Supplementary material

Effect of elevated nitrate and sulfate concentrations on selenate removal by mesophilic anaerobic granular sludge bed reactors

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Method for FISH imaging

The microbial communities present in the sludge samples were qualitatively analyzed using fluorescence in situ hybridization (FISH). Vigorous biomass mixing within the reactor was done first by increasing the liquid upflow velocity to ensure a good representation of biomass sampling. All microbial cells were visualized by DAPI straining with specific oligonucleotide probes ARCH915 [1] and EUB I-III [2] for archaeal and bacterial cells, respectively. Two subgroups of the proteobacteria (beta-proteobacteria and gamma-proteobacteria) were included using BET42a and GAMMA42a probes. [3] Sulfate-reducing bacteria (SRB) were visualized using the *Desulfobulbaceae* DBB60 [1] and *Desulfovibrionales* SRB385 [4] probes. The inoculum anaerobic granular sludge was used as the control.

Table S1 Batch test results with standard deviation after 5 days incubation under different molar ratios of NO_3^- and SO_4^{2-} to SeO_4^{2-}.

Condition/molar ratio		Lactate		*NO_3^-		SO_4^{2-}		TDS	SeO_4^{2-}	
		Initial conc. (mM)	% Removal	Initial conc. (mM)	% Removal	Initial conc. (mM)	% Removal	Produced (mM)	Initial conc. (µM)	% Removal
Sterilized biomass		21±1	0±0	2±1	0±0	3±1	0±0	0.0±0.0	179±0	0±0
No lactate added		n/a***		2±0	0±0	3±0	0±0	0.0±0.0	163±1	0±0
NO_3^- only		12±1	100±0	4±0	100±0	n/a	n/a	n/a	n/a	n/a
SO_4^{2-} only		14±1	100±0	n/a	n/a	25±1	15±1	4.0±0.0	n/a	n/a
NO_3^- + SO_4^{2-}		14±0	100±0	4±1	100±0	24±0	6±2	3.0±0.0	n/a	n/a
SeO_4^{2-} only		25±4	83±0	n/a	n/a	n/a	n/a	n/a	108±0	67±2
**NO_3^- / SeO_4^{2-}	1	13±0	100±0	0.5±0.0	100±0	n/a	n/a	n/a	410±0	62±12
	20	17±2	85±3	2±0	100±0	n/a	n/a	n/a	96±0	100±0
	40	17±2	85±3	4±0	100±0	n/a	n/a	n/a	141±0	91±12
	70	19±2	90±1	7±0	100±0	n/a	n/a	n/a	117±0	81±10
	100	18±)	92±1	10±0	100±0	n/a	n/a	n/a	98±0	59±4
SO_4^{2-} / SeO_4^{2-}	1	13±0	91±2	n/a	n/a	0.5±0.0	63±9	0.3±0.2	410±0	71±4
	50	24±4	83±2	n/a	n/a	4±0	39±10	0.5±0.2	236±0	85±0
	100	24±5	92±0	n/a	n/a	9±1	28±1	1.0±0.2	237±0	79±1
	150	24±5	86±1	n/a	n/a	14±1	23±1	1.0±0.2	194±0	40±0
	200	24±3	92±1	n/a	n/a	20±1	25±7	0.9±0.3	235±0	52±0
	300	22±4	89±1	n/a	n/a	28±1	16±1	1.7±0.9	247±0	39±1
SO_4^{2-} / SeO_4^{2-} with co-exposure of 4 mM NO_3^-	1	13±0	92±1	0.5±0.0	100±0	0.5±0.0	27±4	0.1± 0.0	500±0	100±0
	50	25±3	86±0	3±1	100±0	4±0	30±2	0.3±0.1	201±0	92±1
	100	24±5	84±1	3±1	100±0	9±1	25±5	0.7±0.3	197±0	91±1
	150	24±4	86±0	3±1	100±0	14±1	25±6	0.6±0.2	217±0	61±2
	200	24±5	92±1	3±1	100±0	19±1	20±4	0.4±0.0	240±)	61±2
	300	24±5	92±1	3±1	100±0	31±1	19±3	0.5±0.1	221±0	32±0

*Note: results are all reported as mean ± standard deviation, n = 4 replicates (total); *NO_3^- concentration reached 100% removal for all concentration at 48 h; n/a – not applicable*

