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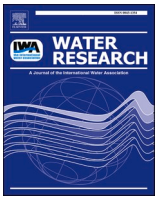

Selenium treatment via integrating flow electrode capacitive deionization (FCDI) and bio-electrochemical systems (BES)

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ABSTRACT

Selenium pollution in aquatic environments poses a major global challenge, with a significant gap in effective treatment technologies. In this study, we explored a novel approach integrating flow-electrode capacitive deionization (FCDI) with bio-electrochemical systems (BES) for the removal and reduction of selenate and selenite ions in one compact reactor. Our integrated system was electricity-driven, eliminating chemical usage. Up to 76 % selenium removal from the waste streams was achieved, followed by up to 66 % and 54 % reduction of selenate and selenite to elemental selenium respectively. The addition of acetate, a carbon source, enhanced selenate reduction by 14 % but lowered selenite reduction by 21 %, suggesting the substrate-dependent and bio-electrochemical-driven nature of selenate and selenite reduction respectively. Metagenomic sequencing revealed that *Geobacter sulfurreducens* and *Pseudomonas stutzeri* two known Se-reducing species, likely contributed to both selenite and selenate reduction through up-regulating functional genes related to sulfide reductase, fumarate reductase, and multi-heme c-type cytochromes. *Thauera* spp. and *Alishewanella* spp., two species not previously associated with selenium reduction, were likely involved in selenite reduction via the up-regulation of genes related to sulfite reductase and selenium reductase.

1. Introduction

Selenium (Se) is a naturally occuring non-metal trace element, with a narrow range between being an essential nutrient (< 40 μg/day) and a toxin (> 400 μg/day) (Institute of Medicine, Food and Nutrition Board, 2000). Selenium pollution was reported to affect 40 million people in 15 countries (Malhotra et al., 2020) and aquatic habitats across five continents (Devi et al., 2017; Lemly, 2004). In light of new evidence on selenium bioaccumulation, the U.S. Environmental Protection Agency (USEPA, 2019) recently reduced the maximum selenium contaminant level in several water bodies, including industrial wastewater (70 μg/L) (USEPA,2019), lentic (1.5 μg/L), and lotic (3.1 μg/L) aquatic systems (USEPA, 2014).

In the aquatic environment, selenium predominantly exists as inorganic Se (IV) (selenite) and Se (VI) (selenate), both of which have high solubility and toxicity (Sharma et al., 2019). It is preferable for treatment technologies to not only remove them from waste streams but also reduce them to the non-soluble and non-toxic elemental selenium (Chapman et al., 2010; Gebreeyessus and Zewge, 2019; Staicu et al., 2015). To this end, physicochemical technologies, such as chemical precipitation, adsorption, ion exchange, and reverse osmosis, can effectively remove selenate from waste streams, but generate a mix of chemical and selenate-rich residuals that need further treatment (Ali and Shrivastava, 2021; He et al., 2018). Biological methods are effective at reducing dissolved selenium species to elemental selenium, (Eswayah Abdurrahman et al., 2016; Staicu and Barton, 2021; Zhang et al., 2022, 2018) but typically suffer from slow removal rates (Tan et al., 2016).

The best solution recommended by the USEPA is the combination of chemical precipitation and low hydraulic residence time biological reduction (USEPA, 2019). However, this two-step process has considerable drawbacks, including reliance on chemical usage, a large operational footprint, and inhibitive capital and operating costs (Chu, 2010). Its implementation in the US steam electric power industry is estimated to incur a total annual compliance cost of $102 million (US EPA, 2020,

Abbreviations: FCDI, Flow-electrode capacitive deionization; BES, Bio-electrochemical system; GAC, Granular activated carbon.

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2023).

In this study, we investigated the feasibility of coupling flow-electrode capacitive deionization (FCDI) with bio-electrochemical systems (BES) for selenate removal and reduction in a single reactor. FCDI is an electrochemical technology that utilizes a voltage gradient to drive ion sorption into electrodes based on charge (Oren, 2008). While FCDI has not been specifically studied for selenate or selenite removal, it has achieved 83–93 % removal of oxyanions such as NO_3^-, PO_4^{3-}, and SO_4^{2-} at hydraulic residence times of 7–12 hours, considerbly shorter than biological methods (Bian et al., 2019; Linnartz et al., 2017; Zuo et al., 2018). It is also potentially more energy-efficient than reverse osmosis, a leading alternative in certain applications (Siekierka et al., 2018; Welgemoed and Schutte, 2005). BES, on the other hand, is a technology that uses electroactive bacteria to catalyze electrochemical reactions, either driving chemical transformation or converting chemical energy into electrical energy (Sydow et al., 2014). Previous BES studies reported near-complete (>99 %) selenate reduction to elemental selenium at steady state, (Zhang et al., 2018) in the form of recoverable extracellular selenium nanoparticles (Zhang et al., 2022).

We hypothesized that integrated FCDI-BES could harness the synergies between FCDI and BES: the rapid removal of selenate ions by FCDI and the complete reduction of selenate to elemental selenium by BES, all in a compact reactor. We used a three-chamber, two-stage design: in the first stage (i.e., FCDI), a voltage was applied to enable the adsorption of selenate into the electrode. In the second stage (i.e., BES), the voltage was reversed, enabling the adsorbed selenate to be reduced to elemental selenium utilizing the biomass on the electrode. The integrated FCDI-BES was completely electricity-driven, eliminating chemical usage, which, combined with the compact footprint, could offer an economical and sustainable alternative to current selenium treatment options.

