenged with a higher concentration of Cr (VI).

In comparison, the Cr (VI) removal efficiency of the siderite- hydrochar group decreased from 100% to 88.55±1.77%, followed by slight fluctuations, and stabilised at a high Cr (VI) removal rate (93.48±1.87%) by the 18th week. The combined effect of hydrochar and Fe^{2+} may promote the removal of Cr (VI) by microorganisms through both abiotic and biotic mechanisms. Liu *et al*. immobilised a Cr (VI)-tolerant Bacillus sphaericus (LD513) on modified hydrated charcoal (Fe3-SNHC), and found that the adsorption and reduction of Cr (VI) by Fe3-SNHC was greatly improved, and that Fe3-SNHC was able to promote the synthesis of proteins and metabolites by LD513 to cope with the pressure of the heavy metal Cr (VI), and Fe3-SNHC also act as an electron carrier to enhance bioreduction performance and promote Cr (VI) removal [25]. Wei *et al*. synthesised two types of ball-milled carbon Fe-MHBC (modified with $FeCl_3$) and MHBC (not modified with $FeCl_3$) from straw, it was found that Fe-MHBC was superior to MHBC for Cr (VI) removal, the presence of Fe enhanced the adsorption capacity of Cr (VI) and electron transfer flux, the hydrochar removed Cr (VI) effectively by electrostatic adsorption, redox and complexation reactions [26].

In summary, the siderite-hydrochar group showed the best Cr (VI) removal at both lower and higher Cr (VI) concentrations, consistently above 88.55±1.77% ($p< 0.001$).

Changes of TOC and TN in Effluent

The changes in TOC and TN in the three groups of experimental columns are shown in Figure 2. At the beginning of the experiment, the initial concentration of effluent TOC in the three groups of siderites, hydrochar, siderite-hydrochar was the highest in their respective experimental columns, which were 11.36 mg/L, 149.7 mg/L and 122.3 mg/L, respectively. Subsequently, there was a slight fluctuation accompanied by an overall decreasing trend. This decline can be attributed to the proliferation of Cr (VI)-tolerant heterotrophic reducing bacteria, which utilized the available TOC as a carbon and energy source for microbial metabolism. Under Cr (VI) stress, these bacteria likely enhanced their metabolic activity, driving the reduction of Cr (VI) while simultaneously consuming organic carbon for growth and energy production [27]. The decrease in TOC thus reflects not only microbial assimilation but also the potential role of organic matter as an electron donor in Cr (VI) reduction processes. At the beginning of Week 12th, the effluent TOC content in all three experimental column groups showed a slight increase compared to previous levels, followed by stabilization. This observed rise in TOC may be explained by the increased influent Cr (VI) concentration (1 mmol/L) implemented in Week 12. The elevated Cr (VI) levels likely inhibited heterotrophic microbial activity, resulting in reduced TOC degradation and consequently leading to the measured increase in effluent TOC concentration. By the end of the 18th week, the effluent TOC content in the three groups of experimental columns of siderite, hydrochar, siderite-hydrochar were 1.95 mg/L, 2.23 mg/L, and 2.13 mg/L, respectively. This stabilization reflected a shift in microbial community dynamics, The dominance of autotrophic microorganisms, which utilize inorganic carbon and derive energy from Cr (VI) reduction or other inorganic reactions, likely reduced organic carbon dependence [26], leading to decreased TOC consumption and stable effluent levels.

The TN concentration in the effluent water of the three groups siderite, hydrochar, siderite-hydrochar showed a similar pattern to the change of TOC. with a high initial effluent TN concentration, followed by a fluctuating downward trend. At the 11th week, the TN in the three groups of experimental columns dropped to the lowest, which were 0.03 mg/L, 0.12 mg/L and 0.02 mg/L, respectively. This process may be due to the action of nitrate-reducing bacteria, which results in a denitrification reaction [28]. At the beginning of the 12th week, the influent Cr (VI) concentration increased to 1mmol/L, and the TN concentration in the effluent water of the three groups of experimental columns showed a slight upward trend, probably as the increased Cr (VI) inhibited the action of nitrate-reducing bacteria. Chovanec *et al*. (2012) found that in a nitrate medium containing 100 μM Cr (VI), the growth of *Geobacter metallireducens* was inhibited entirely. In contrast, the growth rate of *Desulfovibrio desulfuricans* decreased by half, and the presence of Cr (VI) inhibited the growth of Cr (VI)-reducing bacteria [29].

