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Original Research

Groundwater chromate removal by autotrophic sulfur disproportionation

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ABSTRACT

Chromate [Cr(VI)] contamination in groundwater is a global environmental challenge. Traditional elemental sulfur-based biotechnologies for Cr(VI) removal depend heavily on the synthesis of dissolved organic carbon to fuel heterotrophic Cr(VI) reduction, a bottleneck in the remediation process. Here we show an alternative approach by leveraging sulfur-disproportionating bacteria (SDB) inherent to groundwater ecosystems, offering a novel and efficient Cr(VI) removal strategy. We implemented SDB within a sulfur-packed bed reactor for treating Cr(VI)-contaminated groundwater, achieving a notable removal rate of 6.19 mg L^{-1} h^{-1} under oligotrophic conditions. We identified the chemical reduction of Cr(VI) via sulfide, produced through sulfur disproportionation, as a key mechanism, alongside microbial Cr(VI) reduction within the sulfur-based biosystem. Genome-centric metagenomic analysis revealed a symbiotic relationship among SDB, sulfur-oxidizing, and chromate-reducing bacteria within the reactor, suggesting that Cr(VI) detoxification by these microbial communities enhances the sulfur-disproportionation process. This research highlights the significance of sulfur disproportionation in the cryptic sulfur cycle in Cr(VI)-contaminated groundwater and proposes its practical application in groundwater remediation efforts.
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1. Introduction

Chromium, recognized as a highly toxic metal, poses substantial risks to both human health and the broader ecosystem. It is frequently identified as a pollutant in groundwater, especially in mining areas. The World Health Organization has set a stringent threshold for the total chromium limit in groundwater at 50 ug L^{-1}, underscoring the critical need to mitigate chromium contamination and safeguard the quality of groundwater resources. However, its concentration has frequently been reported to exceed this limit [1]. For instance, near the marble processing plant in Limpopo Province of South Africa, the chromium concentration in groundwater was reported to be 2.30–6.49 mg L^{-1}. In the United States, 4% of groundwater systems contained chromium with concentrations ranging from 10 to 97 μg L^{-1} [2]. Hexavalent [Cr(VI)] and trivalent [Cr(III)] are the two stable forms of chromium in nature. Cr(VI) is soluble, teratogenic, and carcinogenic, while Cr(III) is less toxic and usually exists in an insoluble form (e.g., $Cr(OH)_3$) under neutral conditions.

Microbial reduction of Cr(VI) into Cr(III) to form an insoluble form of $Cr(OH)_3$ has been demonstrated to be a promising approach for Cr(VI)-contaminated groundwater remediation [3–5]. In such processes, Cr(VI) bio-reduction is primarily mediated by heterotrophs (e.g., *Geobacter*, *Pseudomonas*, *Desulfovibrio*, *Leucobacter*) with organic carbon as the primary electron donor [6–9]. However, groundwater is deficient in bioavailable organics, suggesting that exogenous organic substrates are required to support efficient Cr(VI) bio-reduction [10]. Thus, such substrate-dosing processes are costly and likely result in secondary pollution caused by the residual organics.

Autotrophic elemental sulfur (S^0) oxidation has recently been demonstrated as a new alternative, as S^0 is an insoluble, cheap, and

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environmentally friendly mineral. In this process, autotrophs (e.g., *Thiobacillus, Ferrovibrio*) can synthesize dissolved organic carbon (DOC) via bicarbonate reduction coupled with sulfur oxidation to provide organic carbon for heterotrophic Cr(VI) reduction [11]. Notably, DOC production was a rate-limiting step in such a process. The synthesis of DOC via autotrophic bacteria is thermodynamically unfavorable and necessitates the symbiosis between sulfur-oxidizing bacteria (SOB) and Cr(VI)-reducing bacteria (CRB). This symbiotic relationship leads to a low Cr(VI) reduction rate, thereby impeding the scalability of Cr(VI)-contaminated wastewater and groundwater treatment.

In S^0-based biotechnology, autotrophic chromate reduction driven by microbial sulfur disproportionation (SD) could be a more efficient pathway for Cr(VI)-contaminated groundwater remediation, which has yet to be explored. SD is mediated by sulfur-disproportionating bacteria (SDB) and can disproportionately convert S^0 into sulfate and sulfide (equation (1)). In the biogeochemical sulfur cycle present in groundwater ecosystems, chemoautotrophic SD is an active process [12–14]. In theory, the sulfide generated by SDB could facilitate sulfide-oxidizing chromate reduction and could chemically reduce Cr(VI) at a higher rate than microbial Cr(VI) reduction [15,16]. Such *in situ* sulfide production circumvents safety concerns associated with the transportation, handling, and storage of sulfide chemicals. It is worth noting that while the SD process is thermodynamically unfavorable under standard conditions, it has been frequently observed to occur in both natural and engineered ecosystems [17–19]. The low aqueous solubility of sulfur (~5 μg L^{-1} at 25 °C) raises significant uncertainties regarding the extent to which SD can occur. This aspect is crucial in determining the practical feasibility of such a process.

$$4S^0 + 4H_2O \rightarrow {SO_4}^{2-} + 3HS^- + 5H^+ \quad (1)$$

Therefore, this study investigated the potential of chromate reduction mediated by the SD process. A sulfur-packed bed reactor (S^0-PBR) was established to (1) assess the long-term feasibility of Cr(VI) removal from Cr(VI)-contaminated groundwater and determine the final products of Cr(VI); (2) characterize the underlying mechanisms underpinning Cr(VI) reduction in the S^0-PBR; and (3) reconstruct the metabolic pathways of draft genomes related to functional bacteria population via genome-centric metagenomics analysis to infer their contributions for maintaining system stability. The findings obtained in this work would contribute to advancing our understanding of SD-driven autotrophic Cr(VI) reduction. This could provide valuable insights for optimizing the design and operation of sulfur-based biotechnologies aimed at remediating Cr(VI)-contaminated groundwater.

