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Carbon Source Competition in Biological Selenate Reduction under Other Oxyanions Contamination

Hyun-Woo Kim [1], Seong Hwan Hong [2] and Hyeoksun Choi [3,*]

[1] Soil Environment Research Center, Department of Environment and Energy, Division of Civil, Environmental, Materials Resource, and Energy Engineering, Jeonbuk National University, Jeonju 54896, Korea; hyunwoo@jbnu.ac.kr
[2] Shinsung Engineering Co., Ltd., Seoul 06779, Korea; hmh5hot@naver.com
[3] Department of Civil and Environmental Engineering, Wonkwang University, Iksan 54538, Korea
* Correspondence: choihs@wku.ac.kr; Tel.: +82-63-850-6713

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Abstract: Selenate removal in drinking water is being vigorously debated due to the various health issues concerned. As a viable treatment option, this study investigated a fixed-bed biofilm reactor (FBBR) with internal recycling. The experimental design tested how hydraulic loading rate and electron donor affect selenate reduction together with other oxyanions. The tested accompanying oxyanions were nitrate and perchlorate and experiments were designed to test how an FBBR responded to the limited electron donor condition. The results showed that the reactor achieved almost complete selenate reduction with the initial hydraulic loading rate of 12 m^3/m^2/day (influent concentration of 1416 μg SeO_4^{2-}/L). Increasing the hydraulic loading rates to 16.24 and 48 m^3/m^2/day led to a gradual decline in selenate removal efficiency. A sufficient external carbon source (C:N of 3.3:1) achieved an almost complete reduction of nitrate as well as selenate. The FBBR acclimated to selenate instantaneously and reduced nitrate via synergistic denitrification. An experiment with another oxyanion addition, perchlorate (459 μg ClO_4^-/L), revealed that perchlorate-reducing bacteria were more strongly associated with carbon limitation than selenate-reducing bacteria, which can help us to understand parallel reactions in FBBRs. This research provides a framework to further study the use of electron donor-controlled FBBRs for simultaneous reduction of selenate and other oxyanions threatening the drinking water-related environment and public health.

Keywords: biological reduction; selenate; nitrate; perchlorate; fixed-bed biofilm reactor

1. Introduction

Selenium (Se) is an essential element for human and animal nutrition but exposure to an excess amount of Se is toxic to living organisms and can cause adverse health effects in humans, such as hair loss, nail discoloration, damage to the kidney, liver congestion and problems with the nervous and circulatory systems [1,2]. The US Environmental Protection Agency set the maximum concentration limit (MCL) of total Se as 50 μg/L and national regulations set the MCL of Se in primary drinking water as <5 μg/L [3].

Selenium is released into the environment from both natural and anthropogenic sources. Soils, rocks, natural water, atmosphere and so on are the natural sources while the anthropogenic sources are photocells, semiconductor pigments, pesticides and metal processing industries [4,5].

Although inorganic selenium may show a wide range of oxidation states, from +6 to −2, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are abundant in surface water [5,6]. One important source of Se in water may be agricultural irrigation or drainage and thus high concentrations of nitrate and sulfate may be cocontaminants. Many countries, including the US, have given great attention to these

multiple contaminants because of their adverse impact on and toxicity with regard to the ecosystem [7]. Wastewaters generated from mining are another important source of selenate contamination in surface or groundwater that can be a potential source of drinking water from a water recycle viewpoint [8].

Although various physicochemical methods, such as membranes, ion exchange, reducing agents, metal powders, green rusts, etc., may be applicable to selenate reduction, the existence of other oxyanions, problematic byproducts generation, slow reaction kinetics, low pH, etc., make the process costly [9,10]. Meanwhile, selenate can be biologically reduced to insoluble elemental Se (Se^0) in an economical way under an anaerobic environment [6,9]. Although low concentration makes the biological reduction slow due to microbial kinetics, microorganisms eventually utilize such electron acceptors in anaerobic heterotrophic respiration, autotrophic hydrogen oxidation and so on. If other oxyanions, such as nitrate and sulfate, are abundant, nitrate-reducing and sulfate-reducing microorganisms can compete for biodegradable dissolved organic carbon (DOC) with selenate-reducing bacteria for carbon and energy sources. This makes the overall reaction limiting and bacterial growth slow.