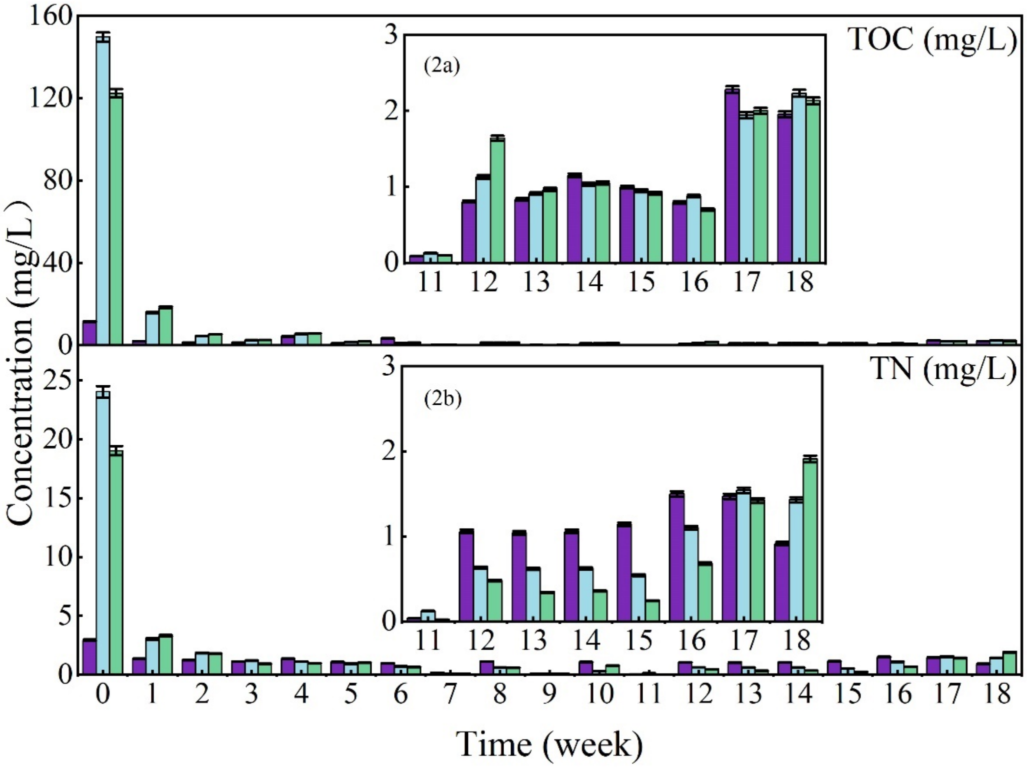

Figure 2. shows Changes in TOC and TN from the effluent of the siderite group (🟪), hydrochar group (🟦), siderite-hydrochar group (🟩). The initial concentration of TOC in the effluent of the siderite, hydrochar, and siderite-hydrochar groups peaked at 11.36 mg/L, 149.7 mg/L, and 122.3 mg/L, respectively. In the 18th week, the TOC concentrations in the effluent of the siderite, hydrochar, and siderite-hydrocha groups decreased to 1.95 mg/L, 2.23 mg/L, and 2.13 mg/L, respectively (Figure 2a). Initially, there was a higher TN concentration in effluent water, and in week 11, the TN in the effluent water of the three groups dropped to 0.03 mg /L, 0.12 mg /L and 0.02 mg /L (Figure 2b), and in week 12, the Cr (VI) concentration in the influent water increased to 1 mmol/L, the TN concentration in the effluent water of the three groups of experimental columns showed a slight upward trend and then maintained a relatively stable decreasing trend

X-ray Diffraction Analysis (XRD)

The XRD spectrum of the products from the three experimental columns (Figure 3) revealed significant diffraction peaks for Cr_2O_3 and Fe (CrO_4) OH in the siderite group, and the siderite-hydrochar group. This observation indicates that siderite not only participates in the reduction of Cr (VI) but may also facilitate the further stabilization of Cr (III) to form Fe (CrO_4) OH. In contrast, the hydrochar group lacked detectable Fe (CrO_4) OH peaks, likely due to insufficient iron availability for co-precipitation and the dominance of adsorption mechanisms. Bibi *et al*. (2018) reported that siderite possesses a strong capacity to remove and reduce Cr (VI) in anoxic aqueous environments, leading to the formation of a stable and insoluble mixed Cr (III)-Fe (III) oxidation phase [9]. The formation of Cr_2O_3 and Fe (CrO_4) OH correlates with higher Cr (VI) removal efficiency in the siderite-containing groups, as these phases represent irreversible immobilization of chromium via reductive precipitation and co-precipitation.