2. Materials and methods

2.1. Bioreactor setup and operation

A laboratory-scale S^0-PBR with a working volume of 1.2 L (5.0 cm diameter × 75 cm height) was employed and packed with approximately 832 g of sulfur lumps (Fig. S1). Activated sludge with enriched SDB from a previously reported sulfur-based autotrophic denitrification bioreactor was used as the inoculum of the S^0-PBR. The details can be found in our previous study [17]. Synthetic groundwater was prepared according to Zhang et al. [20]. The S^0-PBR was continuously operated for 198 days at a constant room temperature (25 ± 1.0 °C), which was divided into five stages depending on the influent Cr(VI) concentrations. In stage I (days 1–70) without Cr(VI), the S^0-PBR was only fed with synthetic groundwater to achieve stable SD. In stage II (days 71–89), stage III (days 90–113), stage IV (days 114–151), and stage V (days 168–198), different Cr(VI) concentrations (5, 10, 20, and 30 mg L^{-1}) were present in the synthetic groundwater to assess the capacity of S^0-PBR for Cr(VI) removal. The hydraulic retention time (HRT) was maintained at 5 h during the entire operational period.

2.2. Batch tests

The S^0-PBR produced sulfide, which could contribute to Cr(VI) reduction. Bio-reduction of Cr(VI) may also be involved in Cr(VI) removal inside the bioreactor. Thus, two sets of batch tests were conducted to unravel the Cr(VI) reduction pathways in the S^0-PBR.

Batch test I was conducted in 50-mL serum bottles to investigate whether the sulfide produced from the SD-driven autotrophic system could reduce Cr(VI) efficiently. Different weight ratios of sulfide to Cr(VI) (1:0, 1:2, 1:1, and 2:1) were studied by adjusting initial sulfide concentrations but fixing the initial Cr(VI) concentration (8.6 mg L^{-1}). $Na_2S{\cdot}9H_2O$ was used to prepare sulfide-containing solution. A batch test without initial sulfide was conducted as the control. The batch tests were performed for 120 min, during which samples were taken at 0, 5, 30, 60, and 120 min to measure Cr(VI), sulfate, sulfite, thiosulfate, and sulfide. All batch tests were performed in duplicates.

Batch test II was performed to demonstrate Cr(VI) bio-reduction with the sludge collected from the bioreactor during the last stage. Five different Cr(VI) concentration levels (0, 1, 3, 5, and 10 mg L^{-1}) were tested in triplicates. 1 g L^{-1} sulfur and 1000 mg L^{-1} $NaHCO_3$ were added into all bottles. The batch test was conducted with the sludge concentration in each bottle being 0.89 g MLVSS (mixed liquor volatile suspended solids) L^{-1}. The serum bottles were purged with pure nitrogen gas to maintain anaerobic conditions before being sealed with rubber stoppers. The batch tests lasted for 240 min, in which water samples were collected every 60 min to determine the variations in concentrations of sulfide, sulfate, sulfite, thiosulfate, and Cr(VI).

2.3. Chemical analyses

Cr(VI) concentration in water samples was quantified by the 1,5 diphenyl carbazide method using an Ultraviolet–visible spectrophotometer (DR 6000, HACH) at 540 nm [21,22]. Total Cr in water samples was determined by flame atomic absorption spectrometry (TAS-990, Beijing Puxi Instrument Factory). Total dissolved sulfide (H_2S, HS^-, and S^{2-}) was measured using methylene blue method [23]. The sulfate, sulfite, and thiosulfate concentrations were analyzed with an ion chromatograph (IC-16, Shimadzu, Japan) equipped with a conductivity detector and an SI-52 4E (Shodex)-slow analytical column after filtration. MLVSS were measured according to the Standard Methods [23]. Cytochrome *c* (cyt *c*) was extracted from the biomass and measured following the protocols described by Kang et al. [24]. In addition, the morphology of the precipitates was visualized by scanning electron microscope (SEM, Zeiss Sigma 300, UK) with ingredient analysis equipped with energy dispersion spectrum (EDS, Quantax XFlash SDD 6|30, Bruker, Germany). X-ray photoelectron spectroscopy (XPS) (Thermo Fisher Scientific K-Alpha, USA) was employed for characterizing the valence state of chromium precipitate and the X-ray pattern was then analyzed by Avantage software (v5.9921). pH, oxygen, and temperature were measured using the portable meters (HQ40D, Hach). Elemental sulfur in the sludge samples was quantified according to our previous study [25,26].

2.4. Sludge sampling, DNA extraction, 16S rRNA gene amplicon sequencing, and data processing

Sludge samples were collected at the end of each stage during

the operation of S^0-PBR (days 70, 89, 113, 151, 198). MOBIO PowerSoil® DNA isolation Kit (MOBIO Laboratories, CA, USA) was used to extract the total genomic DNA as per the instructions provided by the manufacturer. The concentration and purity of DNA were determined with Thermo NanoDrop One (Thermo Fisher Scientific, MA, USA). The 16S rRNA was amplified with the 515F-907R primer pair to target the hypervariable V4 and V5 regions of the bacteria [17]. Amplicon sequencing analysis was conducted using the Illumina Nova 6000 platform at Magigene Biotechnology Co., Ltd., Guangzhou, China. The data processing followed the pipeline described by Zhang et al. [27].

2.5. Genome-resolved metagenomics analysis

Sludge samples were collected from the bottom and top layers of the bioreactor at the last stage and used for total DNA extraction. The Illumina sequencing service was provided by Novogene Biotech. Co., Ltd. (Beijing, China). A total of 40.7 GB of sequences were retrieved from the two samples. The detailed procedures for metagenome assembly, genome binning, and phylogenetic analysis can be found in Zhang et al. [28] and Zhang et al. [29]. The open reading frames (ORFs) prediction of these metagenome-assembled genomes (MAGs) was performed with Prodigal (v2.6.3) [30]. The predicted ORFs were annotated using both KofamScan (https://github.com/takaram/kofam_scan) and KAAS (KEGG Automatic Annotation Server) webserver with the 'Complete or Draft Genome' setting [31]. The predicted ORFs were also annotated against the pfam database using HMMER (v3.3.2).

3. Results and discussion

3.1. Efficient Cr(VI) removal in the long-term trial of S^0-PBR

Sulfide production and Cr(VI) removal in the S^0-PBR is presented in Fig. 1, showing the excellent capacity of the S^0-PBR to remove Cr(VI) from groundwater in the long-term trial. During stage I, where Cr(VI) was absent, 64.1 ± 17.4 and 75.3 ± 9.6 mg S L^{-1} of sulfide and sulfate were produced, respectively. Since organics were absent in the influent, the stable sulfide production with high concentration was not likely attributed to heterotrophic sulfate/sulfur reduction. A batch test conducted under strictly anaerobic conditions showed that the S^{2-}/SO_4^{2-} ratio (3.2 ± 0.4) was slightly higher than the stoichiometric ratio of SD reaction (3.0), suggesting that sulfide could be primarily generated from autotrophic SD (the experimental design was described in Text S1 and the result was showed in Fig. S2). Other sulfide producers could exist (see the results of microbial community analysis). The lower S^{2-}/SO_4^{2-} ratio in the S^0-PBR at stage I (0.93 ± 0.2) could be due to the potential sulfide oxidation driven by a possible oxygen leak into S^0-PBR and the influent. Lower pH values in the effluent than in the influent were observed (Fig. S3), following the proton-generating SD process. SD was also reported in sulfur-packed reactors treating nitrate-contaminated wastewater [17] or pretreated acid mine drainage via pH amelioration [18].