Previous research demonstrated simultaneous bioreduction of selenate and nitrate in sequencing batch reactors cultivating acclimated selenate-reducing bacteria [11]. To reduce selenate at very low concentrations, a fixed-bed biofilm reactor (FBBR) might be more suitable from the engineering point of view than a suspended growth reactor because it is designed to prevent microbial washout while keeping the biocatalysts, i.e., selenate reducing bacteria, in the system longer. Moreover, the potential of biofilm reactors can be extended via a long acclimation period [12], which can simultaneously reduce selenate and other anions, such as nitrate and perchlorate, even in diluted agricultural or coal-mining drainage [13]. However, such simultaneous reductions make it difficult to rationalize how the microbial acclimation affects selenate reduction with other oxyanions and how the carbon and energy source limitations alter the reduction of selenate and other oxyanions in the biofilm reactor. Guided by experimental design, this study further verifies the microbial competition for carbon between oxyanion-reducing bacteria in the biofilm reactor. This research, therefore, set out to gain mechanistic insight into the anaerobic heterotrophic respiration of microorganisms for reducing the adverse environmental effects of oxyanions on the ecosystem.

2. Materials and Methods

2.1. Experimental Set-Up of the FBBR

Figure 1 presents a schematic of the FBBR used in this study. The reactor was constructed using an acrylic column (40 cm length and 8 cm internal diameter) body with an empty bed volume of 2 L. The flow pattern and internal recycling in the reactor are represented by arrows. High porosity plastic rings, whose specifications were a specific gravity of 0.9 kg/m^3 and a specific surface area of 361 m^2/m^3, were packed to make the media-bed depth 40 cm. A rigid acrylic screen was located above 2.0 cm from the bottom to provide a mixing zone for homogeneous distribution of influent. A bolted flange with a rubber O-ring, located at the top of the column, minimized oxygen diffusion into the reactor.

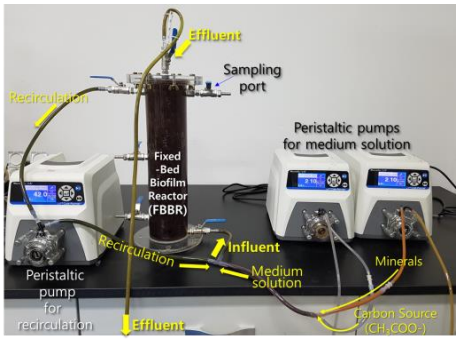


Figure 1. Schematic diagram on a picture of the fixed-bed biofilm reactor (FBBR) system.

2.2. Inoculum and Culture Media

As a seed sludge, activated sludge was obtained from a local municipal wastewater treatment plant of I-city, South Korea. To prepare the inoculum of the FBBR, selenate reducing bacteria was selectively precultured before inoculation in a bench-scale sequencing batch reactor (SBR), following previous studies [11]. Under the anoxic condition, the acclimation period was maintained for more than a month. Using selenate as a sole electron acceptor, selective pressure was supplied, keeping the temperature at 30 °C [14]. The feed solution for acclimated inoculum contained SeO_4^{2-} (50 mg/L or 0.35 μM), CH_3COO^- (200 mg/L or 3.39 μM), $(NH_4)_2SO_4$ (46 mg/L or 0.35 μM), K_2HPO_4 (13.7 mg/L or 0.08 μM), $NaHCO_3$ (84 mg/L or 1.0 μM), $MgSO_4{\cdot}7H_2O$ (51.3 mg/L or 0.21 μM), $CaSO_4{\cdot}2H_2O$ (43 mg/L or 0.25 μM) and $FeSO_4{\cdot}7H_2O$ (2.5 mg/L or 0.009 μM). CH_3COO^- was used as a sole source of organic carbon. We monitored SeO_4^{2-} concentration as an indicator of the acclimation.

Synthetic groundwater, culture media, containing selenate was prepared using distilled water, Na_2SeO_4, CH_3COOH, $(NH_4)_2SO_4$ and K_2HPO_4 following the experimental design. To supply alkalinity of 100 mg/L as $CaCO_3$, $NaHCO_3$ was added. The groundwater also contained 5 mg/L of Mg^{2+}, 10 mg/L of Ca^{2+} and 0.5 mg/L of Fe^{2+} by adding 51.3 mg/L $MgSO_4{\cdot}7H_2O$, 43.0 mg/L $CaSO_4{\cdot}2H_2O$ and 2.5 mg/L $FeSO_4{\cdot}7H_2O$ as inorganic macronutrients. It was presumed that other trace minerals required for biofilm growth were sufficient due to the inoculation step bringing such micronutrients.

