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1 **Microbial Selenate Detoxification Linked to Elemental Sulfur Oxidation:**
2 **Independent and Synergic Pathways**

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21 **ABSTRACT**

22 Elevated selenium levels in the environment, with soluble selenate [Se(VI)] as
23 the common chemical species, pose a severe threat to human health. Anaerobic Se(VI)
24 bioreduction is a promising approach for selenium detoxification, and various
25 organic/inorganic electron donors have proved effective in supporting this bioprocess.
26 Nevertheless, autotrophic Se(VI) bioreduction driven by solid inorganic electron
27 donors is still not fully understood. This work is the first to employ elemental sulfur
28 [S(0)] as electron donor to support Se(VI) bioreduction. A batch trial with mixed
29 culture demonstrated the feasibility of this bioprocess, with Se(VI) removal efficiency
30 of $92.4 \pm 0.7\%$ at an initial Se(VI) concentration of 10 mg/L within 36 h. Continuous
31 column tests showed that increased initial concentration, flow rate, and introduction
32 of NO_3^- -N depressed Se(VI) removal. Se(VI) was mainly bioreduced to solid
33 elemental Se with trace selenite in the effluent, while S(0) was oxidized to SO_4^{2-} .
34 Enrichment of *Thiobacillus*, *Desulfurivibrio*, and *Sulfuricurvum* combined with
35 upregulation of genes *serA*, *tatC*, and *soxB* indicated Se(VI) bioreduction was coupled
36 to S(0) oxidation. *Thiobacillus* performed S(0) oxidation and Se(VI) reduction
37 independently. Intermediate metabolites as volatile fatty acids, hydrogen, and methane
38 from S(0) oxidation were utilized by heterotrophic Se(VI) reducers for Se(VI)
39 detoxification, indicative of microbial synergy.

40 **Keywords:** Elemental sulfur; Selenate; Microbial reduction; Biotoxification

41 1. Introduction

42 Selenium has an unusually narrow gap between its benefits and toxicity to
43 humans (Lai et al., 2016). For a typical adult person, 40 to 55 µg/day of selenium is
44 needed to support their basic nutritional needs. However, adverse impact occurs
45 through selenosis if total selenium intake exceeds 400 µg/day (Navarro-Alarcon and
46 Cabrera-Vique, 2008). The World Health Organization defines the selenium intake
47 value of 40 µg/L as reference, whereas the United States Environmental Protection
48 Agency rules that the maximum concentration of selenium must not exceed 50 µg/L
49 in drinking water (Zhu et al., 2017; Nancharaiah et al., 2018). However, excessive
50 concentrations of selenium are found in the environment. For example, selenium at
51 6000 µg/L was reported for groundwater in Utah, USA, whereas up to 9000 µg/L
52 selenium was detected in certain areas of Colorado, USA (Ji and Wang, 2017). It is
53 obviously important to reduce such high selenium levels below the safety threshold,
54 especially in regions where drinking water is abstracted.

55 Selenate [Se(VI)] and selenite [Se(IV)] are the main forms of selenium species
56 found in the natural aqueous environment (Latorre et al., 2013). Both Se(VI) and
57 Se(IV) can be transformed to elemental selenium [Se(0)] enabling selenium removal
58 due to its lower solubility, toxicity, and bioavailability (Lai et al., 2016; Song et al.,
59 2021; Wang et al., 2021). With eco-friendly, cost-effective and in situ application
60 advantages, microbial reduction of Se(VI) to Se(0) is preferred among remediation
61 methods (Lenz et al., 2009; Fu et al., 2014; Qiao et al., 2018), especially with
62 indigenous microorganisms (Li et al., 2021). Bioreduction of Se(VI) can be achieved

63 through either heterotrophic or autotrophic processes (Ontiveros-Valencia et al., 2016;
64 Zhang et al., 2019). Compared with heterotrophic metabolism, autotrophic reduction
65 is advantageous in that it prevents secondary pollution and produces less biomass,
66 making it a promising option that is also practicable and efficient (Liu et al., 2016;
67 Zhang et al., 2018).

68 Electron donors are absolutely essential in the process of autotrophic Se(VI)
69 bioreduction. For example, hydrogen (H₂) is used in gas-supported bioreduction of
70 Se(VI) (Zhou et al., 2018), but requires considerable care in its usage and storage
71 from a safety perspective. **Solid inorganic electron donors that support autotrophic**
72 **bioreduction for metal oxyanion detoxification have also been reported (Shi et al.,**
73 **2019; Lu et al., 2020; He et al., 2021), of which elemental sulfur [S(0)], an insoluble,**
74 **stable and low-cost industrial product, has gained increasing scrutiny (Wang et al.,**
75 **2021). S(0) is able to serve as an electron donor for autotrophic bioprocesses**
76 (Sahinkaya et al., 2014; Li et al., 2019). Many studies have explored the performance
77 of S(0) in assisting in the bioreduction of contaminants such as nitrate (NO₃⁻-N),
78 perchlorate (ClO₄⁻), vanadate [V(V)], and chromate [Cr(VI)] (Zhang et al., 2018;
79 Ucar et al., 2019). **In spite of this, autotrophic microbial Se(VI) reduction supported**
80 **by S(0) has been little researched, and the mechanism of Se(VI) bioreduction driven**
81 **by S(0) remains largely unknown.**

82 Herein, bioreduction of Se(VI) supplemented by S(0) is investigated through
83 both batch and column trials, with analysis of product characterization, microbial
84 community dynamics, functional genes, and intermediate metabolites. The objectives

85 of this paper are: (1) to examine the feasibility and performance of S(0)-supported
86 Se(VI) bioreduction; (2) to reveal microbial dynamics at community, gene, and
87 metabolic levels; and (3) to analyze the mechanisms related to S(0)-driven Se(VI)
88 bioreduction. The present study focuses on bioremediation as a potentially practicable
89 technology for Se(VI)-polluted environments.

90 **2. Materials and methods**

91 *2.1. Batch trial*

92 Three groups of 250 mL volume plexiglass bottles were employed as biotic,
93 sterilized, and abiotic batch reactors. Strict anaerobic conditions were preserved by
94 sealing each bottle with butyl rubber stoppers and aluminum crimp caps. Before being
95 transferred to the bottles, simulated groundwater was flushed with nitrogen gas for 30
96 min to remove oxygen. The biotic group with 50 mL aquifer sediment from a smelting
97 site in China was combined with 5 g S(0) and 200 mL simulated groundwater
98 containing the following basic constituents (g/L): CaCl₂ 0.2464; NH₄Cl, 0.1557;
99 MgCl₂·6H₂O, 1.0572; NaCl, 0.4459; KCl, 0.0283; KH₂PO₄, 0.0299; and NaHCO₃,
100 0.5040. The pH, ORP, and electrical conductivity of aquifer sediment were 7.99, -
101 48.2 mV, and 424.9 μS/cm, respectively. Concentrations of organic matter and
102 available sulphur in aquifer sediment were 34.4 g/kg and 21.9 mg/kg, respectively,
103 whereas the cell dry weight was 6.7 mg/g soil. Concentrations of trace elements were
104 as follows: Fe (84.2 mg/kg), Mn (2.22 mg/kg), Zn (0.19 mg/kg), Pb (0.13 mg/kg), V
105 (0.34 mg/kg), Cr (0.33 mg/kg). The medium was supplemented with bicarbonate as

106 the inorganic carbon source with inorganic carbon concentration at 72 mg/L. Na_2SeO_4
107 was used as the source of Se(VI), at 10 mg/L concentration. All reagents were
108 purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) with
109 analytical grade, and utilized directly without further processing. The sterilized group
110 was supplemented with S(0), simulated groundwater, and 50 mL high temperature
111 sterilized aquifer sediment (20 min at 121 °C). The abiotic group was supplemented
112 with S(0) and simulated groundwater, but without aquifer sediment. All batch reactors
113 were run for two months before data collection. The cycle of 72 h was set to evaluate
114 Se(VI) reduction in different groups. All experiments were conducted at room
115 temperature (22 ± 2 °C) and in triplicate.

116 2.2. Column trial

117 A biological column of 25 cm in length and interior diameter of 5 cm was made
118 of plexiglass. The column was covered by aluminum foil to prevent ingress of
119 sunlight. The column was inoculated with 50 mL aquifer sediment supplemented by
120 200 g S(0). The remaining space was filled with quartz sand. All additives were mixed
121 thoroughly beforehand. The synthetic water component was the same as in the batch
122 experiment. A peristaltic pump (BT100-1L, Longer, UK) drove influent through the
123 column at a prescribed flow rate (Zhong et al., 2011). Se(VI) and other ingredients at
124 specified concentrations were added to the influent. The column experiment took
125 place over 258 consecutive days divided into five phases in order to investigate the
126 effects of different hydrodynamic and hydrochemical factors on S(0)-based Se(VI)
127 removal (Table 1). Data were then obtained on Se(VI) removal performance, reaction

128 products, microbial communities, functional genes and intermediate metabolites.