From stage II, Cr(VI) was introduced into the influent. Cr(VI) was efficiently removed during the long-term operational period even when the Cr(VI) concentration was increased to 30 mg L^{-1} in stage V, except for the first several days of stage IV (Fig. 1a). During that period, 84.5% of the influent Cr(VI) on average were removed, resulting in 3.5 mg L^{-1} Cr(VI) on average in the effluent. It may be due to a sudden increase in Cr(VI) concentration from 10.3 mg L^{-1} at stage III to 25.7 mg L^{-1} at the beginning of stage IV, which could have an inhibitory effect on the microbial community, but the microbial activities quickly recovered. A high Cr(VI) removal rate calculated as 6.19 mg L^{-1} h^{-1} was determined in stage V. The

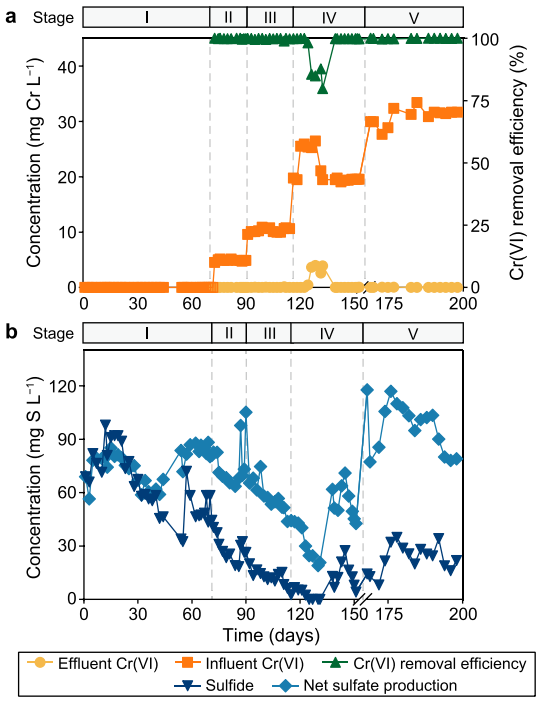

Fig. 1. The long-term performance of S^0-PBR: **a**, Cr(VI) removal; **b**, Sulfide and sulfate production.

lower Cr(VI) removal rate reported in other S^0-based systems could be due to the absence of SD process (Table S1). The results suggest that the SD process could be a promising approach for remediation of Cr(VI)-contaminated groundwater. In addition, sulfide concentrations in the effluent decreased with elevated Cr(VI) concentrations. It may be due to its consumption during Cr(VI) removal. However, even when exposed to ~30 mg L^{-1} Cr(VI), 23.0 ± 7.8 mg L^{-1} sulfide was present in the bioreactor effluent. S^0-PBR could be robust when encountering Cr(VI) loading fluctuation.

3.2. Identification of reduction products of Cr(VI)

Non-detectable dissolved Cr in the effluent revealed that Cr was retained in the bioreactor. The greyish-green of S^0-PBR during the later stage of operation suggests the likelihood of $Cr(OH)_3$ formation (Fig. S4). In order to identify the products of Cr(VI) reduction, biomass samples of the bioreactor were collected for both SEM-EDS and XPS analysis. SEM image showed spheroids widely deposited on the surface of bacterial cells. EDS analysis determined that Cr was the dominant element in the sludge and the spheroids (Fig. 2a–b and S5). It indicates that Cr(VI) could likely be biologically reduced to some extent, which is further confirmed in the following section. Meanwhile, XPS analysis showed two peaks at 586.4 and 577.3 eV in the high-resolution spectrum of Cr 2p (Fig. 2c), which is related to $Cr(OH)_3$ [32]. Non-detectable dissolved Cr in the effluent also confirmed the formation of insoluble products. Thus, Cr(VI) was removed via reduction, followed by forming $Cr(OH)_3$ precipitates.

3.3. Mechanisms towards Cr(VI) reduction

The role of sulfide in Cr(VI) reduction was further investigated. For example, in the presence of 18 mg S L^{-1} sulfide initially, 8.6 mg L^{-1} Cr(VI) was completely reduced via chemical reaction within 30 min (Fig. 3a and S6), with a Cr(VI) reduction rate of

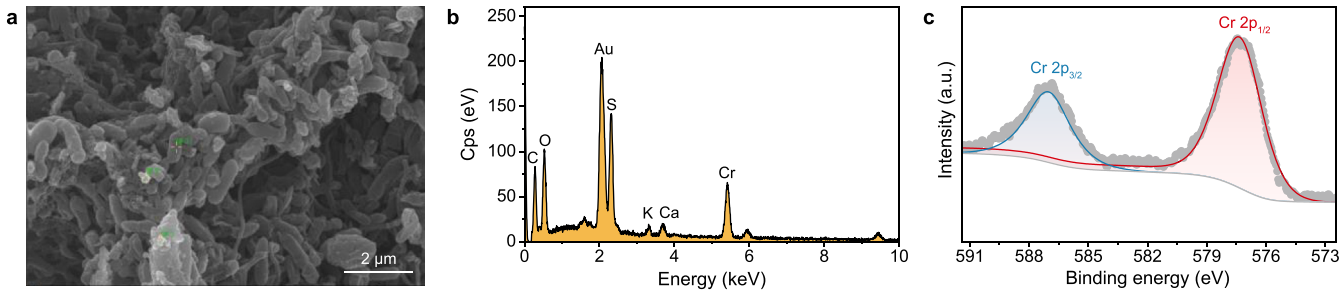

Fig. 2. Characterization of the morphology and composition of biomass with produced precipitates in the biosystem. **a**, SEM image of precipitates-associated biomass. **b**, EDS pattern of precipitates at location 1# shown in the SEM image. **c**, XPS spectrum of precipitates.