2.3. Operating Conditions of Continuous FBBR Experiments for Oxyanions and Carbon Source Experiment

Table 1 summarizes the experimental design of this study. After the acclimation period, the continuous operation of the FBBR was conducted, keeping the substrate conditions of each phase as shown in Table 1. Samples were collected every day and stored at 4 °C before chemical analyses.

Table 1. Experimental design to test the effects of various oxyanions and carbon source conditions on biological selenate reduction. EBCT, empty bed contact time.

Phase	Flowrate (L/d)	EBCT (h)	Hydraulic Surface Loading Rate ($m^3/m^2/d$)	SeO_4^{2-} (μg/L)	NO_3^- (mg/L)	ClO_4^- (μg/L)	CH_3COO^- (mg/L)	SeO_4^{2-} Loading Rate ($g/m^3/d$)	NO_3^- Loading Rate ($g/m^3/d$)	ClO_4^- Loading Rate ($g/m^3/d$)
1	6	8	12	1470 [a]	0	0	10 [d]	4.3	0	0
2	8	6	16	1470 [a]	0	0	10 [d]	5.3	0	0
3	12	4	24	1470 [a]	0	0	10 [d]	8.5	0	0
4	24	2	48	1470 [a]	0	0	10 [d]	18.1	0	0
5	6	8	12	1470 [a]	62 [b]	0	120 [e]	5.8	192	0
6	6	8	12	1470 [a]	62 [b]	0	50 [f]	3.8	178.5	0
7	6	8	12	1470 [a]	0	440 [c]	50 [f]	3.8	0	1.4
8	6	8	12	1470 [a]	0	440 [c]	10 [d]	5.0	0	1.3

[a] ≅ 10 μM; [b] ≅ 1.0 μM; [c] ≅ 4.4 μM; [d] ≅ 0.17 μM; [e] ≅ 2.0 μM; [f] ≅ 0.85 μM.

Continuous FBBR experiments consisted of eight phases. Keeping influent SeO_4^{2-} as 1470 mg/L, the initial four phases tested the effect of the selenate loading rate on the selenate reduction. Phases 5 to 6 verified the impact of organic carbon concentration on the simultaneous selenate and nitrate reduction. The concentration of CH_3COO^- was determined based on the theoretical C:N mass ratio (1.13:1) of biological denitrification using acetate [15]. Then, phases 7 and 8 tested how organic carbon limitation affected the simultaneous selenate and perchlorate reduction. The change of loading was controlled by varying the flowrate from 6 to 24 L/d during phases 1 to 4. The corresponding average empty bed contact time (EBCT) ranged between 8 and 2 h. After finding the flowrate to be in the optimal condition during the initial phases, the contents of the electron acceptors (NO_3^- or ClO_4^-) or the sole electron donor (CH_3COO^-) were varied for phases 5 to 8. The recirculation was kept constant at one-tenth of the influent flowrate throughout the experiment to maintain a relatively homogeneous reaction in the FBBR [16].

2.4. Analytical Methods

Influent and effluent samples were taken every day from the sampling ports of the FBBR and instantaneously filtered using a 0.2 μm syringe filter kept in a refrigerator at 4 °C before physical and chemical analyses. All anions were monitored using an ion chromatograph (Dionex ICS-1100, Sunnyvale, CA, USA). Selenate was determined using 36.5 mM NaOH eluent and a 100 μL sample loop with an IonPac AS15 analytical column and AG15 guard column. Perchlorate was measured using 50 mM NaOH eluent and a 1000 μL sample loop with an IonPac AS16 analytical column and AG16 guard column. For nitrate and acetate concentrations, 9 mM Na_2CO_3 eluent was prepared and a 25 μL sample loop was used with an IonPac AS9-HC analytical column and AG9-HC guard column. The detection limits of selenate and perchlorate were both 5 μg/L while those of acetate and nitrate were both 0.5 mg/L. A total organic carbon analyzer (TOC-L, Shimadzu, Japan) determined the DOC to quantify remaining electron donors for bacterial growth.