129 2.3. Chemical analysis and characterization

130 Aqueous samples were filtered through 0.22 μm filter membrane, then Se(0) and
131 the suspended microbial cells were removed by centrifuging at 12580 g for 20 min.
132 pH, ORP, and conductivity were monitored by a multifunctional meter
133 (SevenExcellenceS400, Mettler-Toledo, Switzerland) (Zhu et al., 2021). Inductively
134 coupled plasma-mass spectrometry (ICP-MS, X series, Thermo Fisher, Germany) was
135 used to determine total Se concentration. Se(IV) concentration was analyzed using a
136 spectrophotometer (UV2300, Shanghai, China) as previously described (Li et al.,
137 2014; Jain et al., 2015). Se(VI) was determined after being reduced to Se(IV) by
138 hydrochloric acid (Brimmer et al., 1987; Xie et al., 2017). NO_3^- -N, nitrite (NO_2^- -N)
139 and ammonium (NH_4^+ -N) concentrations were also determined using
140 spectrophotometry (Shi et al., 2020a). Ion chromatography monitored sulfate (SO_4^{2-}),
141 sulfite (SO_3^{2-}) and thiosulfate ($\text{S}_2\text{O}_3^{2-}$) concentrations (Basic IC 792, Metrohm,
142 Switzerland) (Shi et al., 2020b).

143 Analyses of the surface morphology and composition of the biomass samples
144 were conducted using a scanning electron microscope (SEM) (JEOL JAX-840,
145 Hitachi, Japan) equipped with energy dispersive X-ray spectroscopy (EDS). X-ray
146 photoelectron spectroscopy (XPS) measurement was carried out to identify
147 components of the produced deposits, using a Kratos XSAM-800 spectrometer (UK)
148 (Zhang et al., 2012). Surface crystallographic structures of these deposits were

149 determined by X-ray diffraction (XRD) (Rigaku-D/MAX-PC 2500, Rigaku, Japan).

150 *2.4. Microbial and metabolic analyses*

151 Microbial community analysis was conducted for both inoculum and biomass
152 obtained from each stage of the biological column. Samples were collected for DNA
153 extraction, amplification, and purification, then sent to Shanghai Majorbio
154 Technology (Shanghai, China) for high-throughput 16S rRNA gene sequencing using
155 MiSeq (Illumina, USA) (Zhang et al., 2019; Wang et al., 2020). Prior to analysis,
156 centrifugation was used to remove moisture from the samples. Operational taxonomic
157 units (OTUs) were clustered from sequences with a 0.03 distance limit (equivalent to
158 97% similarity). Rarefaction curves and alpha diversity indexes were acquired by
159 Mothur (version v.1.30.1) software. Non-metric multi-dimensional scaling (NMDS)
160 analysis was performed using Bray-Curtis similarities to cluster the microbial
161 community composition.

162 Real-time quantitative PCR (qPCR) was chosen to quantify the functional genes
163 with previously reported primers (Table S1, Supporting Information) during the S(0)-
164 driven Se(VI) bioreduction process (Throbäck et al., 2004; Bru et al., 2007; Ma et al.,
165 2007; Meyer et al., 2007; Wen et al., 2016). Electron transfer compounds, such as
166 cytochrome c (Cyt c) on cell surfaces and intracellular nicotinamide adenine
167 dinucleotide (NADH), were also examined, and their measurements normalized to
168 volatile suspended solids (VSS) according to previously documented procedure
169 (Zhang et al., 2019; Shi et al., 2020a; Zhang et al., 2021). Intermediate metabolites

170 were also monitored. Volatile fatty acids (VFAs) were measured by a gas
171 chromatograph (Agilent 4890, J&W Scientific, USA) equipped with a flame
172 ionization detector (Zhang et al., 2018). A gas chromatograph (GC) (Agilent, 4890,
173 J&W Scientific, USA) was utilized to analyze gas concentrations (including H₂ and
174 CH₄) through a thermal conductivity detector.

175 **3. Results and discussion**

176 *3.1. Feasibility of Se(VI) bioreduction driven by S(0)*

177 Fig. 1a shows that a gradual, progressive decrease in Se(VI) of the biotic group
178 occurred during the batch trial. Removal efficiency of Se(VI) reached $92.4 \pm 0.7\%$ by
179 36 h. Hardly any Se(VI) concentration was detected at the end of a 72 h cycle
180 indicating almost complete removal of Se(VI). The mean reduction rate of Se(VI) was
181 1.1 ± 0.08 mg/L·h, corresponding to a pseudo first-order reaction kinetics process
182 with a kinetics rate constant of 0.061 d⁻¹ and R² of 0.99 (Fig. 1b). Temporal dynamics
183 of Se(VI) in the control groups was also examined comparatively (Fig. S1, Supporting
184 Information). The highest removal efficiency of Se(VI) in the sterilized group was
185 only $18.1 \pm 0.2\%$, much lower than that in the biotic group, suggesting Se(VI)
186 reduction was microbially mediated. A limited Se(VI) removal efficiency of $19.1 \pm$
187 0.2% was obtained after one cycle in the abiotic group, implying that S(0) made
188 negligible contribution to abiotic reduction of Se(VI). **Taken the aforementioned into**
189 **consideration, it appears quite feasible to use S(0) as an electron donor to bioreduce**
190 **and biodetoxify Se(VI).**

191 3.2. Long-term Se(VI) removal dynamics

192 Efficient Se(VI) elimination was observed in the column trial during 258-d
193 operation (Fig. 1c). During Stage 1 (Days 0-94), with 10 mg/L of Se(VI) initial
194 concentration and 0.56 mL/min constant flow rate, Se(VI) was completely removed and
195 the corresponding Se(VI) removal capacity was 41.5 ± 4.1 (g/m³·d) (Table 1). No
196 accumulation of Se(IV) was observed during this phase. The Se(VI) removal efficiency
197 decreased slightly to $94.1 \pm 6.2\%$ when the initial concentration of Se(VI) was raised
198 to 50 mg/L during Stage 2 (Days 95-135), accompanied by an increase in Se(VI)
199 removal capacity to 193.0 ± 11.9 (g/m³·d). Se(VI) removal efficiency decreased to 84.2
200 $\pm 5.1\%$ and removal capacity rose to 346.8 ± 30.2 (g/m³·d) when the initial
201 concentration of Se(VI) was further increased to 100 mg/L during Stage 3 (Days 136-
202 176). This implied that functional microorganisms had gradually adapted to an
203 environment characterized by high Se(VI) loading. Notably, accumulation of Se(IV)
204 occurred for a mean concentration of 9.8 ± 15.2 mg/L, followed by almost sudden
205 disappearance of accumulated Se(IV) at the end of Stage 3 (Fig. 1c). This phenomenon
206 was similar to a previous study where Se(IV) accumulation was observed in Se(VI)
207 reduction using bacterium *Bacillus sp.* SF-1 with Se(IV) removal subsequently
208 occurring (Kashiwa et al., 2000). In Stage 4 (Days 177-217), the flow rate was adjusted
209 to 1.68 ml/min with Se(VI) concentration returned to 10 mg/L. During this stage, Se(VI)
210 removal efficiency increased to $89.7 \pm 10.3\%$ but the removal capacity fell to $117.0 \pm$
211 12.7 (g/m³·d). In Stage 5 (Days 218-258), during which 10 mg/L NO₃⁻-N was
212 introduced as co-acceptor to the bioreactor, the Se(VI) removal efficiency dropped to

213 $82.8 \pm 3.7\%$, suggesting that NO_3^- -N was the preferred electron acceptor used by the
214 microorganisms for metabolism, as also reported by He et al. (He et al., 2021). In a
215 previous study, Lucas and Hollibaugh showed that Se reduction was depressed by NO_3^-
216 -N when both co-existed at equal levels owing to physiological, kinetic, or enzymatic
217 factors involved in the NO_3^- -N and Se(VI) reductases (Oremland et al., 1999; Lucas
218 and Hollibaugh, 2001).

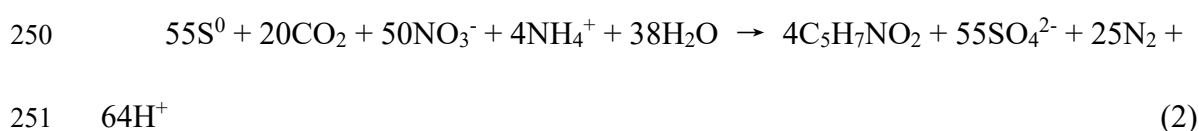
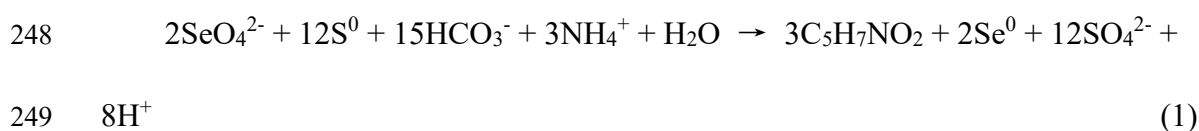
219 The evolution of pH and conductivity during long-term study were monitored.
220 Changes in pH were in the range of 7.6 to 8.4 during the Se(VI) bioreduction process
221 (Fig. S2, Supporting Information), with conductivity varying from 3000 to 3700 $\mu\text{S}/\text{cm}$.
222 This ambient condition was suitable for microbial activity to achieve diverse
223 bioprocesses (Zhang et al., 2015).