2. Materials and methods

2.1. Reactor setup

A three-chamber reactor (Changsha Spring New Energy Technology Co. LTD, China) (Figure S2) was used. The reactor comprises a 40 mL middle chamber, separated from the anode (10 mL) and cathode (10 mL) chambers by anion and cation exchange membranes (AEM and CEM, Membranes International Inc., USA), respectively. Side chambers contained graphite electrodes with carved flow channels, through which granular activated carbon (GAC) (General Carbon Corp, USA) flowed as the flow electrodes.

The operation phase comprised two stages: The first stage was FCDI for selenate removal and the second stage was BES for selenate/selenite reduction (Figure S2). To prevent faradaic reactions, such as water electrolysis (He et al., 2016; Kim et al., 2015), a constant voltage of 1.2 V was applied for 24 hrs. This voltage was in the range of the voltages used for NO_3^-, PO_4^{3-}, and SO_4^{2-} removal in FCDI (Bian et al., 2020, 2019; Ge et al., 2018; Zhang et al., 2020).

During the second stage, the voltage was reversed, and a constant 2.0 V was applied for 72 hrs. This voltage exceeds the theoretical minimum for selenate reduction, avoids limitations, and provides sufficient energy to overcome the activation barrier (Bouroushian, 2010; Hageman et al., 2013; Maslennikov et al., 1999; Zhang et al., 2018).Previous studies under similar conditions demonstrated successful long-term selenate reduction (Zhang et al., 2018).

2.2. Experimental design

The effects of selenium ions (selenate vs. selenite), biomass, and carbon source (11mg C/L sodium acetate) were assessed as detailed in Table 1. The cultivation of biotic GAC is detailed in the Supporting Information (Text S1).

Table 1
FCDI-BES abiotic and biotic conditions.

Conditions	Middle Chamber		Reduction Chamber
	Feed solution	Biomass	Carbon Source (Acetate)
SeO_4^{2-} Bio	10 mg/L SeO_4^{2-}	GAC with pre-cultivated biofilm	NO
SeO_4^{2-} Bio C			(11 mg C/L)
SeO_4^{2-} Abio		Plain GAC	NO
SeO_4^{2-} Abio C			(11 mg C/L)
SeO_3^{2-} Bio	10 mg/L SeO_3^{2-}	GAC with pre-cultivated biofilm	NO
SeO_3^{2-} Bio C			(11 mg C/L)
SeO_3^{2-} Abio		Plain GAC	NO
SeO_3^{2-} Abio C			(11 mg C/L)

Note: "C" in biotic and abiotic conditions represents the carbon source (acetate).

2.3. Mass balance of Se species in FCDI-BES

Se species included dissolved Se, adsorbed Se, and elemental Se^0. Dissolved Se was measured directly using Ion Chromatography (Dionex Aquion Ion Chromatography System, USA) as detailed in the Supporting Information (Text S3). Adsorption was estimated using a separate beaker test with 10 mg/L selenate/selenite and GAC loading 5wt% in 250 mL DI water, replicating the conditions present in the FCDI-BES reactors. Beaker tests were carried out at ambient temperature (25 °C) and 850 rpm mixing with a magnetic stirrer. Samples were collected at 0, 2, and 8 hrs. until a steady state was reached. Liquid samples were analyzed and desorption from GAC was carried out using the FCDI-BES reactor by reversing the cell voltage. This desorption process was monitored for 1 hr. (Figure S6). All adsorption tests were biologically replicated. Adsorption was estimated based on interpolation (Figure S7). Results were then applied to the FCDI-BES test to obtain the mass of adsorbed Se. Elemental Se^0 was estimated using a mass balance approach, i.e., Total Elemental Se^0 solid = Influent Se (selenate or selenite) – Dissolved Se after filtration - adsorbed Se in GAC.

2.4. Calculations, chemical analysis, and metabolic network analysis

Electric current and potential data were recorded every thirty seconds using a Keithley datalogger (model 2700, USA). Faradaic efficiency (%) was calculated based on the standard method detailed in the Supporting Information (Text S4).

Surface characterization of GAC was conducted using scanning electron microscopy (SEM, Apreo Thermo Fisher Scientific, USA). Analysis of dissolved selenium species and water quality parameters followed standard protocols, with all the procedural details outlined in the Supporting Information (Text S3).

For microbial analyses, biofilm samples were collected from the biotic GAC at three key time points: immediately after cultivation (post-cultivation), after 5 days of selenium ion acclimatization (post-acclimatization), and following the operation of the FCDI-BES system (post-FCDI-BES). DNA extraction, metagenomic sequencing, and data analysis were conducted using standard protocols, with further details available in the Supporting Information (Text S5).