3. Results and Discussion

3.1. Selenate Reduction in a Continuous FBBR Operation

Figure 2 shows the dynamics of effluent selenate in the FBBR during phases 1 to 4. Table 2 summarizes the average FBBR performance for effluent selenate concentration and selenate reduction efficiency according to the selenate loading rate and EBCT during phases 1 to 4. Although the influent selenate concentration fluctuated due to the man-made influent solution as well as complex pump linings, relatively high selenate concentration compared to that found in the literature was maintained at 1470 μg/L on average with a standard deviation of 223 μg/L.

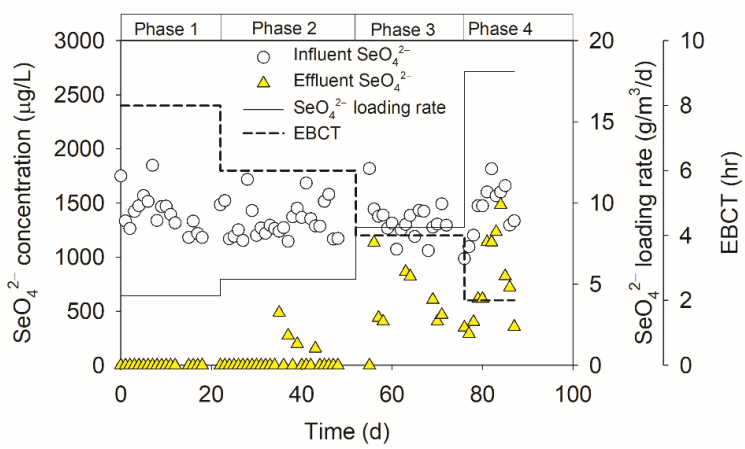


Figure 2. Variation of effluent selenate concentration according to the selenate loading rate and EBCT during phases 1 to 4.

Table 2. Effluent selenate concentration and selenate removal efficiency obtained in an FBBR according to the selenate loading rate.

Division	Operating Conditions of FBBR			
Phase	Phase 1	Phase 2	Phase 3	Phase 4
EBCT (hours)	8	6	4	2
Hydraulic loading rate ($m^3/m^2/d$)	12	16	24	48
Selenate loading rate ($g/m^3/d$)	4.3	5.3	8.5	18.1
Influent selenate (μg/L)	1470 ± 223			
Effluent selenate (μg/L)	BDL [a]	41	245	356
Selenate removal efficiency (%)	99.9	97.2	83.3	75.8

[a] Below detection limit.

At phase 1, almost complete selenate reduction was observed under the selenate loading of 4.3 g/m^3/d, thus all the determination was recorded to be below the detection limit (BDL). This indicates that the acclimated biofilm successfully reduced most of the selenate as an electron acceptor to form elemental selenate [15] and the reduction must have initiated instantaneously right after the start of continuous operation. When the selenate loading rate was increased to 5.3 g/m^3/d, the FBBR did not show a significant change in the effluent selenate. Despite the selenate detection of ~486 µg/L in the middle of phase 2, it eventually decreased to BDL afterward.

This result demonstrates that the FBBR could effectively withstand the selenate loading rate despite shorter EBCT (6 h), recording a selenate removal efficiency of 97.2% on average (Table 2). When the selenate loading was further increased to 8.5 g/m^3/d at phase 3 and 18.1 g/m^3/d at phase 4, the effluent selenate concentration drastically increased up to ~1134 µg/L (phase 3) and ~1484 µg/L (phase 4), though it demonstrated a decreasing trend down to around 400 µg/L at the end of phases 3 and 4, respectively. Average removal efficiencies of phase 3 and phase 4 were recorded as 83.3% and 75.8% despite the 4 h and 2 h EBCTs, respectively (Table 2).

3.2. *Effect of Organic Carbon on the Simultaneous Selenate and Nitrate Reduction*

After phase 4, the FBBR returned to the operating condition of phase 1, which showed the best stable performance, until the recovery of the selenate reduction performance. Then, the operation was readjusted to phase 5 after the performance recovery. Following the experimental design, the acetate concentration was increased to 120 mg/L, making the C:N mass ratio of influent to be 3.3:1 (=4.1:1 as molar ratio), with a nitrate concentration of 62 mg/L. Phase 6 used a decreased acetate concentration of 50 mg/L resulting in a C:N ratio of 1.3:1 (=1.7:1 as molar ratio). Since the C:N mass ratio of 2:1 has been reported to be sufficient for denitrification [17], this study compared the performance of an FBBR under substrate sufficient (phase 5) and limitation (phase 6) conditions (Table 1).