224 3.3. Bioprocess description

225 By the end of the experiment, the biological column had become dark red (Fig.
226 S3, Supporting Information), indicating precipitation of solid Se(0) from the Se(VI)
227 bioreduction process. The speciation percentages of selenium in the effluent were not
228 entirely dominated by Se(VI) and Se(IV) (Fig. 2a), suggesting generation of
229 unaccounted dissolved Se and/or organic selenium (Tan et al., 2018; Xia et al., 2019).
230 Residual Se(VI) and Se(IV) could be further removed by adsorption or co-
231 precipitation (Gezer et al., 2011; Li et al., 2018). To provide further confirmation of
232 the product morphology, characterization analyses were conducted for the precipitate.
233 In Fig. 2b, the SEM image shows spheroids observed on the surface of the biomass.
234 EDS analysis revealed Se(0) was present in the spheroids, with a corresponding

235 spectral peak (Fig. S4, Supporting Information). The 56.1 eV peak signal obtained
236 from XPS spectra relates to the 3d orbit of Se (Fig. 2c), attributed to Se(0) (Li et al.,
237 2014). XRD demonstrated the presence of elemental Se with various crystal planes
238 (Fig. 2d). These results collectively confirm that Se(0) was the final product in the
239 Se(VI) bioreduction process.

240 SO_4^{2-} accumulated in the biosystem as a result of S(0) oxidation (Fig. 1c),
241 indicating Se(VI) bioreduction coupled with oxidation of S(0) following Eq. (1). It
242 should be noted that S(0) has previously been reported to act as an electron donor in
243 the reduction of other heavy metals, accompanied by SO_4^{2-} production (Peng et al.,
244 2016; Shi et al., 2019). SO_3^{2-} and $\text{S}_2\text{O}_3^{2-}$, the intermediates related to S(0) oxidation,
245 were hardly detected in the present system. Moreover, hardly any NO_3^- -N, NO_2^- -N,
246 and NH_4^+ -N were detected at the end of Stage 5 (Fig. S5, Supporting Information),
247 implying complete denitrification according to Eq. (2) (Zhang et al., 2015).



252 The mass balance is calculated for Stage 4, as an example. During operation,
253 0.49 ± 0.05 mmol Se(VI) was removed, leading to theoretical production of $2.94 \pm$
254 0.29 mmol SO_4^{2-} according to Eq. (1). In fact, 5.15 ± 0.41 mmol SO_4^{2-} was detected,
255 implying over-consumption of S(0), which might be ascribed to other bioactivities,
256 such as biomass growth and the accumulation of metabolites (Shi et al., 2019).

257 *3.4. Microbial community evolution*

258 The evolution process of microbial community structure was examined through
259 16S rRNA gene sequencing for both inoculum and treated samples. OTUs in the
260 bioreactor were lower than inoculum, indicating that the microbial community has a
261 procedure for selection and adaptation (Table S2, Supporting Information). The
262 decreasing total number of species (Sobs indexes) in treated samples reflected
263 reduced community richness, which was also indicated by the Chao1 and Ace
264 indexes. Perfect community diversity was confirmed by the lower Shannon and higher
265 Simpson indexes in Stages 1-5, suggesting highly selected microbial communities
266 were received (Table S2, Supporting Information). The coverage index was invariably
267 close to unity, suggesting that almost all sequences were covered. Smooth rarefaction
268 curves were obtained (Fig. S6, Supporting Information), confirming that the
269 sequencing sample numbers were sufficient.

270 NMDS analysis showed that compared to other stages, biomass in Stage 1 was
271 similar to the inoculum, whereas biomass from Stage 4 and Stage 5 were quite distinct
272 from the inoculum (Fig. 3a). The microbial community composition cultivated in
273 Stage 2 and Stage 3 were similar, which may be attributed to increased Se(VI) loading
274 in Stage 2 that subsequently affected microorganisms in Stage 3. The Venn diagram
275 (Fig. 3b) shows that 906, 188, 31, 188, and 449 of OTUs were found separately in
276 five treated stages, whereas a total of 722 OTUs were common to all cultivated stages.
277 The presence of OTUs shared by all groups implies that such OTUs had adapted
278 simultaneously to the situations of Se(VI) bioreduction, S(0) oxidation, and NO₃⁻-N

279 removal.

280 Significant changes occurred in the microbial community structure of biomass
281 from the bioreactor, in comparison with the inoculum (Fig. 3c). The class related to
282 *Gammaproteobacteria* increased substantially through Stages 1 to 5, and it became
283 the predominant species (75.2%) in Stage 3. Known as NO₃⁻-N reducing bacteria,
284 *Gammaproteobacteria* was previously found to be the majority class in an S(0)-based
285 Cr(VI) bioreduction bioreactor (Shi et al., 2019). The *Deltaproteobacterian* species
286 increased in Stages 1 (11.1%), 2 (23.2%), 4 (34.9%), and 5 (11.3%) compared to the
287 inoculum (8.9%), but decreased in Stage 3 (7.62%). In Stage 4 especially,
288 *Deltaproteobacterian* accounted for the majority of species present. Recently, Tan et
289 al. reported that *Deltaproteobacterian* was the main community observed in their
290 study of selenium pollution wastewater microbial treatment (Tan et al., 2018).
291 Declines in both *Alphaproteobacteria* and *Actinobacteria* were observed through
292 Stages 1 to 5. *Anaerolineae* were found to be present at all treated stages. This is
293 consistent with a previous finding that bacteria related to the *Anaerolineae* family
294 have the capacity to reduce Se(VI) owing to the presence of Se(VI) reductases in
295 *Anaerolineae* (Fakra et al., 2018).

296 To develop a more profound understanding of the alteration in microbial
297 community structure, we also analyzed the relative abundance of key genera (Fig. 3d),
298 and found that the relative abundance of *Thiobacillus* increased considerably during
299 the experiment. Compared to its 0.83% abundance in the inoculum, *Thiobacillus*
300 accounted for 4.86% in Stage 1. The relative abundance of *Thiobacillus* rose to 36.8%

301 in Stage 2 and even higher to 72.5% in Stage 3 with elevated Se(VI) loading, and so
302 *Thiobacillus* became the most dominant species. Although its abundance later
303 declined, the proportion of *Thiobacillus* was 13.8% in Stage 4 and 7.31% in Stage 5.
304 As a chemoautotrophic genus, *Thiobacillus* could oxidize S(0) to release electrons and
305 synthesize metabolites, realizing the removal of oxidized contaminants (Yang et al.,
306 2010; He et al., 2021). Therefore, *Thiobacillus* was believed to promote S(0)
307 oxidation and Se(VI) reduction independently. **Known as a sulfur-oxidizing**
308 **bacterium, *Thiobacillus* is widely distributed in natural water, mud and soil**
309 **(Fjerdingsstad, 1969; Zhai et al., 2016), and thus could be utilized for the**
310 **bioremediation of contaminants in practice, with high microbiological safety.** Species
311 related to *Desulfurivibrio* increased from Stages 1 to 5, with 20.8% abundance
312 reached in Stage 4. *Desulfurivibrio* has demonstrable capability of reducing Se(VI)
313 and Se(IV) to Se(0) without growth or respiration, using organic electron donors
314 (Tomei et al., 1995; Lucas and Hollibaugh, 2001). And NO₃⁻-N can act as the terminal
315 electron acceptor of *Desulfurivibrio* in NO₃⁻-N dissimilatory reduction (Keith and
316 Herbert, 1983). Other SO₄²⁻ reducing bacteria such as *Desulfococcus* and
317 *Desulfocapsa* were detected during operation, which might be related to Se(VI)
318 reduction and sulfur circulation (Qian et al., 2015; He et al., 2021). The relative
319 abundance of *Pseudomonas* first declined from Stages 1 to 3, then increased in Stages
320 4 and 5 with proportions of 4.04% and 19.6%, respectively. Previously, reduction of
321 Se(VI) to Se(0) was achieved using a bacterial strain belonging to *Pseudomonas*,
322 through anaerobic respiration (Kuroda et al., 2011; Subedi et al., 2017). Although

323 almost none was detectable in inoculum, *Sulfuricurvum* was present in Stages 1 to 5
324 with respective percentages of 2.71%, 2.63%, 0.40%, 3.98% and 0.66%. As a sulfur
325 oxidizer, *Sulfuricurvum* is known to extract energy from S(0) oxidation when
326 synthesizing organic metabolites from an inorganic carbon source (Ontiveros-
327 Valencia et al., 2016). *Sulfuricurvum* has also been reported capable of ClO_4^-
328 reduction using S(0) as the electron donor (Sahu et al., 2009). *Clostridium* and
329 *Methanobacterium* genera were also detected, with relatively lower abundances; these
330 genera could produce H_2 and CH_4 through their metabolisms (Kamalaskar et al.,
331 2016; He et al., 2021).