3. Results and discussion

3.1. Detected selenium species

During the removal stage (i.e., FCDI, 24 hours), 70 % of selenate and 76 % of selenite ions were consistently removed from the middle chamber to the anode chamber (Fig. 1). Abiotic conditions increased the removal by ~20 % compared to biotic conditions, likely due to higher adsorption by clean GAC as demonstrated elsewhere (Wasewar et al.,

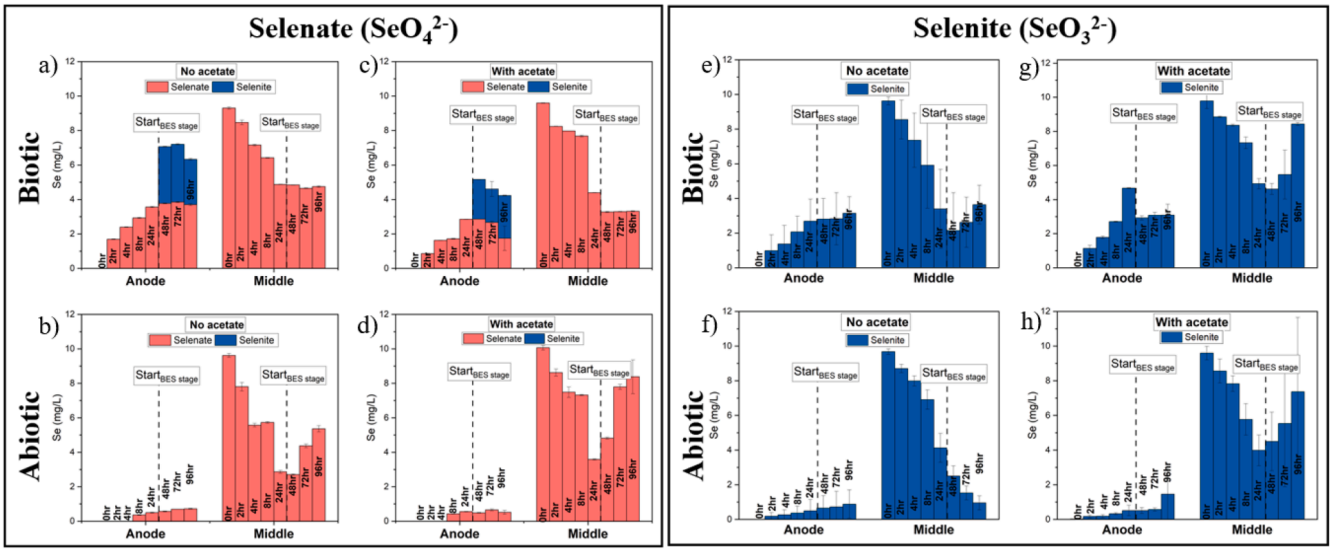

Fig. 1. Dissolved selenium ion concentration in the anode and middle chambers. The dashed lines indicate the end of the FCDI removal stage and the beginning of the BES reduction stage.

2009; Zhao et al., 2020). The presence of acetate had mixed effects, with biotic conditions recording a 5 % increase and a 14 % decrease in removal rates for selenate and selenite respectively (Fig. 1,a,1c,1e,1g), and abiotic conditions recording a consistent 4 % decrease in removal rates (Fig. 1b,1d,1f,1h).

To date, there are no studies using FCDI to remove selenate or selenite ions. Therefore, a comparison of removal efficiency was made with ions of similar structure such as PO_4^{3-} and SO_4^{2-} (Table 2). Our system achieved a removal efficiency of 70–76 % for selenate and selenite ions within 24 hours, while other studies reported removal efficiencies ranging from approximately 40 % to 93 % for PO_4^{3-} and SO_4^{2-} ions (Table 2). Our removal rate was achieved with lower electrolyte concentration (0.2 g/L of Na_2SO_4 in Table 2) but longer hydraulic residence time (HRT) than others. We anticipate potential to further improve selenium removal through increasing the electrolyte concentrations, which has been demonstrated to significantly impact on the cell ohmic resistance and overall system efficiency (Liang et al., 2017; Yang et al., 2016).

Specific energy consumption for selenate and selenite removal was calculated as kWh/kg SeO_4 and kWh/kg SeO_3, respectively, with detailed calculations in the Supporting Information (Text S4). For the FCDI phase (24 hours at 1.2 V), energy consumption for selenate was 1.35 kWh/kg without acetate and 1.55 kWh/kg with acetate, while for selenite, it was 0.54 kWh/kg and 0.36 kWh/kg, respectively. Our system demonstrated lower energy consumption than previous studies, which reported up to 16.9 kWh/kg for PO_4^{3-} and 8.7 kWh/kg for NO_3^- (Table 2).

During the selenate reduction stage (i.e., BES 72 hrs.), selenite, the intermediate reduction product of selenate was only detected under biotic conditions (Fig. 1a and c). These results are consistent with previous findings that selenate reduction requires biomass (Zhang et al., 2022, 2018). Although selenate (or selenite) reduction is thermodynamically favorable ($E^0 = 0.95$ V) (Zou and Mauter, 2021), it is hindered by the substantial energy required to break the strong Se=*O* double bond and alter the overall molecular structure of the anion (Maslennikov et al., 1999). Bacteria serve as catalysts to overcome this activation energy barrier (Hageman et al., 2013; Ma et al., 2007).