At phase 5, the FBBR experienced sufficient carbon and energy conditions to enable better bacterial growth, though the existence of nitrate may have led to sharing of electron donors between selenate-reducing and nitrate-reducing bacteria. Moreover, some microorganisms may reduce both selenate and nitrate simultaneously [18]. Figure 3 presents the variations of selenate and nitrate in the FBBR. Enough of the carbon source in the influent may contribute to almost complete reduction of selenate and nitrate. Although the influent selenate concentration slightly fluctuated, sufficient electron donors made the effluent selenate concentration BDL. The recorded nitrate concentration was also as low as ~2 mg/L. Remaining effluent DOC (9.8~20.6 mg C/L) was clear evidence supporting the steady-state performance of the FBBR in selenate and nitrate reduction without limitation.

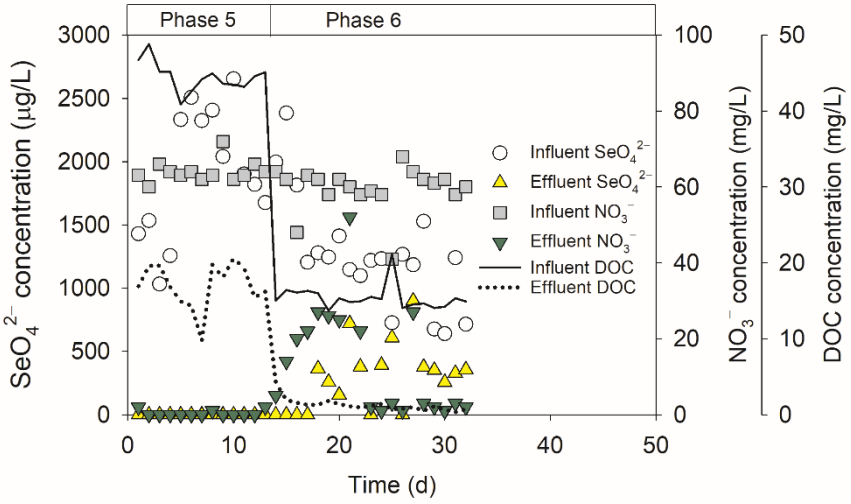


Figure 3. Effluent selenate and nitrate profiles in the FBBR reactor. The C:N mass ratio was controlled, changing from 3.3:1 (phase 5) to 1.3:1 (phase 6).

As soon as the influent acetate concentration was decreased to 50 mg/L (C:N mass ratio of 1.3:1) at phase 6, effluent nitrate concentrations sharply increased to 13.6 mg/L NO_3^-. Selenate reduction slowly responded to the shortage of electron donors by increasing between day 18 (287 μg/L SeO_4^{2-}) and day 27 (900 μg/L SeO_4^{2-}). After a significant fluctuation period over about 15 days, microorganisms presented acclimation keeping effluent concentration stable at 330 μg/L SeO_4^{2-}. Average removal efficiencies of nitrate (77.3%) and selenate (77.1%) drastically declined and fluctuated significantly while that of DOC increased to over 90%.

These results indicate that keeping enough electron donors was essential for the FBBR to conduct simultaneous nitrate and selenate reduction and that the nitrate reducing mechanism was more sensitive to carbon limitation.

3.3. *Effect of Organic Carbon on the Simultaneous Selenate and Perchlorate Reduction*

Right after the end of phase 6, the rehabilitation of the FBBR was conducted again under the phase 1 conditions. After confirming performance recovery, phase 7 started with another oxyanion addition of 440 μg/L ClO_4^- together with 1470 μg/L SeO_4^{2-} to investigate any different inhibitory or synergistic effects (Table 1). Phase 7 used 50 mg/L CH_3COO^- to achieve an excess supply of electron donors for the simultaneous perchlorate and selenate reduction, following previous research [19].

As illustrated in Figure 4, monitoring results showed almost complete reduction of both selenate and perchlorate at phase 7 with 20% DOC consumption under the 8 h of EBCT. Inevitable fluctuations in influent selenate and acetate concentration did not create any difference in their effluent concentration. The literature indicates that biological perchlorate reduction can be coupled with cocontaminants reduction. The results of this study reveal that biological reduction of both selenate and perchlorate is possible in an FBBR.