332 3.5. Metabolic pathway interpretation

333 Se(VI) was firstly reduced to Se(IV) through specialized selenate reductase
334 (Staicu and Barton, 2021), followed by subsequent reduction of Se(IV) to Se(0) by
335 nitrite reductase, sulfite reductase, or hydrogenase I (Harrison et al., 1984; Yanke et
336 al., 1995; Basaglia et al., 2007). Fig. 4a shows that both *serA* and *tatC* genes were
337 greatly enriched during Stage 1-5 despite of their concentrations were below limit of
338 detection in the inoculum. Located in the periplasmic space, *serA* encoded specific
339 reductase for Se(VI) bioreduction (Schröder et al., 1997; Wen et al., 2016). *tatC* was
340 reported to encode specific enzyme capable of reducing Se(VI) (Ma et al., 2007).
341 About twenty years ago, enzyme encoded by gene *napA* was observed to reduce
342 Se(VI) (Sabaty et al., 2001), whereas it increased in the bioreactor rather than the
343 inoculum in the present study. Herein, the abundance of *nirS* gene increased during
344 Stages 1-5. Se(IV) reduction to Se(0) was attributed to the *nirS* gene because of its

345 ability to reduce Se(IV) (DeMoll-Decker and Macy, 1993); this was consistent with
346 the previously mentioned finding that there was no accumulation of Se(IV) in the
347 bioreactor. Studies also showed that nitrate/nitrite reductase genes were able to
348 accomplish the microbial reduction of vanadate (Zhang et al., 2019; He et al., 2021).
349 Encoding enzymes, gene *soxB*, related to the oxidation of S(0) to SO₄²⁻ exist at all
350 stages during operation, suggesting efficient S(0) oxidation during Se(VI)
351 bioreduction.

352 Electron transfer compounds, such as Cyt c and NADH, were found in the S(0)-
353 driven Se(VI) bioreduction system (Fig. 4b), suggesting that electron transfer
354 occurred during the S(0) oxidation and Se(VI) bioreduction bioactivities. This
355 phenomenon of electron transfer during Se(VI) bioreduction has also been
356 reported previously (Li et al., 2021). Metabolites, including VFAs, H₂ and CH₄, were
357 also detected in the constructed biosystem (Fig. 4b). It is likely that the autotrophs
358 utilized either carbon dioxide or bicarbonate as the sole carbon source to synthesize
359 VFAs during bioreduction, with energy gained from S(0) oxidation (Estelmann et al.,
360 2011; Zhang et al., 2020). Meanwhile, VFAs have been commonly detected when
361 using H₂ and CH₄ to reduce Se(VI) (Lai et al., 2016; Ontiveros-Valencia et al., 2016).
362 VFAs, generated as the mediate product, can be utilized by heterotrophic
363 microorganisms for reduction of high-valence pollutants (Lu et al., 2020). Notably, H₂
364 and CH₄ were also detected during operation, indicating these two gases were
365 involved in the formation and/or transformation of VFAs during Se(VI) bioreduction.
366 Furthermore, these gaseous intermediate metabolites could also be utilized by Se(VI)

367 reducers either directly or indirectly (Lai et al., 2016; Ontiveros-Valencia et al.,
368 2016).

369 3.6. Mechanism summary and practical implication

370 Mechanisms of Se(VI) reduction linked to S(0) oxidation can now be
371 summarized based on the evidence obtained from the present tests (Fig. 5).
372 Independent and synergic pathways existed for Se(VI) reduction. For the former
373 pathway, S(0) oxidation and Se(VI) reduction could be achieved by a single
374 chemoautotrophic genus (e.g., *Thiobacillus*). For the latter pathway, S(0) oxidizers
375 such as *Sulfuricurvum* converted HCO_3^- to intermediate metabolites (e.g., VFAs, H_2 ,
376 CH_4) as a result of S(0) oxidation, which were utilized by heterotrophic Se(VI)
377 reducers (e.g., *Desulfurivibrio* and *Pseudomonas*). In general, dissimilatory reduction
378 and detoxification of selenium are the main mechanisms behind Se(VI) reduction
379 (Eswayah et al., 2016). Se(VI) bioreduction involved initial reduction of Se(VI) to
380 Se(IV), followed by further reduction of Se(IV) to Se(0). Genes were detected that
381 encoded specialized reductase for Se(VI) reduction to Se(IV) (i.e., *serA* and *tatC*),
382 Se(IV) reduction to Se(0) (i.e., *nirS*), and S(0) oxidation to SO_4^{2-} (i.e., *soxB*). Electron
383 transfer by Cyt c and NADH was verified for the Se(VI) bioreduction process.

384 Selenium pollution is of increasing concern due to adverse environmental impact
385 of Se(VI). Bioremediation has been proposed as a favourable method to biotically
386 reduce Se(VI). S(0) is readily available as a byproduct from oil refining. To our
387 knowledge, this study is the first to report on bioreduction of Se(VI) utilizing S(0) as
388 an electron donor, which might be a promising option for in situ bioremediation of

389 **Se(VI) contaminated aquifers.** Our finding elucidated the removal pathway where
390 Se(VI) bioreduction can be coupled to the oxidation of S(0). **In practical application,**
391 **the proposed bioprocesses can be implemented with an S(0)-packed biological**
392 **permeable reactive barrier installed perpendicular to the migration direction of a**
393 **groundwater plume (Gibert et al., 2011).** Further research can be performed to explore
394 geological influences on the bioreduction process. Optimization of S(0) dosage is
395 required and the effect of coexisting pollutants in aquifers, such as V(V) and SO_4^{2-} ,
396 should be considered (Du et al., 2020).

397 **4. Conclusions**

398 This study investigated the chemoautotrophic transformation of Se(VI) driven by
399 S(0) in the simulated aquifer groundwater. Batch trial indicated the feasibility of
400 Se(VI) bioreduction utilizing S(0) as an electron donor under anaerobic conditions,
401 with Se(VI) removal efficiency of $92.4 \pm 0.69\%$ in 36 h operation. A 258-d continuous
402 experiment showed that Se(VI) removal performance varied under different
403 hydrodynamic and hydrochemical conditions in a column reactor. Se(VI) was reduced
404 to insoluble Se(0) and S(0) was transformed to SO_4^{2-} . Detailed mechanisms of Se(VI)
405 bioreduction driven by S(0) were proposed through analysis of microbial
406 communities, functional genes, and intermediate metabolites.

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410 **Reference**

- 411 Basaglia, M., Toffanin, A., Baldan, E., Bottegal, M., Shapleigh, J.P., Casella, S.,
412 2007. Selenite-reducing capacity of the copper-containing nitrite reductase of
413 *Rhizobium sultae*. FEMS Microbiol. Lett. 269, 124-130.
- 414 Brimmer, S.P., Fawcett, W.R., Kulhavy, K.A., 1987. Quantitative reduction of
415 selenate ion to selenite in aqueous samples. Anal. Chem. 59, 1470-1471.
- 416 Bru, D., Sarr, A., Philippot, L., 2007. Relative abundances of *Proteobacterial*
417 membrane-bound and periplasmic nitrate reductases in selected environments[†].
418 Appl. Environ. Microb. 73, 5971-5974.
- 419 DeMoll-Decker, H., Macy, J.M., 1993. The periplasmic nitrite reductase of *Thauera*
420 *selenatis* may catalyze the reduction of selenite to elemental selenium. Arch.
421 Microbiol. 160, 241-247.
- 422 Du, J., Zhang, B., Li, J., Lai, B., 2020. Decontamination of heavy metal complexes by
423 advanced oxidation processes: A review. Chin. Chem. Lett. 31, 2575-2582.
- 424 Estelmann, S., Hügler, M., Eisenreich, W., Werner, K., Berg, I.A., Ramos-Vera,
425 W.H., Say, R.F., Kockelkorn, D., Gad'on, N., Fuchs, G., 2011. Labeling and
426 enzyme studies of the central carbon metabolism in *Metallosphaera sedula*[∇]. J.
427 Bacteriol. 193, 1191-200.
- 428 Eswayah, A.S., Smith, T.J., Gardiner, P.H.E., 2016. Microbial transformations of
429 selenium species of relevance to bioremediation. Appl. Environ. Microb. 82,
430 4848-4859.
- 431 Fakra, S.C., Luef, B., Castelle, C.J., Mullin, S.W., Williams, K.H., Marcus, M.A.,

432 Schichnes, D., Banfield, J.F., 2018. Correlative cryogenic spectro-microscopy to
433 investigate Selenium bioreduction products. *Environ. Sci. Technol.* 52, 503-512.

434 Fjerdingstad, E., 1969. Bacterial corrosion of concrete in water. *Water Res.* 3, 21-30.

435 Fu, Y., Wang, J., Liu, Q., Zeng, H., 2014. Water-dispersible magnetic nanoparticle-
436 graphene oxide composites for selenium removal. *Carbon* 77, 710-721.

437 Gezer, N., Gulfen, M., Aydin, A.O., 2011. Adsorption of selenite and selenate ions
438 onto thiourea-formaldehyde resin. *J. Appl. Polym. Sci.* 122, 1134-1141.