17.2 mg L^{-1} h^{-1}. To demonstrate Cr(VI) bio-reduction, batch tests with different initial Cr(VI) concentrations were performed (Fig. 3). In the absence of Cr(VI), sulfide started producing after 1-h incubation, indicating that SD process occurred (Fig. 3d). Although SD did not occur within 1 h, Cr(VI) was substantially reduced accompanying with sulfate being generated in the presence of Cr(VI), (Fig. 3b and c). In detail, with initial Cr(VI) concentrations of 1 and 3 mg L^{-1}, Cr(VI) was quickly reduced to a non-detectable level within 1 h. Meanwhile, 2.5 and 2.6 mg S L^{-1} sulfate, on average, were generated within 1 h in the two batch tests, respectively. Since SD did not occur within 1 h, likely due to the low SDB activity, we could infer that sulfate was derived from sulfur oxidation. In this light, sulfur oxidation mediated by sulfur-oxidizing bacteria (SOB) could contribute to Cr(VI) removal (equation (2)), which is consistent with the findings reported by Shi et al. [11]. They reported that DOC can be synthesized by autotrophic S^0 oxidation to sulfate along with bicarbonate reduction.

In addition, 5 mg L^{-1} of Cr(VI) was completely reduced within 4 h. Even for the initial Cr(VI) concentration of 10 mg L^{-1}, 64.9% of the spiked Cr(VI) were reduced within 4 h (Fig. 3). These results indicate that in addition to chemical reduction, Cr(VI) bio-reduction participate in Cr(VI) removal. Cytochrome *c* is a heme protein located in the cell membrane and could play an important role in Cr(VI) reduction through electron transfer in the respiratory chain [8]. In this study, it was observed that cytochrome *c* concentrations increased with elevated Cr(VI) concentrations during the long-term operation of S^0-PBR (Fig. 4), which further demonstrated the occurrence of Cr(VI) bio-reduction.

During the batch test, the Cr(VI) bio-reduction rate was calculated as 1.3–3.0 mg L^{-1} h^{-1}, which is slower than the chemical reduction rate, suggesting the greater role of chemical reduction in Cr(VI) removal. Although chemical Cr(VI) reduction was faster than bio-reduction, the latter could not be excluded. Taking the Phase V of the S^0-PBR as an example, the amount of sulfide was stoichiometrically insufficient (equation (2)), while the influent Cr(VI) was completely retained in the bioreactor, suggesting the presence of Cr(VI) bio-reduction. Notably, sulfide was present in all batch reactors after approximately 24 h by the end of the experiment (data not shown). Hence, the inhibitory effect of Cr(VI) on SDB might be reversible. The presence of SOB could also alleviate Cr(VI) toxicity via a reduction process.

$$2CrO_4^{2-} + 3HS^- + 7H^+ \rightarrow 2Cr(OH)_3 + 3S^0 + 2H_2O \quad (2)$$

3.4. Microbial community structures

To study the effects of Cr(VI) on microbial community structure and determine the functional populations, five sludge samples were collected from the five stages of S^0-PBR. At the phylum level, Proteobacteria (55.2%), Epsilonbacteraeota (16.8%), Bacteroidetes (12.6%), Chloroflexi (8.7%), Caldiserica (3.4%), and Spirochaetes (2.7%) were the dominant phyla in the absence of Cr(VI). Although their abundance had different variation patterns, they were still the predominant phyla in the presence of Cr(VI) (Fig. 5).

The bacterial compositions at the genus level are present in Fig. 5. The functional bacteria related to sulfur cycling were highly diverse. For instance, the species affiliated with *Dissulfurimicrobium* has been reported to be SDB [33], and its relative abundance remained stable over the entire period (4.0–8.7%), ensuring the stable and sustainable performance of the system. The relative abundance of *Desulfurella* increased from 0.01% in the absence of Cr(VI) to 0.08% in the presence of 20 mg L^{-1} Cr(VI), followed by a

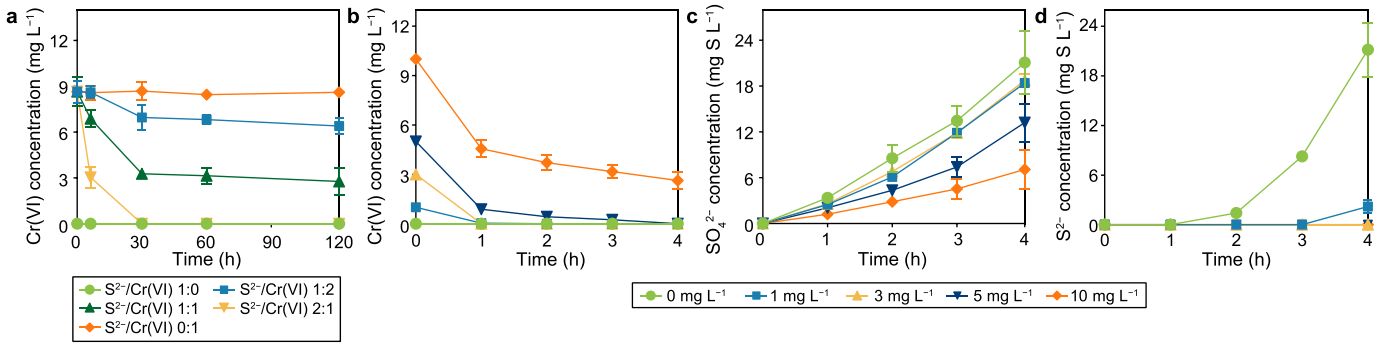

Fig. 3. **a**, Variations of Cr(VI) concentrations in the chemical batch tests with different weight ratios of sulfide to Cr(VI) ratio (1:0, 1:2, 1:1, 2:1, and 0:1). **b**–**d**, Variations of Cr(VI) (**b**), sulfate (**c**), and sulfide (**d**) concentrations in the batch tests with different initial Cr(VI) dosages (0, 1, 3, 5, and 10 mg L^{-1}).

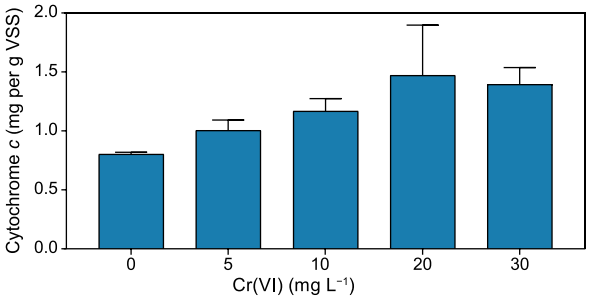

Fig. 4. Variations of cytochrome *c* in the S^0-PBR dosed with 0, 5, 10, 20, and 30 mg L^{-1} Cr(VI).

decrease to 0.03% in the presence of 30 mg L^{-1} Cr(VI). This genus was capable of SD and sulfur reduction [34]. Heterotrophic Cr(VI)-reducing genera (i.e., *Acidithiobacillus*, *Thermomonas*) were also detected [35,36].