3.2. *Estimated elemental selenium production*

Under biotic conditions, the yield of elemental Se^0 ranged from 52 % to 66 % for selenate and 33 % to 54 % for selenite (Fig. 2). While these yields are lower than the previous BES studies (>90 %, Table 3), they were obtained within 72 hours, much shorter than the reported operating periods of 16 to 90 days in those studies. For instance, one study reached 96 % Se^0 yield after the 16th day of operation but the yield was only ~38 % by day 10 (Zhang et al., 2022, 2018), whereas we observed a yield of 66 % within 72 hours. BES's capability of efficient selenium reduction, coupled with GAC's large surface area (Jiang and Li, 2009; Logan et al., 2007), likely contributed to the high Se^0 yield in this study. In terms of the removal rate, FCDI-BES achieved maximum removal rates of 10.01 mg-Se/L/day for selenate and 11.98 mg-Se/L/day for selenite, compared to 3.38 mg-Se/L/day and 4.49 mg-Se/L/day repectively, in the previous studies (Table 3).

Acetate improved the elemental selenium Se^0 yield by 14 % in selenate reduction, but inhibited the yield by 21 % in selenite reduction

Table 2
Summary of anion removal and operating conditions in related FCDI studies.

Process	Ion	Removal Rate	Voltage (V)	Electrolyte	Carbon Loading	HRT	Energy Consumption	Reference
FCDI-BES	SeO_4^{2-}	59~64 %	1.2	0.2 g/L Na_2SO_4	GAC 5 wt.%	Batch 24 hrs.	1.35~ 1.55 kWh/kg SeO_4^{2-}	This Study
FCDI-BES	SeO_3^{2-}	56~70 %	1.2	0.2 g/L Na_2SO_4	GAC 5 wt.%	Batch 24 hrs.	0.36~ 0.56 kWh/kg SeO_3^{2-}	This Study
FCDI	SO_4^{2-}	93 %	1.2	3.33 g/L $MgCl_2$	Charcoal 5 wt.%	Batch 4 hrs.	N/A	Linnartz et al. (2017)
FCDI	PO_4^{3-}	77 %	1.2	3.55 g/L Na_2SO_4	GAC 5 to 15 wt.%	Batch 7 hrs.	16.9 kWh/kg PO_4^{3-}	Bian et al. (2019)
FCDI	NO_3^-	93 %	1.2	3.55 g/L Na_2SO_4	GAC 5 to 15 wt.%	Batch 7 hrs.	8.7 kWh/kg NO_3^-	Bian et al. (2019)
FCDI	NO_3^-	91 %	1	1.0 g/L NaCl	GAC 10 wt.%	Single pass 0.73~2.94 min.	N/A	(Song et al., 2019)
FCDI	PO_4^{3-}	~40 %	1.5~2.1	1.2 g/L NaCl	GAC 5 wt.%	Single pass 0.73~2.94 min.	N/A	(Xu et al., 2021)

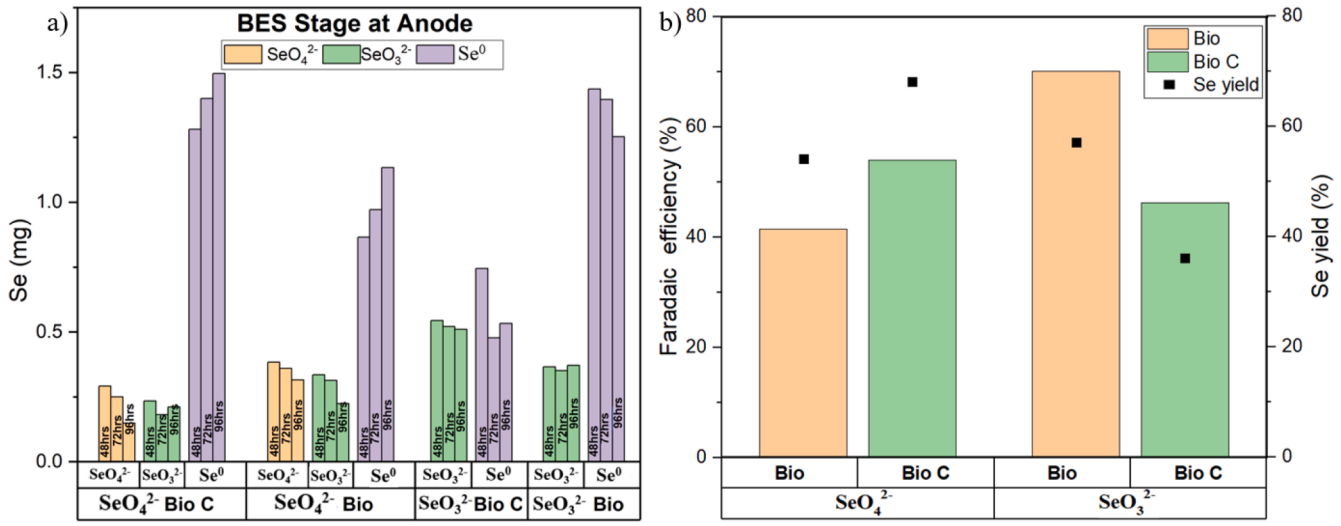

Fig. 2. a) Selenium speciation during the reduction stage (BES) in the anode chamber, generated from mass balance calculations. b) Faradaic efficiency during BES stage. Bio and Bio C represent biotic conditions, where" C" indicates the presence of an additional carbon source.