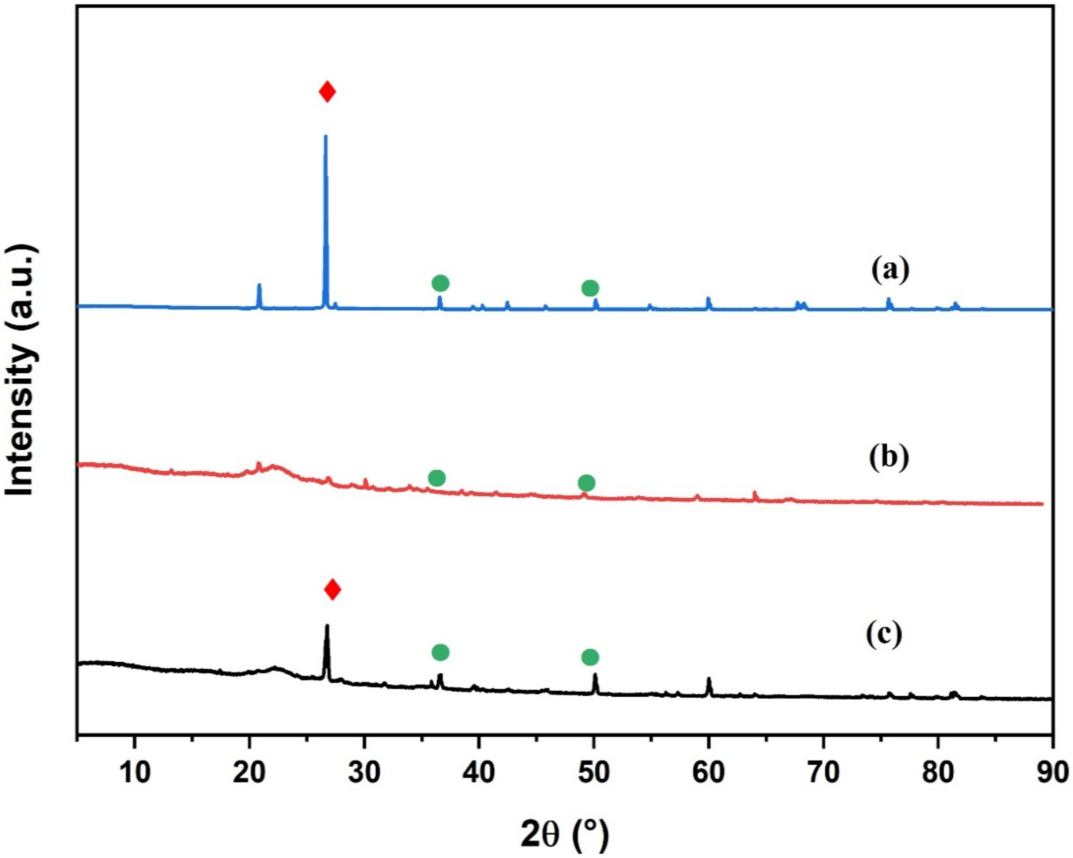

Figure 3. XRD spectrum of the solid samples the siderite (a), hydrochar (b), siderite-hydrochar (c) shows prominent diffraction peaks corresponding to Cr_2O_3 (●) and $Fe(CrO_4)OH$ (◆) in both the siderite and the siderite-hydrochar groups