Interestingly, sulfur-oxidizing genera dominated the microbial community regardless of Cr(VI) concentrations tested in this study, such as *Ferritrophicum*, *Sulfurovum*, *Sulfurimonas*, *Sulfuricurvum*, and *Thiobacillus*. *Ferritrophicum* could not tolerate the toxicity of Cr(VI), resulting in its relative abundance gradually decreasing from 24.7% in the absence of Cr(VI) to 1.0% in the presence of 30 mg L^{-1} Cr(VI). On the contrary, *Sulfurovum*, *Sulfurimonas*, *Sulfuricurvum*, and *Thiobacillus* were substantially enriched in the presence of Cr(VI) (Fig. 5), suggesting that SOB could be involved in Cr(VI) reduction. Zhang et al. [37] found that *Thiobacillus* spp. harbors the genes [e.g., N-ethylmaleimide reductase (*nemA*), FMN-dependent NADH-azoreductase (*azoR*)] related to Cr(VI) bio-reduction, suggesting its ability of direct Cr(VI) reduction, which needs to be further demonstrated. The present results indicate that the co-existence of diverse SDB, SOB, and CRB ensured the highly efficient Cr(VI) removal in the S^0-PBR.

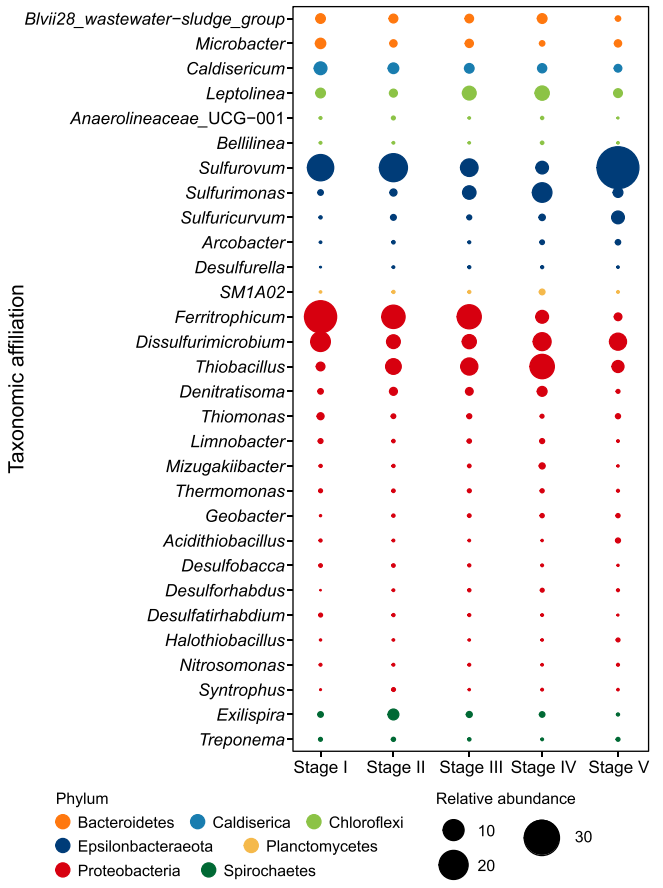

Fig. 5. Bubble plot showing the relative abundance of the top 30 genera in the sludge samples collected at different stages. For a comprehensive understanding of the color references in this figure legend, readers should consult the web version of this article.

3.5. Metabolic potentials of symbiotic microorganisms

Since the co-existed SDB, SOB, and CRB were expected to contribute to Cr(VI) reduction simultaneously, genome-centric metagenomics analysis was performed to decipher the metabolic potentials of the putative representative SDB, SOB, and CRB. Their metabolic potentials and detoxification mechanisms towards Cr(VI) were also discussed and inferred. A total of 79 high-quality MAGs with estimated completeness > 75% and contamination < 5% were retrieved from the samples (Fig. 6, Tables S2 and S3). According to the taxonomic assignment from GTDB-Tk (v.0.3.2), these MAGs were phylogenetically affiliated with Caldisericota (n = 3), Spirochaetota (n = 4), Actinobacteriota (n = 2), Armatimonadota (n = 1), Chloroflexota (n = 6), Hydrogenedentota (n = 1), Verrucomicrobiota_A (n = 1), Gemmatimonadetes (n = 1), Planctomycetota (n = 1), Bacteroidota (n = 16), Campylobacterota (n = 7), Desulfobacterota (n = 5), and Proteobacteria (n = 31).

The corresponding metabolic maps of these MAGs were then reconstructed. *Dissulfurimicrobium* sp. Bin.11 harbored the reductive dissimilatory sulfite reductase (*dsrAB*) genes (Fig. 7), suggesting that this MAG could produce sulfide. This MAG also encoded polysulfide reductase (*psrA*), which is involved in polysulfide reduction to sulfide. Meanwhile, adenylylsulfate reductase-associated electron transfer complex (*Qmo*) was identified in *Dissulfurimicrobium* sp. Bin.11, which was reported to likely participate in sulfur oxidation to sulfite. Frederiksen and Finster [38] also found that sulfite was the key intermediate during the SD process via the enzyme assays. Nevertheless, sulfite was not observed during the experiment. It may be attributed to its high oxidization rate, as sulfite could be converted into sulfate via relevant enzymes. The presence of sulfide: quinone oxidoreductase (*apr*) and ATP sulfurylase (*sat*) genes in *Dissulfurimicrobium* sp. Bin.11 indicates that it could have the capacity to oxidize sulfite to sulfate.

In addition, *Dissulfurimicrobium* sp. Bin.11 was phylogenetically close to *D. hydrothermale* SH68 (Fig. 6), previously demonstrated to be SDB [33]. In this light, it can be inferred that *Dissulfurimicrobium* sp. Bin.11 could be capable of SD. Of note, in addition to *Dissulfurimicrobium* sp. Bin.11, reductive *dsrAB* genes were detected in *Desulfurella* sp. Bin.128, *Deltaproteobacterium* sp. Bin.50, and *Syntrophobacter* sp. Bin.183 (Fig. 7). They could also contribute to sulfide production in this bioreactor, but they may play a minor role. This is because that sulfide was primarily attributable to the SD process, as indicated by the batch test discussed above (Fig. S2).