439 Gibert, O., Rötting, T., Cortina, J.L., Pablo, J., Ayora, C., Carrera, J., Bolzicco, J.,
440 2011. In-situ remediation of acid mine drainage using a permeable reactive
441 barrier in Aznalcóllar (Sw Spain). *J. Hazard. Mater.* 191, 287-295.

442 Harrison, G., Curie, C., Laishley, E.J., 1984. Purification and characterization of an
443 inducible dissimilatory type sulphite reductase from *Clostridium pasteurianum*.
444 *Arch. Microbiol.* 138, 72-78.

445 He, C., Zhang, B., Lu, J., Qiu, R., 2021. A newly discovered function of nitrate
446 reductase in chemoautotrophic vanadate transformation by natural mackinawite
447 in aquifer. *Water Res.* 189, 116664.

448 Jain, R., Jordan, N., Weiss, S., Foerstendorf, H., Heim, K., Kacker, R., Hübner, R.,
449 Kramer, H., Hullebusch, E.D.V., Farges, F., Lens, P.N.L., 2015. Extracellular
450 polymeric substances govern the surface charge of biogenic elemental selenium
451 nanoparticles. *Environ. Sci. Technol.* 49, 1713-1720.

452 Ji, Y., Wang, Y.T., 2017. Selenium Reduction by Batch Cultures of *Escherichia coli*
453 Strain EWB32213. *J. Environ. Eng.* 143, 1-7.

454 Kamalaskar, L., Kapse, N., Pore, S., Dhakephalkar, A.P., Ranade, D.R.,
455 Dhakephalkar, P.K., 2016. Genome sequence and gene expression studies reveal
456 novel hydrogenases mediated hydrogen production by *Clostridium*
457 *biohydrogenum* sp. nov., MCM B-509T. Int. J. Hydrogen Energ. 41, 11990-
458 11999.

459 Kashiwa, M., Nishimoto, S., Takahashi, K., Ike, M., Fujita, M., 2000. Factors
460 affecting soluble selenium removal by a selenate-reducing bacterium *Bacillus* sp.
461 SF-1. J. Biosci. Bioeng. 89, 528-533.

462 Keith, S.M., Herbert, R.A., 1983. Dissimilatory nitrate reduction by a strain of
463 *Desulfovibrio desulfuricans*. FEMS Microbiol. Lett. 18, 55-59.

464 Kuroda, M., Notaguchi, E., Sato, A., Yoshioka, M., Hasegawa, A., Kagami, T.,
465 Narita, T., Yamashita, M., Sei, K., Soda, S., Ike, M., 2011. Characterization of
466 *Pseudomonas stutzeri* NT-I capable of removing soluble selenium from the
467 aqueous phase under aerobic conditions. J. Biosci. Bioeng. 112, 259-264.

468 Lai, C.Y., Wen, L.L., Shi, L.D., Zhao, K.K., Wang, Y.Q., Yang, X., Rittmann, B.E.,
469 Zhou, C., Tang, Y., Zheng, P., Zhao, H.P., 2016. Selenate and nitrate
470 bioreductions using methane as the electron donor in a membrane biofilm
471 reactor. Environ. Sci. Technol. 50, 10179-10186.

472 Latorre, C.H., Garcia, J.B., Martin, S.G., Crecente, R.M.P., 2013. Solid phase
473 extraction for the speciation and preconcentration of inorganic selenium in water
474 samples: a review. Anal. Chim. Acta 804, 37-49.

475 Lenz, M., Enright, A.M., O'Flaherty, V., van Aelst, A.C., Lens, P.N.L., 2009.

476 Bioaugmentation of UASB reactors with immobilized *Sulfurospirillum barnesii*
477 for simultaneous selenate and nitrate removal. Appl. Microbial. Biot. 83, 377-
478 388.

479 Li, D.B., Cheng, Y.Y., Wu, C., Li, W.W., Li, N., Yang, Z.C., Tong, Z.H., Yu, H.Q.,
480 2014. Selenite reduction by *Shewanella oneidensis* MR-1 is mediated by
481 fumarate reductase in periplasm. Sci. Rep. 4, 3735.

482 Li, Y., Guo, X., Dong, H., Luo, X., Guan, X., Zhang, X., Xia, X., 2018. Selenite
483 removal from groundwater by zero-valent iron (ZVI) in combination with
484 oxidants. Chem. Eng. J. 345, 432-440.

485 Li, K., Guo, J., Li, H., Han, Y., Chen, Z., Song, Y., Xing, Y., Zhang, C., 2019. A
486 combined heterotrophic and sulfur-based autotrophic process to reduce high
487 concentration perchlorate via anaerobic baffled reactors: Performance
488 advantages of a step-feeding strategy. Bioresource Technol. 279, 297-306.

489 Li, L., Zhang, B., He, H., Zhang, H., 2021. Hydrodynamics- and hydrochemistry-
490 affected microbial selenate reduction in aquifer: Performance and mechanisms.
491 Sci. Total Environ. 768, 145331.

492 Liu, Y., Peng, L., Ngo, H.H., Guo, W., Wang, D., Pan, Y., Sun, J., Ni, B.J., 2016.
493 Evaluation of nitrous oxide emission from sulfide- and sulfur-based autotrophic
494 denitrification processes. Environ. Sci. Technol. 50, 9407-9415.

495 Lu, J., Zhang, B., He, C., Borthwick, A.G.L., 2020. The role of natural Fe(II)-bearing
496 minerals in chemoautotrophic chromium (VI) bio-reduction in groundwater. J.
497 Hazard. Mater. 389, 121911.

498 Lucas, F.S., Hollibaugh, J.T., 2001. Response of sediment bacterial assemblages to
499 selenate and acetate amendments. *Environ. Sci. Technol.* 35, 528-534.

500 Ma, J., Kobayashi, D.Y., Yee, N., 2007. Chemical kinetic and molecular genetic study
501 of selenium oxyanion reduction by *Enterobacter cloacae* SLD1a-1. *Environ. Sci.*
502 *Technol.* 41, 7795-7801.

503 Meyer, B., Imhoff, J.F., Kuever, J., 2007. Molecular analysis of the distribution and
504 phylogeny of the *soxB* gene among sulfur-oxidizing bacteria-evolution of the
505 Sox sulfur oxidation enzyme system. *Environ. Microbiol.* 9, 2957-2977.

506 Nancharaiyah, Y.V., Sarvajith, M., Lens, P.N.L., 2018. Selenite reduction and
507 ammoniacal nitrogen removal in an aerobic granular sludge sequencing batch
508 reactor. *Water Res.* 131, 131-141.

509 Navarro-Alarcon, M., Cabrera-Vique, C., 2008. Selenium in food and the human
510 body: a review. *Sci. Total Environ.* 400, 115-141.

511 Ontiveros-Valencia, A., Penton, C.R., Krajmalnik-Brown, R., Rittmann, B.E., 2016.
512 Hydrogen-fed biofilm reactors reducing selenate and sulfate: community
513 structure and capture of elemental selenium within the biofilm. *Biotechnol.*
514 *Bioeng.* 113, 1736-1744.

515 Oremland, R.S., Blum, J.S., Bindi, A.B., Dowdle, P.R., Herbel, M., Stolz, J.F., 1999.
516 Simultaneous reduction of nitrate and selenate by cell suspensions of selenium-
517 respiring bacteria. *Appl. Environ. Microb.* 65, 4385-4392.

518 Peng, L., Liu, Y., Gao, S.H., Chen, X., Ni, B.J., 2016. Evaluating simultaneous
519 chromate and nitrate reduction during microbial denitrification processes.

520 Water Res. 89, 1-8.

521 Qian, J., Liu, R., Wei, L., Lu, H., Chen, G.H., 2015. System evaluation and microbial
522 analysis of a sulfur cycle-based wastewater treatment process for co-treatment of
523 simple wet flue gas desulfurization wastes with freshwater sewage. Water Res.
524 80, 189-199.

525 Qiao, J., Song, Y., Sun, Y., Guan, X., 2018. Effect of solution chemistry on the
526 reactivity and electron selectivity of zerovalent iron toward Se(VI) removal.
527 Chem. Eng. J. 353, 246-253.

528 Sabaty, M., Avazeri, C., Pignol, D., Vermeglio, A., 2001. Characterization of the
529 reduction of selenate and tellurite by nitrate reductases. Appl. Environ. Microb.
530 67, 5122-5126.

531 Sahinkaya, E., Kilic, A., 2014. Heterotrophic and elemental-sulfur-based autotrophic
532 denitrification processes for simultaneous nitrate and Cr(VI) reduction. Water
533 Res. 50, 278-286.

534 Sahu, A.K., Conneely, T., Nüsslein, K.R., Ergas, S.J., 2009. Biological perchlorate
535 reduction in packed bed reactors using elemental sulfur. Environ. Sci. Technol.
536 43, 4466-4471.

537 Schröder, I., Rech, S., Krafft, T., Macy, J.M., 1997. Purification and characterization
538 of the selenate reductase from *Thauera selenatis*. J. Biol. Chem. 272, 23765-
539 23768.