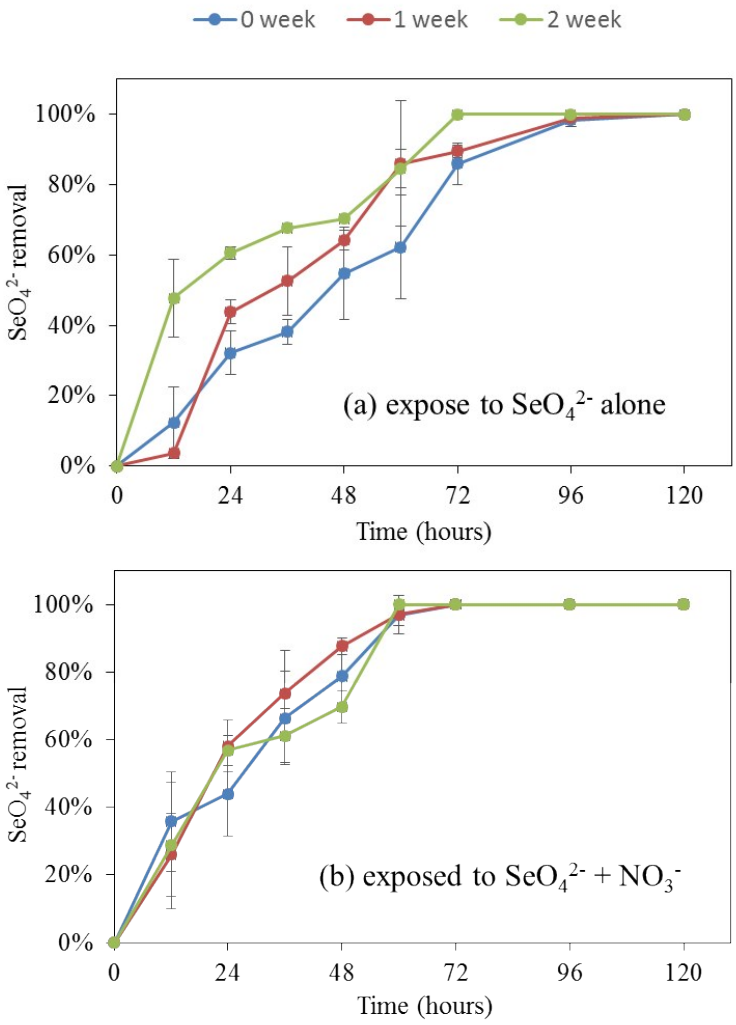
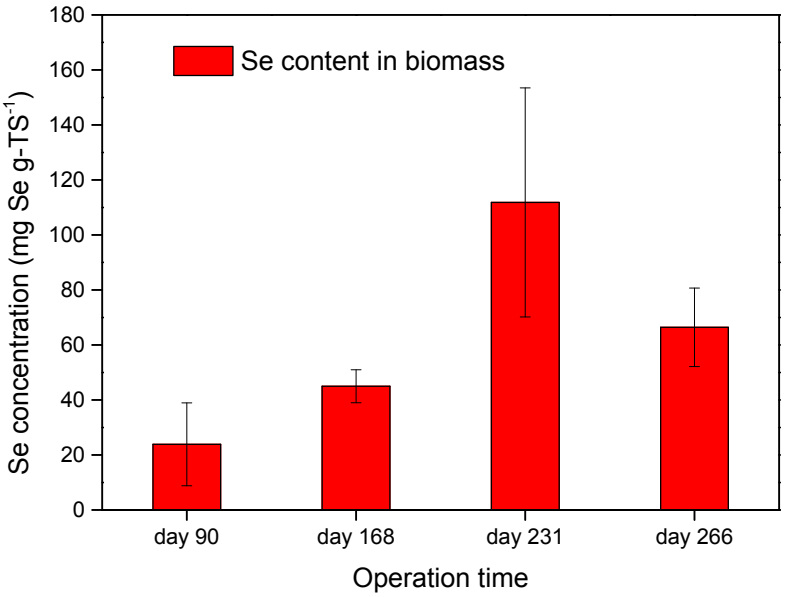


Fig. S1 SeO_4^{2-} reduction using untreated sludge and treated sludge (reused from the previous week) (a) expose only to SeO_4^{2-} and (b) expose to both NO_3^- and SeO_4^{2-}. Batch experiments were conducted under excess lactate (10mM), 30°C, 200 rpm and pH 7.0.



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38 **Fig. S2** Se concentration detected in the biomass at various operation times (n=3 biomass

39 sample replicate at each time period).

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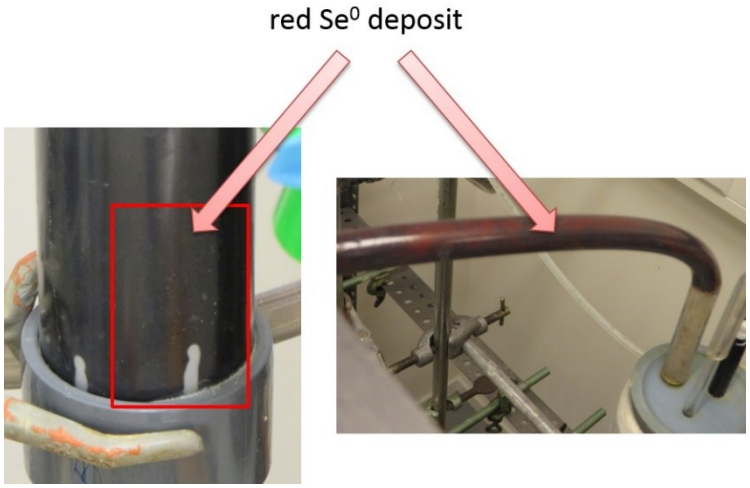

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43 **Fig. S3** Red-color formation in the reactor walls and tubings due to Se^0 production.