Table 3
Summary of elemental Se^0 yields and operating conditions in related studies.

Process	Ion	Elemental Se^0 yield	Se Removal rate (mg-Se removed/L/d)	Se loading (mg/L)	C: Se ratio	Carbon source	HRT	Biocathode material	Reference
BES-FCDI Bio	SeO_4^{2-}	52 %	9.66	10	N/A	None	Batch 3 days	GAC	This Study
BES-FCDI Bio C	SeO_4^{2-}	66 %	11.98	10	~ 2:1	Acetate	Batch 3 days	GAC	This Study
BES	SeO_4^{2-}	96 %	3.38	5	2:1	Acetate	Continuous 1.45 days	Carbon Cloth	Zhang et al. (2018)
BES	SeO_4^{2-}	97 ~ 99 %	3.38	5	2:1	Acetate	Continuous 1.45 days	Carbon Cloth	Zhang et al. (2022)
BES-FCDI Bio	SeO_3^{2-}	54 %	10.01	10	N/A	None	Batch 3 days	GAC	This Study
BES-FCDI Bio C	SeO_3^{2-}	33 %	4.42	10	~ 2:1	Acetate	Batch 3 days	GAC	This Study
BES	SeO_3^{2-}	99 %	0.23	0.7 ~ 5	3:1 ~ 22:1	Acetate	Continuous 3.05 days	GAC	Jugnia et al. (2021)
BES	SeO_3^{2-}	26 %	4.49	51	~ 31:1	Glucose	Batch 3days	Graphite	Sravan et al. (2020)
BES	SeO_3^{2-}	~ 30 %	0.10	~ 423	~ 51:1	Lactate	3days	Graphite felt	Nguyen et al. (2016)

Note: The Se removal rate for this study has been calculated based on the Se concentration in the anode at the beginning of the reduction stage.3.3 Relevant microbial species and functional genes.

(Fig. 2a). The disparity suggests that selenate reduction to selenite was substrate-driven and possibly the rate-limiting step, while selenite reduction to elemental selenium was bio-electrochemically driven. The Faradaic Efficiency (FE) results align with this finding: addition of acetate increased the FE of selenate reduction from 41 % to 54 %, whereas for selenite reduction, acetate addition lowered the FE from 70 % to 46 % (Fig. 2b). This aligns with the previous finding that selenate reduction was a heterotrophic process catalyzed by *Pseudomonas* (Lusa et al., 2017), and acetate can serve as the electron donor to promote selenate reduction in BES (for instance, C: Se ratio of 2:1 was recommended to avoid carbon limitation (Zhang et al., 2022). In contrast, selenite reduction can occur through electrotrophic pathways without organic carbon (Nguyen et al., 2016). Under abiotic conditions, the element selenium yield was minimal (selenite ~1 % and selenate ~4 %), likely due to uncontrollable variations in experimental precision and non-biological processes such as adsorption by GAC.

Pseudomonas_A. stutzeri, Desulfovibrio desulfuricans and *Azospira oryzae*, species reported to use both selenium oxyanions (SeO_4^{2-} and SeO_3^{2-}) as terminal electron acceptors (Hunter, 2007; Ike et al., 2000; Kuroda et al., 2011; Tomei et al., 1995), were detected under all the conditions. The relative abundance of *Pseudomonas_A. stutzeri,* notably, reached 23 % in the selenite post-FCDI-BES condition (Fig. 3). In contrast, *Geobacter sulfurreducens, Shewanella putrefaciens* and *Pseudomonas fluorescens* and strain S44 of *Comamonas testosterone*, species reported to only use selenite (SeO_3^{2-}) as an electron acceptor (Belzile et al., 2006; Jiang et al., 2012; Nancharaiah and Lens, 2015; Pearce et al., 2009; Suzuki et al., 2014; Tan et al., 2018; Zheng et al., 2014) or more effective in reducing selenite compared to selenate (Pearce et al., 2009), were detected in lower abundances (<5 %) except for *Geobacter sulfurreducens* under the selenite post-acclimation condition, in which it reached 10 % (Fig. 3). This was likely a result of *Geobacter's* competitive advantage under favorable electrochemical conditions and selenite availability.

Based on the relative abundances, the genes related to selenium reduction in BES-FCDI likely included those encoding fumarate reductase (*frdA, frdB, frdD*), sulfite reductase (*cysD, cysH*), selenium reductase (*serA, serD)*, and multi-heme c-type cytochromes (*mtrA, omcB, omcS*) (Fig. 4). The first three were identified as the functional genes in selenium reduction (Krafft et al., 2000; Wang et al., 2022; Xu et al., 2023), while the last one was unique to the bioelectrochemical setup of this study. Unlike previous findings, genes encoding fumarate reductase