The oxidative *dsrAB* genes were identified in seven *Betaproteobacteria* species, such as *Thiobacillaceae* sp. Bin.2, *Rhodocyclaceae* sp. Bin.33, *Sulfuricella* sp. Bin.37, *Rhodocyclaceae* sp. Bin.147, *Thiobacillaceae* sp. Bin.167, *Burkholderiales* sp. Bin.179, and *Burkholderiales* sp. Bin.181 (Fig. 7), suggesting that they could be involved in sulfur oxidation. These seven MAGs also possessed flavocytochrome c cytochrome subunit genes (*fccAB*), indicating their potential for sulfide oxidation (Table S4). In addition, *fccAB* genes were detected in the other seven MAGs (*Tibeticola sediminis* sp. Bin.8, *Thiotrichales bacterium* sp. Bin.42, *Halothiobacillus neapolitanus* sp. Bin.62, *Thiomonas* sp. Bin.64, *Rhodocyclaceae* sp. Bin.86, *Halothiobacillus* sp. Bin.108, and *Vitreoscilla_A* sp. Bin.136). The presence of SOX system in 11 MAGs (*Tibeticola sediminis* sp. Bin.8, *Thiotrichales* sp. Bin.42,

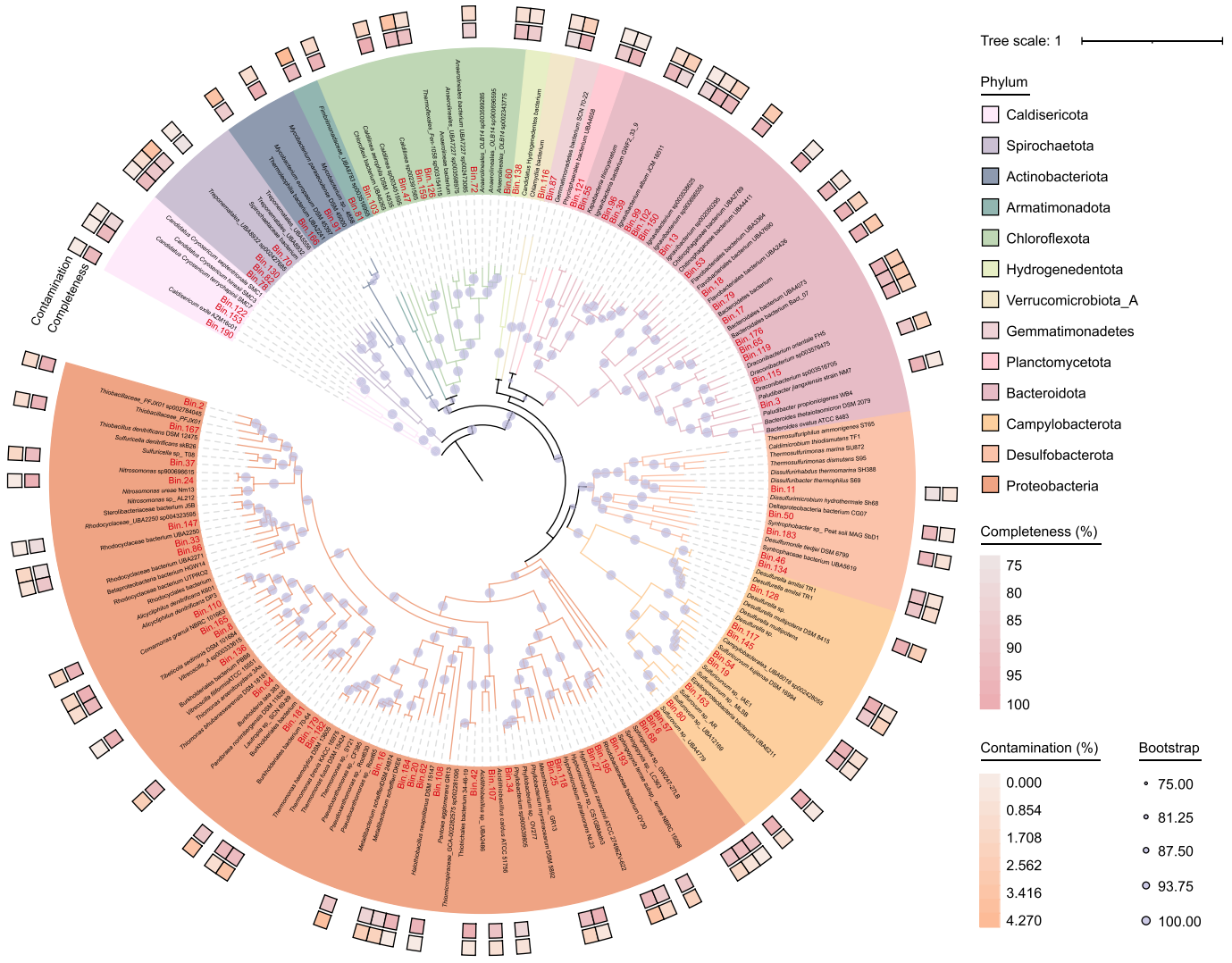

Fig. 6. Phylogenomic placement of assembled high-quality bins with completeness >75% and contamination <5%. Bootstrap values were based on 100 replicates, and percentages ≥75% are shown with light purple circles.

Halothiobacillus neapolitanus sp. Bin.108, *Burkholderiales* sp. Bin.181, etc.) revealed that they could proceed thiosulfate oxidation to sulfate (Table S4). The results indicate that sulfur oxidation could be an important process in the S^0-PBR, which is consistent with the finding of SOB-mediated Cr(VI) bio-reduction.