540 Shi, C., Cui, Y., Lu, J., Zhang, B., 2020. Sulfur-based autotrophic biosystem for
541 efficient vanadium (V) and chromium (VI) reductions in groundwater. Chem.

542 Eng. J. 395, 124972.

543 Shi, J., Zhang, B., Cheng, Y., Peng, K., 2020a. Microbial vanadate reduction coupled
544 to co-metabolic phenanthrene biodegradation in groundwater. *Water Res.* 168,
545 116354.

546 Shi, J., Zhang, B., Qiu, R., Lai, C., Jiang, Y., He, C., Guo, J., 2019. Microbial
547 chromate reduction coupled to anaerobic oxidation of elemental sulfur or
548 zerovalent iron. *Environ. Sci. Technol.* 53, 3198-3207.

549 Shi, et al., Zhang, B., Wang, Y., Fu, J., 2020b. Effects of hydropower dam
550 construction on sulfur distribution and sulfate-reducing prokaryotes assemblage.
551 *Sci. Total Environ.* 705, 135819.

552 **Song, B., Tian, Z., Weijden, R.D., Buisman, C.J.N., Weijma, B.J., 2021. High-rate**
553 **biological selenate reduction in a sequencing batch reactor for recovery of**
554 **hexagonal selenium. *Water Res.* 193, 116855.**

555 **Staicu, L.C., Barton, L.L., 2021. Selenium respiration in anaerobic bacteria: Does**
556 **energy generation pay off? *J. Inorg. Biochem.* 222, 111509.**

557 Subedi, G., Taylor, J., Hatam, I., Baldwin, S.A., 2017. Simultaneous selenate
558 reduction and denitrification by a consortium of enriched mine site bacteria.
559 *Chemosphere* 183, 536-545.

560 Tan, L.C., Nancharaiah, Y.V., Lu, S., Hullebusch, E.D.V., Gerlach, R., Lens, P.N.L.,
561 2018. Biological treatment of selenium-laden wastewater containing nitrate and
562 sulfate in an upflow anaerobic sludge bed reactor at pH 5.0. *Chemosphere* 211,
563 684-693.

564 Tan, L.C., Papirio, S., Luongo, V., Nancharaiah, Y.V., Cennamo, P., Esposito, G.,
565 Hullebusch, E.D.V., Lens, P.N.L., 2018. Comparative performance of anaerobic
566 attached biofilm and granular sludge reactors for the treatment of model mine
567 drainage wastewater containing selenate, sulfate and nickel. *Chem. Eng. J.* 345,
568 545-555.

569 Throbäck, I.N., Enwall, K., Jarvis, Å., Hallin, S., 2004. Reassessing PCR primers
570 targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying
571 bacteria with DGGE. *FEMS Microbiol. Ecol.* 49, 401-417.

572 Tomei, F.A., Barton, L.L., Lemanski, C.L., Zocco, T.G., Fink, N.H., Sillerud, L.O.,
573 1995. Transformation of selenate and selenite to elemental selenium by
574 *Desulfovibrio desulfuricans*. *J. Ind. Microbiol. Biot.* 14, 329-336.

575 Ucar, D., Sahinkaya, E., Yilmaz, T., Cakmak, Y., 2019. Simultaneous nitrate and
576 perchlorate reduction in an elemental sulfur-based denitrifying membrane
577 bioreactor. *Int. Biodeter. Biodegr.* 144, 104741.

578 Wang, S., Zhang, B., Li, T., Li, Z., Fu, J., 2020. Soil vanadium(V)-reducing related
579 bacteria drive community response to vanadium pollution from a smelting plant
580 over multiple gradients. *Environ. Int.* 138, 105630.

581 Wang, Y., Lai, C.Y., Wu, M., Song, Y., Hu, S., Yuan, Z., Guo, J., 2021. Roles of
582 oxygen in methane-dependent selenate reduction in a membrane biofilm reactor:
583 stimulation or suppression. *Water Res.* 198, 117150.

584 Wang, Z., Zhang, B., He, C., Shi, J., Wu, M., Guo, J., 2021. Sulfur-based mixotrophic
585 vanadium (V) bio-reduction towards lower organic requirement and sulfate

586 accumulation. Water Res. 189, 116655.

587 Wen, L.L., Lai, C.Y., Yang, Q., Chen, J.X., Zhang, Y., Ontiveros-Valencia, A., Zhao,
588 H.P., 2016. Quantitative detection of selenate-reducing bacteria by real-time
589 PCR targeting the selenate reductase gene. Enzyme Microb. Tech. 85, 19-24.

590 Xia, S., Xu, X., Zhou, L., 2019. Insights into selenate removal mechanism of
591 hydrogen-based membrane biofilm reactor for nitrate-polluted groundwater
592 treatment based on anaerobic biofilm analysis. Ecotox. Environ. Safe. 178, 123-
593 129.

594 Xie, Y., Dong, H., Zeng, G., Zhang, L., Cheng, Y., Hou, K., Jiang, Z., Zhang, C.,
595 Deng, J., 2017. The comparison of Se(IV) and Se(VI) sequestration by nanoscale
596 zero-valent iron in aqueous solutions: The roles of solution chemistry. J. Hazard.
597 Mater. 338, 306-312.

598 Yang, Z.H., StoVen, K., Haneklaus, S., Singh, B.R., Schnug, E., 2010. Elemental
599 sulfur oxidation by *Thiobacillus spp.* and aerobic heterotrophic sulfur-oxidizing
600 bacteria. Pedosphere 20, 7-79.

601 Yanke, L.J., Bryant, R.D., Laishley, E.J., 1995. Hydrogenase I of *Clostridium*
602 *pasteurianum* functions as a novel selenite reductase. Anaerobe 1, 61-67.

603 Zhai, J., Rahaman, M.H., Chen, X., Xiao, H., Liao, K., Li, X., Duan, C., Zhang, B.,
604 Tao, G., John, T., Vymazal, J., 2016. New nitrogen removal pathways in a full-
605 scale hybrid constructed wetland proposed from high-throughput sequencing and
606 isotopic tracing results. Ecol. Eng. 97, 434-443.

607 Zhang, B., Cheng, Y., Shi, J., Xing, X., Zhu, Y., Xu, N., Xia, J., Borthwick, A.G.L.,

608 2019. Insights into interactions between vanadium (V) bio-reduction and
609 pentachlorophenol dechlorination in synthetic groundwater. Chem. Eng. J. 375,
610 121965.

611 Zhang, B., Feng, C., Ni, J., Zhang, J., Huang, W., 2012. Simultaneous reduction of
612 vanadium (V) and chromium (VI) with enhanced energy recovery based on
613 microbial fuel cell technology. J. Power Sources 204, 34-39.

614 Zhang, B., Li, Y., Fei, Y., Cheng, Y., 2021. Novel pathway for vanadium(V) bio-
615 detoxification by Gram-positive *Lactococcus raffinolactis*. Environ. Sci.
616 Technol. 55, 2121-2131.

617 Zhang, B., Qiu, R., Lu, L., Chen, X., He, C., Lu, J., Ren, Z.J., 2018. Autotrophic
618 vanadium(V) bioreduction in groundwater by elemental sulfur and zerovalent
619 Iron. Environ. Sci. Technol. 52, 7434-7442.

620 Zhang, B., Tian, C., Liu, Y., Hao, L., Liu, Y., Feng, C., Liu, Y., Wang, Z., 2015.
621 Simultaneous microbial and electrochemical reductions of vanadium (V) with
622 bioelectricity generation in microbial fuel cells. Bioresource Technol. 179, 91-
623 97.

624 Zhang, B., Wang, S., Diao, M., Fu, J., Xie, M., Shi, J., Liu, Z., Jiang, Y., Cao, X.,
625 Borthwick, A.G.L., 2019. Microbial community responses to vanadium
626 distributions in mining geological environments and bioremediation assessment.
627 J. Geophys. Res-Biogeophys. 124, 601-615.

628 Zhang, B., Wang, Z., Shi, J., Dong, H., 2020. Sulfur-based mixotrophic bio-reduction
629 for efficient removal of chromium (VI) in groundwater. Geochim. Cosmochim.

630 Ac. 268, 296-309.

631 Zhang, L., Zhang, C., Hu, C., Liu, H., Bai, Y., Qu, J., 2015. Sulfur-based mixotrophic
632 denitrification corresponding to different electron donors and microbial profiling
633 in anoxic fluidized-bed membrane bioreactors. *Water Res.* 85, 422-431.

634 Zhang, Y., Kuroda, M., Arai, S., Kato, F., Inoue, D., Ike, M., 2019. Biological
635 treatment of selenate-containing saline wastewater by activated sludge under
636 oxygen-limiting conditions. *Water Res.* 154, 327-335.

637 Zhong, C., Zhang, B., Kong, L., Xue, A., Ni, J., 2011. Electricity generation
638 from molasses wastewater by an anaerobic baffled stacking microbial fuel cell. *J.*
639 *Chem. Technol. Biot.* 86, 406-413.