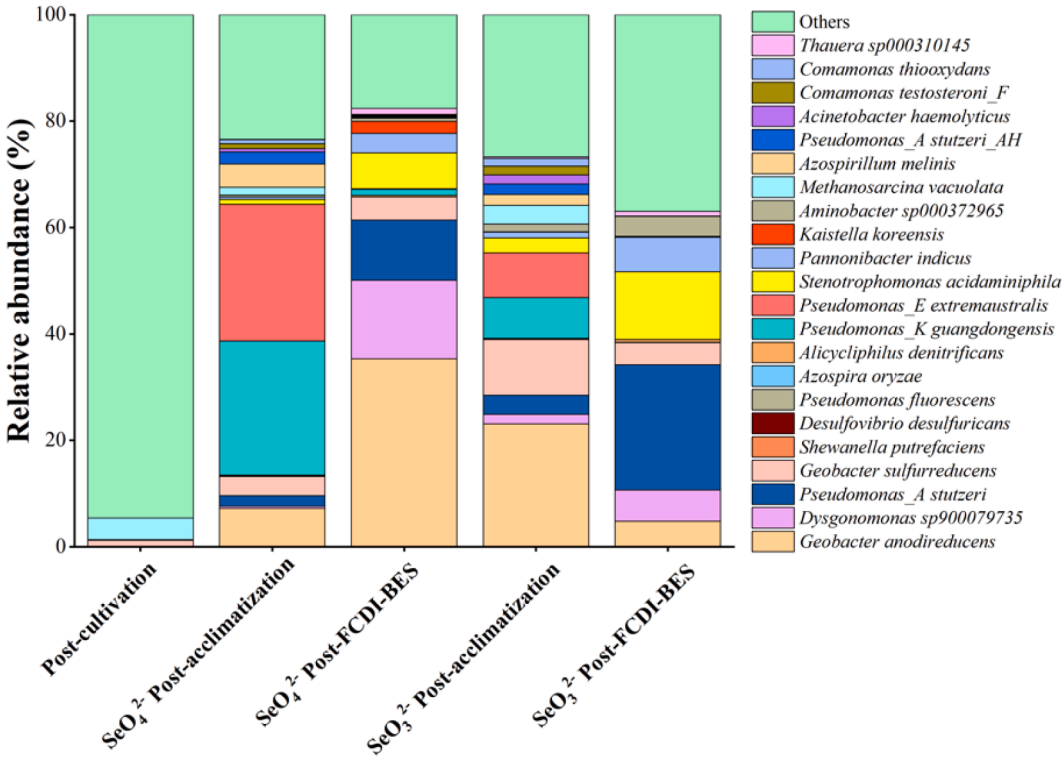

Fig. 3. Microbial community compositions of GAC biofilms during BES cultivation, BES acclimatization, and selenate and selenite reduction.

(*fccA, frdC*), sulfite reductase (*SrrABC*), selenium reductase (*srdBCA)* and nitrite reductase (*nirK)* showed limited abundances and likely did not contribute to selenium reduction in the electrochemical environment used in this study.

Comparing different species, we find that the functional genes in two of the three known selenium-reducing species, namely *Geobacter sulfurreducens* and *Pseudomonas stutzeri*, were present across all the conditions (Fig. 4), suggesting their activity under all the conditions. Notably, under selenite-reducing conditions, the genes encoding sulfite reductase and selenium reductase saw a significant increase in the three species from the last group (Fig. 4). These species, *Thauera aminoaromatica, Alishewanella aestuarii*, and *Alishewanella jeotgali,* though not previously linked explicitly to selenium reduction, are anaerobic and heterotrophic species capable of denitrification and electron transfer (Jung Jaejoon et al., 2012; Kadam et al., 2024; Kłodowska et al., 2018; Xia et al., 2016). The abundant functional genes may be indicative of their adaptation and potential involvement in selenium reduction in this study.

Comparing different conditions, we find that transitioning from the nutrient-rich acclimatization conditions to the nutrient-limited post-FCDI-BES conditions led to significant increases in the relative abundance of the genes encoding fumarate reductase and multi-heme c-type cytochromes in selenate reduction. In contrast, the same change in selenite reduction led to increases in the genes encoding pili, in addition to the previously noted increases in genes encoding sulfite reductase and selenium reduction (Fig. 4). This suggests that nutrient limitation may promote selenite reduction through bio-electrochemical pathways (Li et al., 2020; Lusa et al., 2017; Nguyen et al., 2016; Speers Allison M. and Reguera Gemma, 2012), which notably included *Geobacter*'s cytochrome *c*-mediated electron transfer, and *Pseudomonas's pilA*-mediated electron transfer (Holmes et al., 2016; Liu et al., 2019; Ueki, 2021).

3.3. Future work

This study primarily aimed to establish the proof of concept and probe the underlying biological mechanisms. Future research should explore a few key issues to improve FCDI-BES's viability for real-world applications:

Complicating factors in real-world matrices: For example, wastewater from flue gas desulfurization in coal-fired power plant may contain total dissolved solids (TDS), chloride, sulfate, and nitrates (Staicu et al., 2017) in addition to selenium. For FCDI, ion competition is a significant factor in electro-sorption (Hou and Huang, 2013; Li et al., 2016; Xu et al., 2008) and could lower the selenium removal efficiency. Studies reported that competing anions like SO_4^{2-} and Cl^- led to reductions in NO_3removal by 20 % and phosphate by 30 % (Bian et al., 2019). For BES, TDS, a form of TOC, could differentially impact selenite and selenate reduction. When selenate and selenite co-exist, selenite may be preferentially reduced due to its slightly lower reduction potential (Bouroushian, 2010; Saji and Lee, 2013; Zhang et al., 2018; Zou and Mauter, 2021) Microbial interactions and competition from electron acceptors such as nitrate, sulfate, and organic acids may also hinder selenate reduction (Logan and Rabaey, 2012; Rabaey and Verstraete, 2005; Zhang et al., 2018). While methanogenesis and sulfate typically do not interfere, ammonia was reported to reduce selenate reduction efficiency by up to 15 % (Zhang et al., 2018).