Previous studies reported that some genes (i.e., *chrR, ssuE, acpD, azoR, rutE, nfsA, ribF, nrfA, nemA*) could link with Cr(VI) bio-reduction [39,40]. We observed that 38 MAGs possessed some of these genes (e.g., *Dissulfurimicrobium* sp. Bin.11, *Phyllobacterium* sp. Bin.34, *Thiobacillaceae_PFJX01* sp. Bin.167, *Burkholderiales bacterium 70-64* sp. Bin.179, *Burkholderiales bacterium* sp. Bin.181, *Syntrophobacter* sp. Bin.183) (Table S4), indicating that they could be the Cr(VI) reducers. Cytochrome *c3* was reported to participate in extracellular Cr(VI) reduction [37,41,42], and was identified in 49 of 79 MAGs, including putative SDB, SOB, and CRB (Table S4). It suggests they could reduce Cr(VI) via the electron transfer chain. This result is consistent with the finding that more cytochrome *c* were generated under higher Cr(VI) concentrations (Fig. 4). Previous studies also reported that SOB has the capacity for Cr(VI) reduction [37,43,44]. The identification of a putative Cr(VI) transporter gene (*chrA*) in some recovered MAGs (e.g., *Alicycliphilus denitrificans* sp. Bin.110, *Comamonas granuli* sp. Bin.165) indicates that they could mediate the Cr(VI) efflux across the cytoplasmic membrane (Table S4) [39]. Thus, these MAGs could utilize two strategies (Cr(VI) reduction and chromate efflux) to detoxify the Cr(VI) toxicity.

3.6. Potential mechanisms of Cr(VI) reduction

In this study, we, for the first time, demonstrated that Cr(VI) removal by sulfide that chemoautotrophically generated by SDB as a new pathway could achieve highly efficient Cr(VI)-contaminated groundwater remediation and could overcome the disadvantages during the chemical and biological processes reported previously. With the involvement of SDB, more efficient Cr(VI) reduction was achieved (Table S1). Unlike the previously reported S^0-based bio-systems, where Cr(VI) bio-reduction heavily relies on syntrophic interactions between specific microbial populations (e.g., SOB, CRB), in our study, Cr(VI) was mainly reduced through a chemical way using *in situ* biogenic sulfide as the electron donor.

Microbial Cr(VI) reduction cannot be ignored. The prevalence of SOB and CRB (Figs. 5 and 7) implies that the sulfur-oxidizing DOC

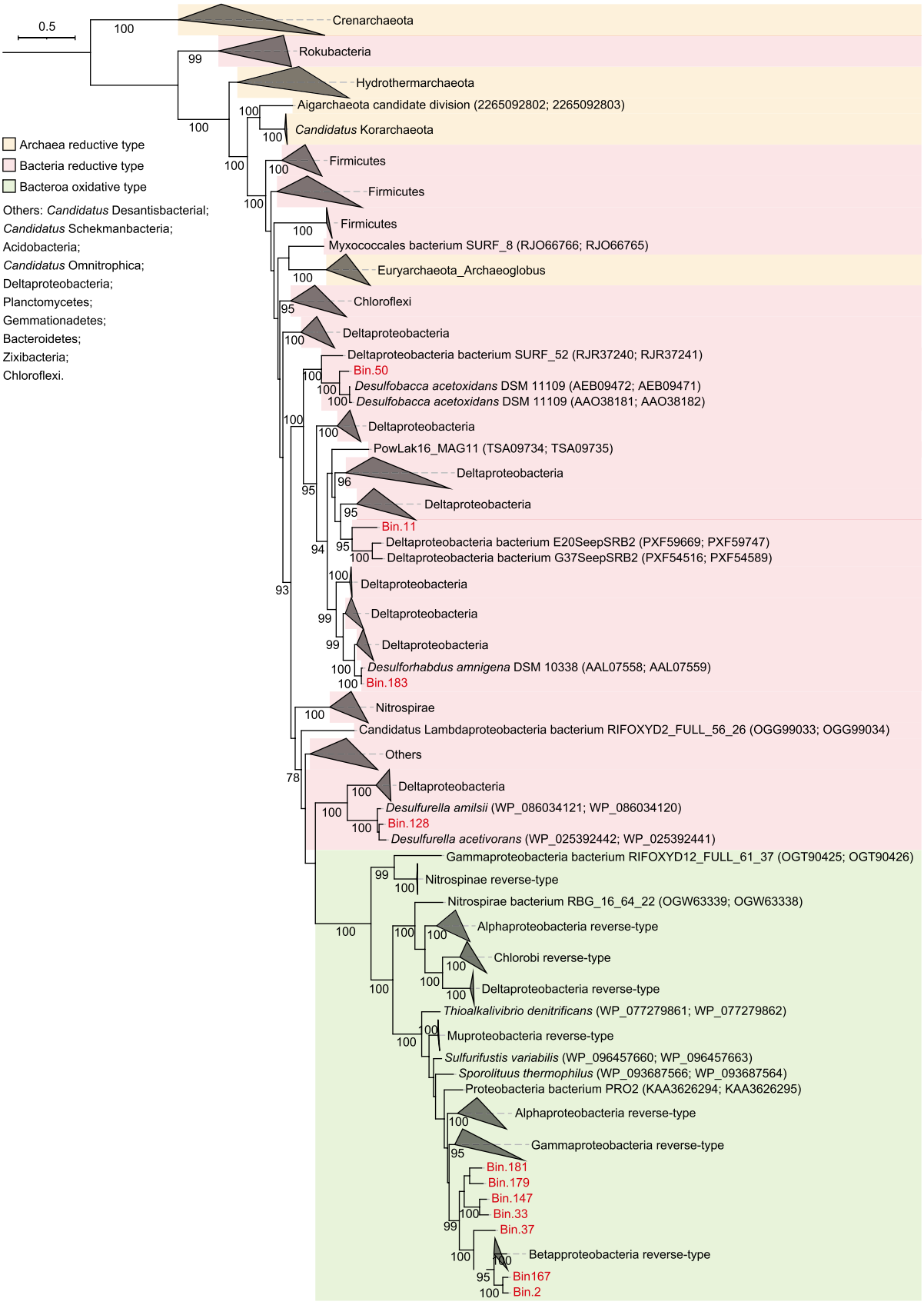

Fig. 7. Phylogenetic analysis of the concatenated DsrAB proteins. The MAGs retrieved from this study were highlighted in red. Bootstrap values based on 100 replicates.