640 Zhou, L., Xu, X., Xia, S., 2018. Effects of sulfate on simultaneous nitrate and selenate
641 removal in a hydrogen-based membrane biofilm reactor for groundwater
642 treatment: Performance and biofilm microbial ecology. *Chemosphere* 211, 254-
643 260.

644 Zhu, L., Zhang, L., Li, J., Zhang, D., Chen, L., Sheng, D., Yang, S., Xiao, C., Wang,
645 J., Chai, Z., Albrecht-Schmitt, T.E., Wang, S., 2017. Selenium sequestration in a
646 cationic layered rare earth hydroxide: a combined batch experiments and EXAFS
647 investigation. *Environ. Sci. Technol.* 51, 8606-8615.

648 Zhu, F., Zheng, Y.M., Zhang, B.G., Dai, Y.R., 2020. A critical review on the
649 electrospun nanofibrous membranes for the adsorption of heavy metals in water
650 treatment. *J. Hazard. Mater.* 401, 123608.

651

652 **Figure Captions.**

653 **Fig. 1.** Se(VI) removal performance in S(0)-driven Se(VI) reduction biosystems. (a)
654 Time histories of Se(VI) removal efficiency during three consecutive operating cycles
655 during batch trial; (b) Pseudo first-order kinetic plots for Se(VI) removal; (c) Time
656 histories of Se(VI), NO₃⁻-N in influent, Se(VI), Se(IV), NO₃⁻-N, and SO₄²⁻ in effluent,
657 corresponding Se(VI) and NO₃⁻-N removal efficiencies, and Se(VI) removal capacities
658 during 258 d operation of the column trial.

659 **Fig. 2.** Characterization of products from Se(VI) bioreduction. (a) Selenium speciation
660 percentages in the effluent. (b) SEM, (c) XPS, and (d) XRD analysis of the precipitates
661 after reaction. SEM: Scanning electron microscope; XPS: X-ray photoelectron
662 spectroscopy; XRD: X-ray diffraction.

663 **Fig. 3.** Microbial community evolution in inoculum and at different stages of column
664 trial. (a) Non-metric multidimensional scaling (NMDS) plot for the distribution of
665 microbial community; (b) Venn plot of microbial richness (b); (c) Class-level relative
666 abundance; (d) Heatmap of functional genera.

667 **Fig. 4.** Metabolic processes of S(0)-driven Se(VI) bioreduction. (a) Abundances of
668 functional genes; (b) Contents of electron transfer compounds and intermediate
669 metabolites. Cyt c: Cytochrome c; NADH: Nicotinamide adenine dinucleotide; VFAs:
670 Volatile fatty acids.

671 **Fig. 5.** Mechanistic pathways of microbial Se(VI) reduction processes linked to S(0)
672 oxidation. VFAs: Volatile fatty acids.

673

Table 1. Ambient conditions and Se(VI) removal performance for each stage during 258-d operation in column trial.

Stage	Period (d)	Flow rate (mL/min)	Initial Se(VI) (mg/L)	Initial NO ₃ ⁻ -N (mg/L)	Se(VI) removal efficiency (%)	Se(VI) removal capacity (g/(m ³ /d))	NO ₃ ⁻ -N removal efficiency (%)	NO ₃ ⁻ -N removal capacity (g/(m ³ /d))
1	0-94	0.56	10	-	98.1 ± 6.3	41.5 ± 4.1	-	-
2	95-135	0.56	50	-	94.1 ± 6.2	193.0 ± 11.9	-	-
3	136-176	0.56	100	-	84.2 ± 5.1	346.8 ± 30.2	-	-
4	177-217	1.68	10	-	89.7 ± 10.3	117.0 ± 12.7	-	-
5	218-258	0.56	10	10	82.8 ± 3.7	35.2 ± 2.1	97.9 ± 0.8	40.6 ± 3.3

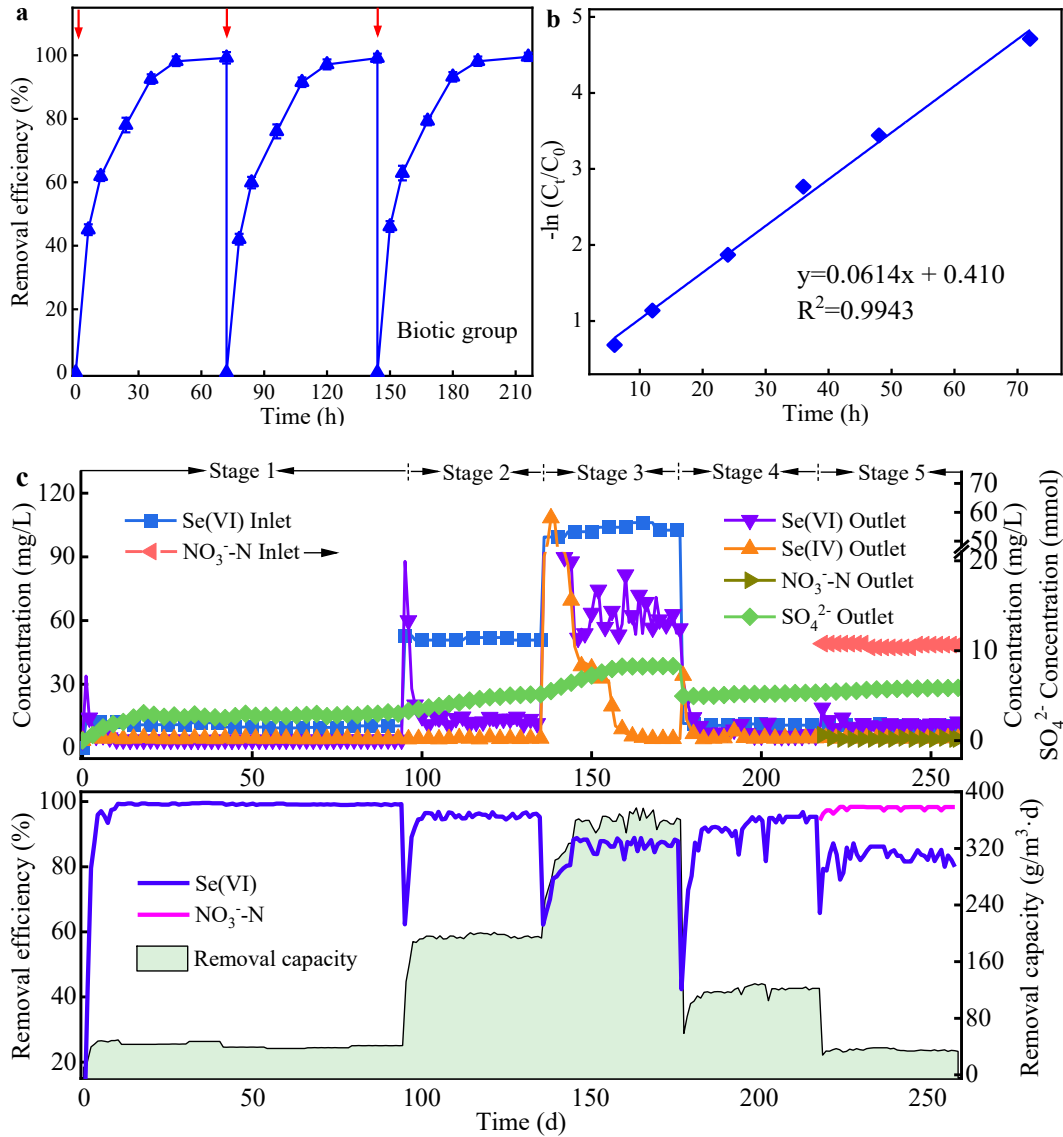


Fig. 1. Se(VI) removal performance in S(0)-driven Se(VI) reduction biosystems. (a) Time histories of Se(VI) removal efficiency during three consecutive operating cycles during batch trial; (b) Pseudo first-order kinetic plots for Se(VI) removal; (c) Time histories of Se(VI), NO₃⁻-N in influent, Se(VI), Se(IV), NO₃⁻-N, and SO₄²⁻ in effluent, corresponding Se(VI) and NO₃⁻-N removal efficiencies, and Se(VI) removal capacities during 258 d operation of the column trial.