Long-term stability: Both biotic GAC and AEM/CEM demonstrated good long-term stability in our study. The biofilm on GAC was subjected to repeated 4-day operational cycles over the course of a year during the preliminary and main experiments. These cycles led to <15 % mass loss, primarily due to mechanical entrapment within tubing and membranes. This loss was effectively mitigated by the periodic incorporation of fresh GAC. Biotic GAC regained efficiency quickly with brief reintroduction to the cultivation conditions. Both AEM and CEM maintained functionality over the same period. Similar membranes in BES systems have shown lifespans of approximately 12,000 hours with minimal voltage degradation, and 4000 hours across 157 start-stop cycles (Hartnig and Schmidt, 2011; Li et al., 2021). Nevertheless, the long-term stability requires further evaluation on real-world water matrices, in which membrane fouling from organic compounds, microbes, or salts was noted as a significant challenge reducing efficiency and increasing resistance (Kokabian and Gude, 2015; San-Martín et al., 2019).

Intermittent carbon dosing: Our results suggest that autotrophic

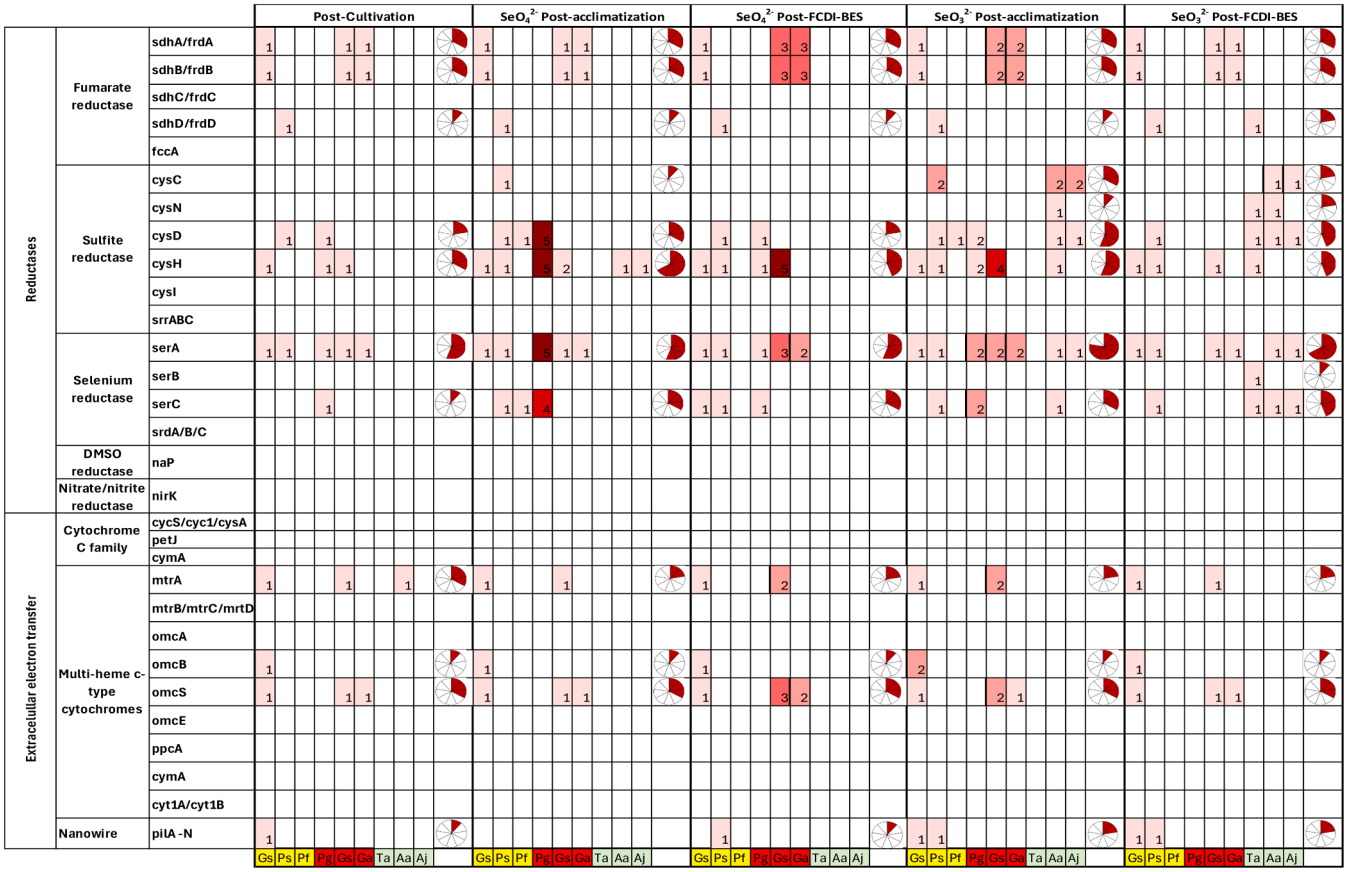

Fig. 4. Heatmap of functional genes related to reduction and extracellular electron transfer in microbial communities on GAC samples under different conditions. Four conditions are presented, namely post-cultivation, post-acclimatization, and post-FCDI-BES for selenate and selenite treatments. Under each condition, three groups of species are presented in columns: (1) known selenium-reducing species with functional genes (yellow), (2) most abundant species in this study but previously not explicitly implicated in selenium reduction (red), and (3) species with abundant functional genes but previously not explicitly implicated in selenium reduction (green). Values 1–5 are assigned based on relative abundance in counts per million.: (1: 0.1–10 cpm, 2: 10–20 cpm, 3: 20–30 cpm, 4: 30–40 cpm, 5: 40–55 cpm). Abbreviations: *Geobacter sulfurreducens* (Gs), *Pseudomonas_A stutzeri* (Ps), *Pseudomonas fluorescens* (Pf), *Pseudomonas guangdongensis* (Pg), *Geobacter soli* (Gso), *Geobacter anodireducens* (Ga), *Thauera aminoaromatica* (Ta), *Alishewanella aestuarii* (Aa), *Alishewanella jeotgali* (Aj).

selenite reduction by *Geobacter sulfurreducens* and heterotrophic selenate reduction by *Pseudomonas stutzeri* may be the key reactions in BES selenium reduction, which are enhanced and suppressed by the presence of organic carbon respectively. Alternating between carbon-rich and carbon-depleted cycles could benefit the overall selenium reduction. Further research is needed to confirm this approach.

Operational optimization: A few engineering parameters can be optimized in future work. For example, voltage is a critical parameter in both FCDI and BES. In FCDI, higher voltages can enhance the rate and extent of ion removal by improving ion migration and increasing the thickness of the electrical double layer. However, a higher voltage also increases the energy consumption (Bard and Faulkner, 2001; Ge et al., 2018; Ma et al., 2023; Qu et al., 2016).In BES, higher voltages could facilitate selenate reduction by providing energy to overcome the activation energy barrier (Hageman et al., 2013; Maslennikov et al., 1999) or adversely affect the metabolic pathways involved in the reduction process (Wang et al., 2021). Ionic strength or electrolyte concentration is