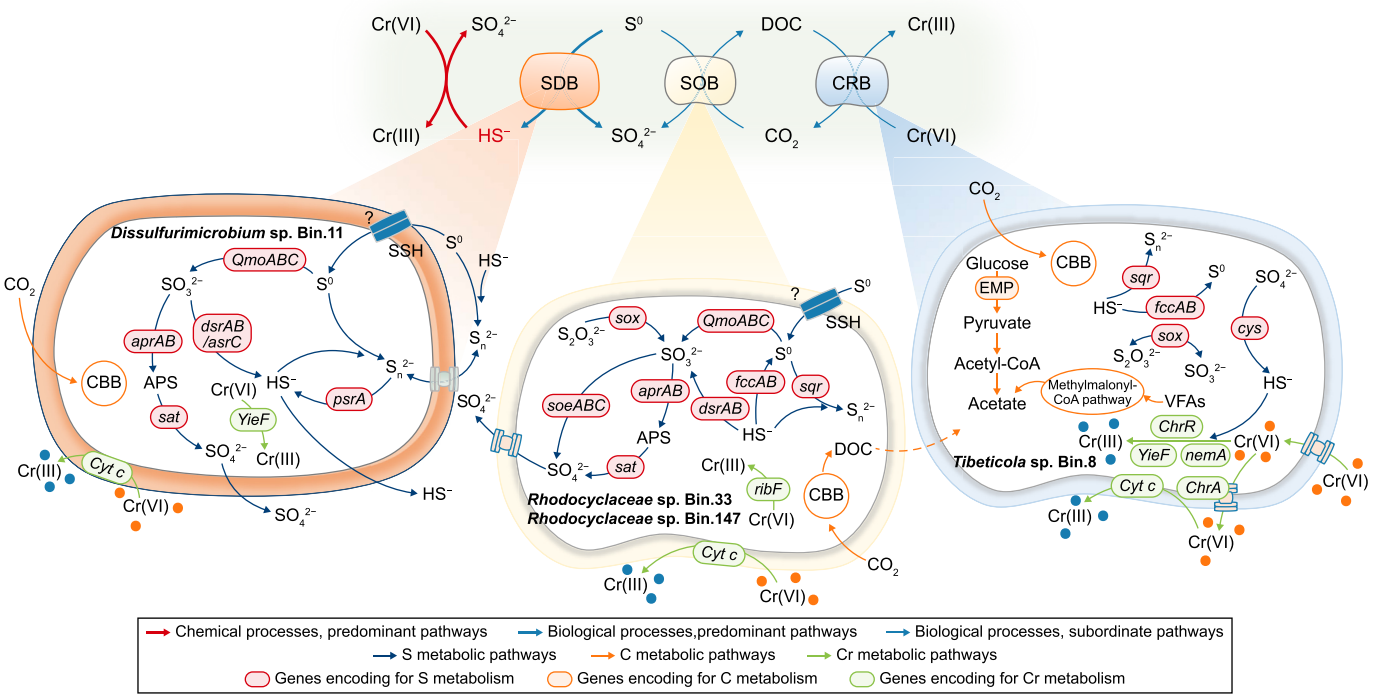

Fig. 8. Putative autotrophic Cr(VI) reduction pathway in the SD-driven ecosystem based on the representative genomes assembled from metagenomic data of the communities.

synthesis may occur in the S^0-PBR, which was partially supported by the detection of organics in the bioreactor effluent (Fig. S7) and can provide electron donors for heterotrophic Cr(VI) reduction. This phenomenon has been previously reported by Shi et al. [32] and Zhang et al. [8]. In this light, the possible mechanisms towards Cr(VI) removal and synergistic interactions among functional bacteria were proposed with the predominant putative SDB, SOB, and CRB as the representatives (Fig. 8). Briefly, the putative SDB *Dissulfurimicrobium* sp. Bin.11 has the ability to disproportionately convert sulfur into sulfide and sulfate. The biogenic sulfide could rapidly reduce Cr(VI) via chemical reaction. Additionally, sulfide could also support the activity of SOB and the presence of abundant SOB, such as *Rhodocyclaceae* sp. Bin.33 and Bin.147 enable the synthesis of DOC through CO_2 reduction to support the activity of heterotrophic CRB (e.g., *Tibeticola sediminis* sp. Bin.8) [11].

3.7. Environmental implications

In this study, we proposed a novel and organic-free S^0-based approach, wherein SDB is incorporated into a S^0-based PBR to induce efficient chemical Cr(VI) reduction. Permeable reactive barrier (PRB) is a sustainable approach for *in situ* groundwater remediation [45]. By inoculating SDB and employing sulfur particles as the filling materials, a PRB dominated by SD can be established. Given that PRB typically has a short HRT, the SD-driven PRB offers a sustainable and promising solution for groundwater remediation, particularly in cases where the groundwater is contaminated with oxidative pollutants such as chromate, nitrate [46,47], and perchlorate [48,49].

Since Cr(VI) may have inhibitory effects on SDB activity, such a process may be preferable for *ex situ* groundwater remediation. In this light, a two-stage system could be designed to separate the sulfidogenic reactor and the Cr(VI) reduction reactor to ensure the sulfide production rate. To make the process more robust and resilient in real applications, the parameters (e.g., HRT, temperature) that influence the system performance need to be determined and optimized in further study. Additionally, the residual sulfide in the effluent may lead to odor and metal corrosion problems. Additional measures (e.g., micro-aeration) should be taken to convert sulfide into benign forms (i.e., S^0, SO_4^{2-}) [50].

4. Conclusions

The newly developed SD-driven bioprocess achieved efficient Cr(VI)-contaminated groundwater remediation. Without external organic substrate supplementation, the S^0-PBR exhibited a remarkable Cr(VI) removal rate of up to 6.19 mg L^{-1} h^{-1}. Cr(VI) was effectively reduced to Cr(III) and retained within the system. *Dissulfurimicrobium* sp. as the dominant SDB maintained highly active in the S^0-PBR regardless of Cr(VI) concentrations, resulting in abundant sulfide produced via the involvement of a series of enzymes (e.g., Qmo, apr, dsr). These enzymes played a significant role in efficiently reducing Cr(VI). Additionally, the coexistence of diverse sulfur-metabolizing bacteria (SOB, SRB) alongside CRB reduces Cr(VI). It facilitates efficient detoxification of Cr(VI), which in return creates a more favorable environment for the growth and respiration of SDB. These results highlight the crucial role of microbial Cr(VI) reduction in maintaining the system performance of the SD process. These findings offer a promising technology for remediating Cr(VI) contaminated groundwater.

CRediT authorship contribution statement

Yan-Ying Qiu: Investigation, Methodology, Formal Analysis, Data Curation, Visualization, Writing - Original Draft. **Juntao Xia:** Methodology, Data Curation. **Jiahua Guo:** Formal Analysis, Data Curation. **Xianzhe Gong:** Formal Analysis, Data Curation. **Liang Zhang:** Methodology, Supervision, Writing - Review & Editing. **Feng Jiang:** Conceptualization, Methodology, Resources, Supervision, Writing - Review & Editing, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2024.100399.

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