Microbial community composition

Figure 4 presents the alpha diversity of the microbial population in the experimental column. The coverage value for the three samples was 0.99, indicating that the sequencing library is reliable and accurately reflects the diversity of the microbial population. Among the three data sets, the hydrochar group had the highest Shannon, Simpson, ACE and Chao. In contrast, the siderite-hydrochar group had the lowest Shannon, Simpson, ACE and Chao, indicating that the hydrochar group had a high microbial diversity and abundance, and the siderite-hydrochar group had low microbial diversity and abundance. This contrast reflects distinct ecological selection pressures: The availability of Fe (II) and Cr (VI) toxicity creates selective pressure favoring Cr (VI)-reducing bacteria while suppressing generalist taxa [30]. Additionally, localized acidic/anaerobic microenvironments generated by iron redox cycling further constrain microbial diversity [31]. Although this reduces overall richness, the siderite-hydrochar system's high Cr (VI) removal efficiency demonstrates the functional dominance of specialized Cr-reducing communities, consistent with known diversity-performance trade-offs in iron-rich environments [32].

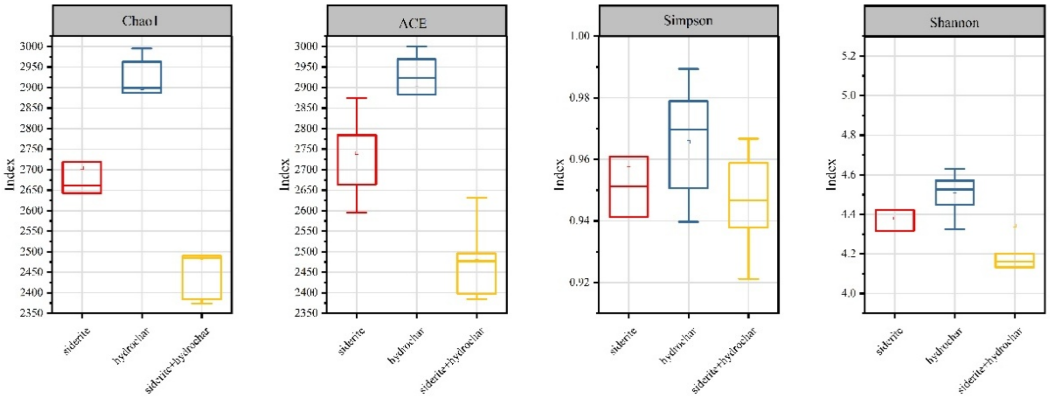

Figure 4. shows the box-and-whisker plot of alpha diversity indices (Chao, ACE, Simpson and Shannon) for the microbial communities in the siderite, hydrochar, and siderite- hydrochar groups. The box represents the interquartile range (IQR), the line inside the box indicates the median, and the whiskers extend to the minimum and maximum values

Based on the phylum classification of the microbial community composition of the samples (Figure 5a), the microbial communities in the three samples; siderite group, hydrochar group, and the combined siderite-hydrochar group—exhibited notable similarities in their dominant bacterial populations. *Proteobacteria* was the main phylum with a relative abundance of 73%, 49% and 50% respectively. The *Firmicutes* are also present in high proportions in the hydrochar and the siderite-hydrochar group at 21% and 39%, respectively; but is only found in the siderite group at very low proportion, *Firmicute* has a strong dependence on organic carbon sources [33]. *Proteobacteria* is usually the dominant phylum in autotrophic denitrification systems, and some *Proteobacteria* have also been reported to be involved in the biotransformation from Cr (VI) to Cr (III) [34]. *Firmicutes* showed high abundance in soils contaminated with heavy metals, especially in chrome-contaminated environments, and were positively correlated with chromium pollution [35].

At the class level (Figure 5b), *Betaproteobacteria* was highly represented in the siderite, hydrochar, and siderite-hydrochar groups. *Alphaproteobacteria* had a high proportion in the siderite and hydrochar groups but a low proportion in the siderite-hydrochar group. *Clostridia* were more abundant in the hydrochar and siderite-hydrochar groups, while *Gammaproteobacteria* was more abundant in the siderite group. The two distinct nutrient sources - hydrochar and siderite - significantly influenced microbial growth. *Betaproteobacteria* was highly enriched in chromium-contaminated soil [36], which can remove Cr (VI) from wastewater and show strong resistance and adaptability under high concentrations of Cr (VI) (125 mg/L) [28]. Previous studies have shown that *Alphaproteobacteria* can play an important role in biological denitrification at high Cr (VI) concentration [28]. *Gammaproteobacteria* and *Clostridia* have the potential to be used in bioremediation technologies for Cr (VI) pollution, multiple bacterial classes have been found by MiSeq sequencing in industrial waste from a chromite mine in Mexico, *Gammaproteobacteria* and *Clostridia* account for 7% and 5% respectively [37]. *Gammaproteobacteria* was a dominant microorganism in the soil polluted by tannage wastewater, it contained chromium reduction-related genes (such as OXR2, OXR3, OXR4, OXR5, OXR7, OXR8, etc.) that can help microorganisms survive in highly chromium-toxic environments and were effective in reducing the chromium concentration [38]. *Clostridia* can participate in the reduction of Cr (VI) in anaerobic environments by regulating the production and composition of EPSs [39].