another critical factor influencing cell ohmic resistance and overall system efficiency in electrochemical processes (Liang et al., 2017; Yang et al., 2021, 2016). In our study, the ionic strength of the electrolyte solution was over 90 % lower than that typically used in similar systems (Table 1). Increasing the ionic strength could enhance our system's efficiency by compressing the electrical double layer, thereby improving ion removal efficiency (Biesheuvel and van der Wal, 2010; Gloukhovski and Suss, 2020; Yang et al., 2021).

Microbial optimization: *Pseudomonas* and *Geobacter* were the most abundant genera in the microbial community, playing pivotal roles in selenate and selenite reduction. Fumarate redutase (*frdA, frdB, frdD*) and sulfite reductase (*cysD, cysH,cysC,cysN*) genes contributed to selenate reduction, while selenium reductase genes (*serA, serC*) showed increased abundance under selenite conditions. Tailoring nutrient availability and electrode potential could enhance the presence of these key genes and improve selenium reduction efficiency. Voltage optimization can further boost the abundance of EET genes such as *mtrA, omcB, omcS and pilA*. In *Geobacter*, these genes are associated with cytochrome *c*-mediated transfer, while in *Pseudomonas, pilA* is implicated in the formation of bacterial nanowires that facilitate long-range electron transport (Kim and Warshel, 2016; Patil et al., 2012).

Additionally, maintaining strict anaerobic conditions and sufficient selenium supply is essential for promoting the activity of *Geobacter* and *Pseudomonas* (Gonzalez-Gil et al., 2016; Semenec et al., 2020). Enriching other selenium-respiring species such as *Thauera selenatis*, which respires selenate via the SerABC system (Butler et al., 2012), could potentially complement *Geobacter* and *Pseudomonas* and improve the overall selenium biomineralization efficiency.

4. Conclusions

This study represents the first integration of flow electrode capacitive deionization and bio-electrochemical systems (FCDI-BES) in a single reactor for selenium treatment. The FCDI stage achieved high selenium removal rates (up to 76 %), while the BES stage effectively reduced Se-

oxyanions to elemental selenium (up to 66 %). The presence of acetate influenced the reduction pathways, favoring substrate-dependent selenate reduction and inhibiting bio-electrochemical-driven selenite reduction. Metagenomic analysis revealed the enrichment of selenium-respiring bacteria *Pseudomonas* and *Geobacter*, as well as a few species not previously linked to selenium respiration. To fully realize the potential of FCDI-BES for selenium removal, further optimization, including but not limited to understanding ion competition and matrix effects from real-world wastewater, evaluating long-term system stability, and optimizing engineering parameters, will be needed.

CRediT authorship contribution statement

Adriana Riveros: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Benhur K. Asefaw:** Writing – review & editing, Formal analysis. **Qingshi Wang:** Formal analysis, Data curation. **Tahir Maqbool:** Writing – review & editing. **Youneng Tang:** Writing – review & editing, Funding acquisition. **Daqian Jiang:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that there are no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122844.

Data availability

Data will be made available on request.

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