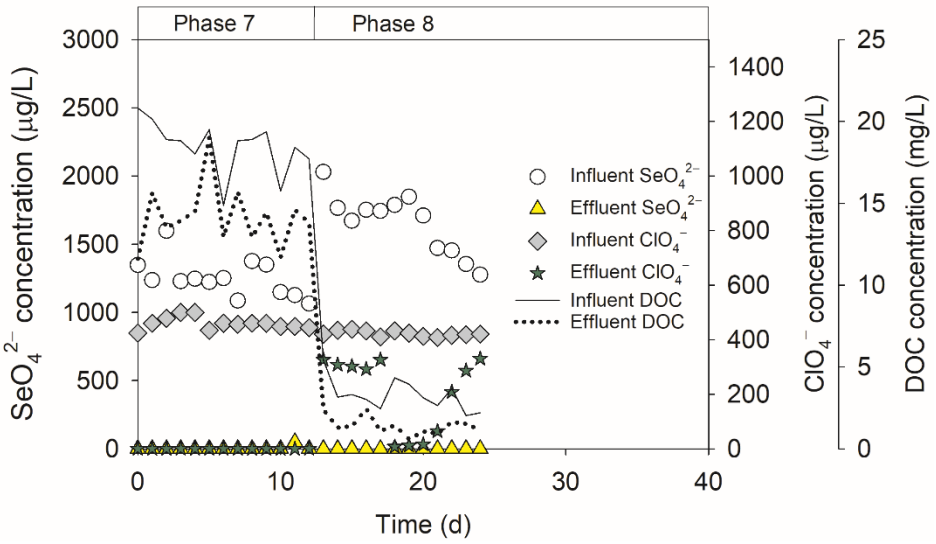


Figure 4. Effluent selenate and perchlorate profiles in the biofilm reactor when carbon substrate was limited.

When the influent acetate concentration was reduced to 10 mg/L CH_3COO^- (phase 8), it was observed that the DOC limitation clearly suppressed the perchlorate reduction mechanism and thus the effluent ClO_4^- increased to 310 μg/L ClO_4^-. On the contrary, selenate reduction demonstrated no metabolic inhibition and was recorded to be BDL throughout phase 8. Average perchlorate removal was as low as 51.1%.

The FBBR showed simultaneous selenate and perchlorate reduction with the excess DOC, which is relevant with regard to previous research [20]. However, only perchlorate reduction was significantly inhibited in the FBBR under DOC-limited conditions. This result demonstrates that the cessation

of perchlorate reduction in the presence of other oxyanions may be linked to the difference in electron donor affinity between heterotrophic selenate- and perchlorate-reducing bacteria [21,22]. Regarding electron donors, the reported high half-rate constants of perchlorate reducers suppressed the perchlorate-reducing mechanisms of the FBBR under carbon limitations. While perchlorate reduction was inhibited by the carbon limitation condition, selenate reduction progressed normally despite the presence of perchlorate and the limited carbon source due to relatively low half-rate constants [23]. In addition, perchlorate reduction may not be a less favorable pathway to yield energy for microorganisms since most perchlorate-reducing bacteria utilize inorganic electron acceptors in preference to perchlorate [24]. The obtained results support the notion that the carbon limitation may selectively harm the reduction mechanisms of perchlorate reducers in an FBBR during the concurrent reduction of other oxyanions.

4. Conclusions

This research investigated the effects of carbon source limitation on the reduction of anions competing for the same electron donor in an FBBR with internal recirculation. Based on the observed data, the following conclusions can be summarized as shown below:

(1) Selenate and nitrate were simultaneously reduced almost completely with excess carbon addition. However, both selenate and nitrate reductions were inhibited under carbon-limited conditions.
(2) An FBBR with internal recirculation can completely reduce selenate at 8 h EBCT (12 $m^3/m^2/d$). Decreasing EBCT reduced the selenate reduction efficiency to as low as 75.8%.
(3) An FBBR acclimated to selenate can instantaneously reduce nitrate. The concurrent reduction was possible under the excess DOC condition (C:N of 3.3:1); otherwise, the reduction of both anions was significantly inhibited.
(4) An FBBR acclimated to selenate and nitrate can conduct immediate reduction of perchlorate. Enough DOC enables the FBBR to achieve a complete reduction of both anions but low DOC conditions limit perchlorate reduction only while maintaining almost complete reduction of selenate.

Overall, with the control of electron donors, an FBBR with internal recirculation can be a viable option for the simultaneous reduction of low oxyanions harmful to the environment and to public health in sources of drinking water.

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