Figure 5c also showed the microbial communities of the top 20 genera in the samples, which were significantly different in the three samples. *Pseudoxanthomonas* was the dominant bacterial group in the siderite group (8.91%), followed by *Brevundimonas* (4.75%), *Reyranella* (4.72%) and A*quabacterium* (4.18%). In the hydrochar group, *Acidovorax* accounted for the highest proportion (9.51%), followed by *Candidatus Cloacimonas* (4.30%), *Acinetobacter* (3.22%), *Clostridium* (2.71%). However, in the combined siderite and hydrochar group, *Acidovorax* had the highest proportion (12.66%), followed by *Ruminococcus* (4.82%), *Ruminiclostridium* (4.33%) and *Pseudoxanthomonas* (4.32%). Iron ion has a dual effect on the growth of microorganisms, not only promoting the growth of some microorganisms, but also inhibiting the growth of others [29]. Organic carbon is a key factor affecting the growth and reproduction of microorganisms [40]. In this experiment, the addition of siderite promoted the growth of *Acidovorax*, and on the contrary hydrochar inhibited the growth of *Pseudoxanthomonas*. *Pseudoxanthomonas* is a group of bacteria that is widely present in a variety of environments, especially in heavy metal and organic contaminant contaminated sites, and these bacteria are capable of surviving under extreme conditions and have demonstrated good ability in pollutant degradation and heavy metal removal [41]. *Pseudoxanthomonas* and *Brevundimonas* microbes also showed the capability of nitrification and denitrification [42]. *Acidovorax* was significantly enriched in iron-biochar wetlands supplemented with pyrite and plays a key role in reducing nitrate and chromium VI [43]. *Acidovorax* was tolerant to Cr (VI) and could proliferate at high Cr (VI) concentrations, and its relative abundance increased with increasing Cr (VI) concentration, from 0 to 20 mg/L Cr (VI) concentration range, the relative abundance of *Acidovorax* increased from 0.01% to 18.5% [44]. *Ruminococcus* promoted the reduction of nitrate and nitrite [28]. In the remediation of heavy metal contamination, *Ruminiclostridium* could degrade organic matter and promote the stabilisation of heavy metals, thus improving the environmental quality of sediments [45].

Thus, In the siderite group, chromium removal was likely mediated in part by microbial reduction, facilitated by taxa such as *Pseudoxanthomonas*, *Aquabacterium, Acidovorax*, and *Reyranella*, which are known for their denitrification capabilities. The relatively lower Cr (VI) removal efficiency in this group may reflect limited microbial metabolic activity due to insufficient bioavailable organic carbon. In the hydrochar group, the addition of hydrochar provided a bioavailable organic carbon source, promoting the dominance of fermentative taxa such as *Acidovorax*, *Candidatus Cloacimonas*, and *Acinetobacter*. This microbial composition suggests that the hydrochar group may function similarly to an anaerobic digestion system. Notably, *Acidovorax*, the most abundant genus in this group, has been widely reported to play a key role in nitrate and Cr (VI) reduction [46]. The second most dominant genus, *Candidatus Cloacimonas*, is commonly associated with biogas reactors, further supporting the anaerobic metabolic potential of this system [47]. Additionally, *Acinetobacter* has demonstrated the ability to reduce both N and Cr (VI) concentrations [48]. Thus, in hydrochar group, Cr (VI) removal likely results from a combination of microbial reduction (mediated by these bacterial genera) and bioadsorption onto the heterogeneous, porous surface of hydrochar. Furthermore, microbial metabolic activity may indirectly influence aqueous TOC and TN dynamics through organic matter degradation and nitrogen transformation processes. In the combined siderite and hydrochar group, the presence of siderite appeared to stimulate the proliferation of key bacterial genera including *Acidovorax*, *Ruminococcus,* and *Ruminiclostridium*, which were also observed in the hydrochar-only system. This group demonstrated the highest Cr (VI) removal efficiency, likely due to the dual effects of siderite supplementation. The iron mineral may serve as both an inorganic carbon source and electron donor for microbial metabolic processes. Notably, these dominant genera (*Acidovorax*, *Ruminococcus*, and *Ruminiclostridium*) have been documented to facilitate not only Cr (VI) reduction but also participate in nitrate and nitrite transformation pathways [29]. Their metabolic activities consequently influenced the dynamics of TOC and TN in the system through organic matter degradation and nitrogen cycling processes.