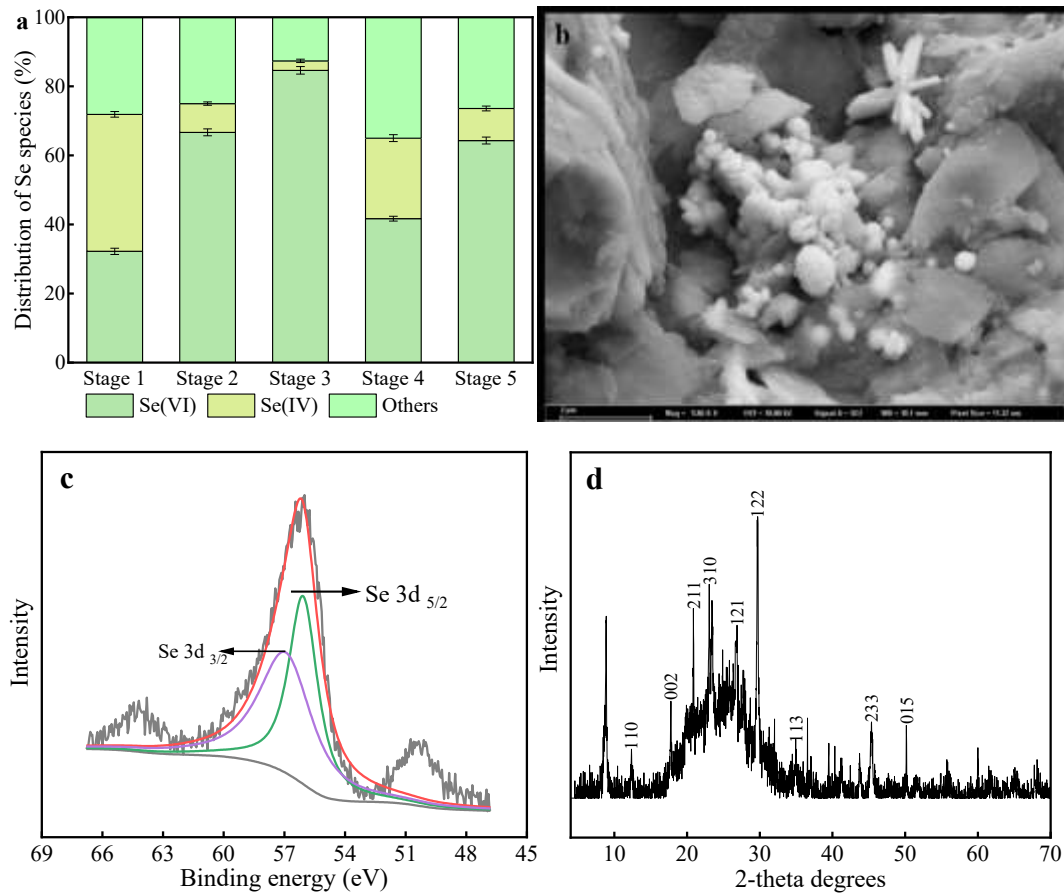


Fig. 2. Characterization of products from Se(VI) bioreduction. (a) Selenium speciation percentages in the effluent. (b) SEM, (c) XPS, and (d) XRD analysis of the precipitates after reaction. SEM: Scanning electron microscope; XPS: X-ray photoelectron spectroscopy; XRD: X-ray diffraction.

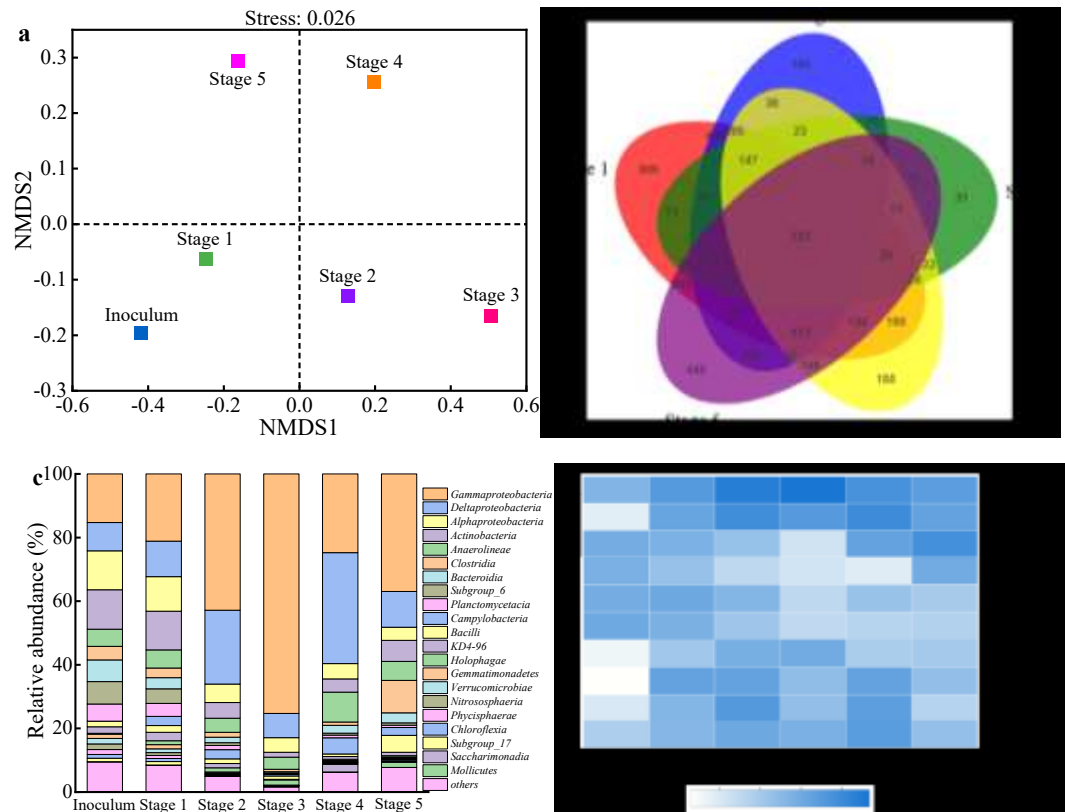


Fig. 3. Microbial community evolution in inoculum and at different stages of column trial. (a) Non-metric multidimensional scaling (NMDS) plot for the distribution of microbial community; (b) Venn plot of microbial richness (b); (c) Class-level relative abundance; (d) Heatmap of functional genera.

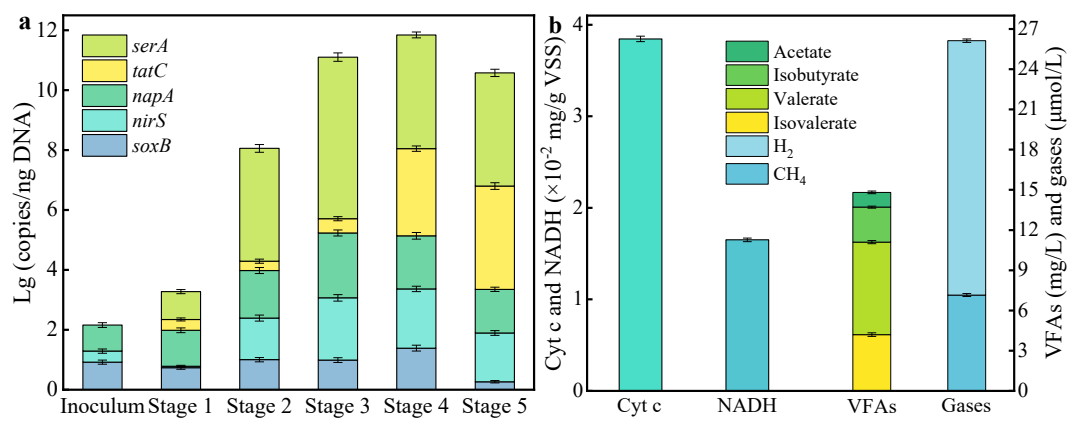


Fig. 4. Metabolic processes of S(0)-driven Se(VI) bioreduction. (a) Abundances of functional genes; (b) Contents of electron transfer compounds and intermediate metabolites. Cyt c: Cytochrome c; NADH: Nicotinamide adenine dinucleotide; VFAs: Volatile fatty acids.

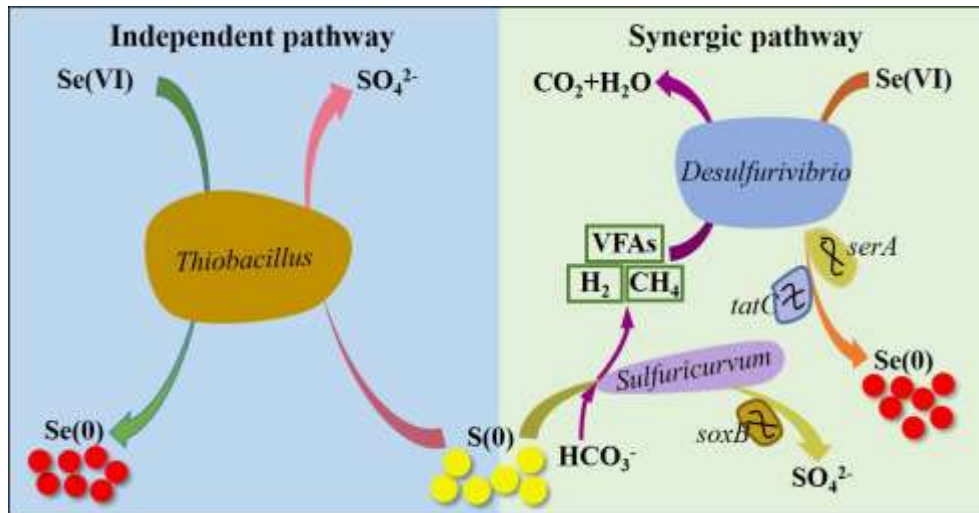


Fig. 5. Mechanistic pathways of microbial Se(VI) reduction processes linked to S(0) oxidation. VFAs: Volatile fatty acids.