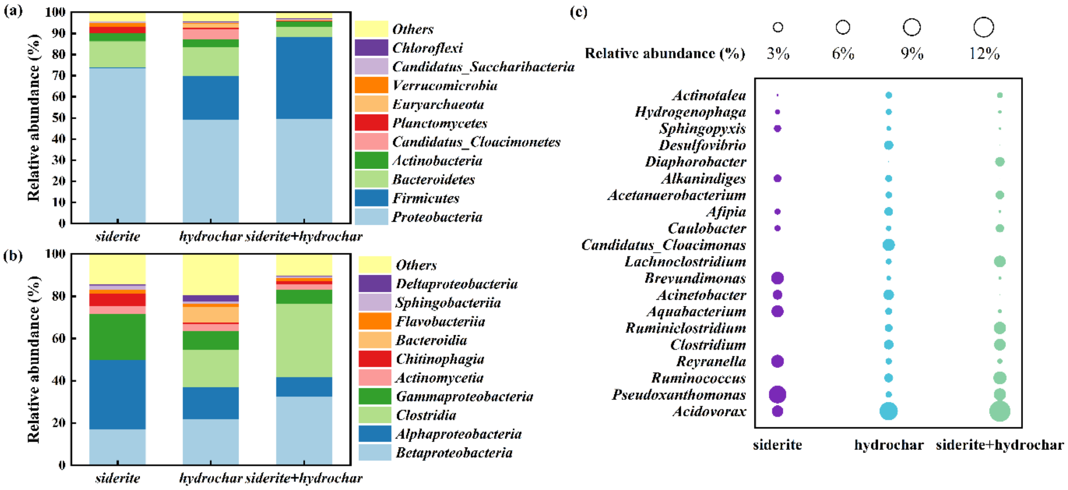

Figure 5. Microbial community structures at phylum (a), class (b), and genus level (c)

Conclusions

This study investigates the performance and potential mechanisms of Cr (VI) removal in a mixed nutrient bioreactor that employs siderite and hydrochar under anaerobic conditions. At an influent Cr (VI) concentration of 0.6 mmol/L, the hydrochar-only group and the siderite-hydrochar group achieved Cr (VI) removal rates of 100%. When the influent Cr (VI) concentration was increased to 1 mmol/L, the siderite and hydrochar group maintained a high removal rate, exceeding 88.55±1.77%. Fluctuations in TOC and TN concentrations in the effluent suggest that the organic carbon source and total nitrogen levels may influence the interactions between autotrophic and heterotrophic microorganisms affecting Cr (VI) removal efficiency. XRD analysis of the reaction products confirmed that the interaction of siderite with Cr (VI) resulted in the formation of precipitated compounds containing Cr (III) and Fe (III). The microbial community structure revealed that *Acidovorax* constituted the largest proportion of bacteria in the hydrochar and the siderite-hydrochar groups, potentially explaining the high Cr (VI) removal rates observed in these experimental columns. Additionally, the system included other bacteria such as *Pseudoxanthomonas*, *Ruminococcus*, and *Ruminiclostridium*, which may play significant roles in denitrification and the removal of Cr (VI). This study demonstrates that the siderite-hydrochar system effectively removes Cr (VI) while enhancing microbial diversity, suggesting strong potential for industrial wastewater remediation. The combined approach offers a scalable and cost-effective solution for high Cr (VI) contamination scenarios. Future research should focus on optimizing reactor design, long-term stability under varying wastewater compositions, and economic feasibility for industrial adoption.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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