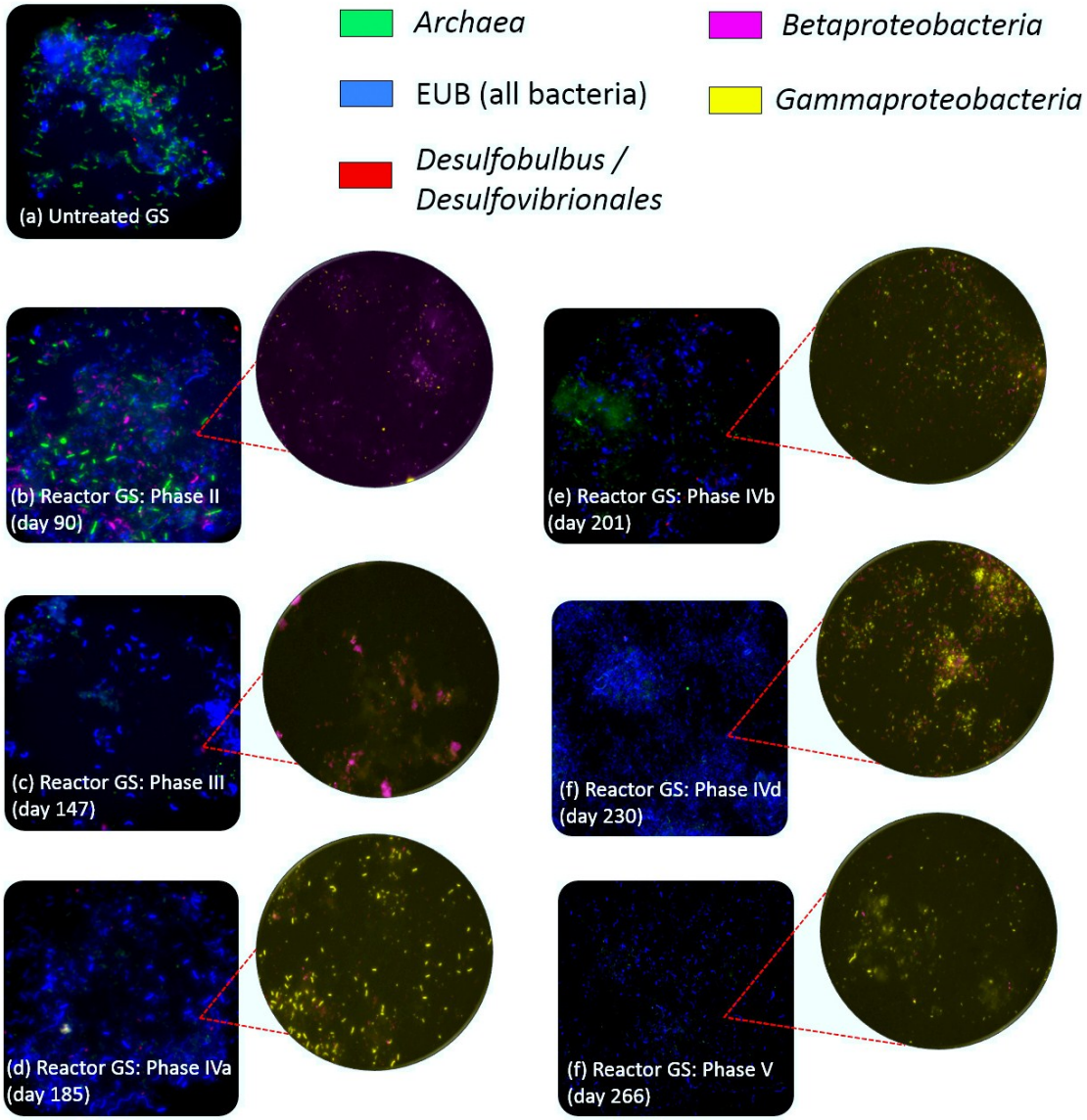

Fig. S4 Microbial community analysis of anaerobic granular sludge sampled from the laboratory-scale UASB reactor using *fluorescence in situ hybridization* (FISH) technique. FISH images are grouped into EUB-SRB-Archaea (box shape) and BETA-GAMMA (circle shape).

References

1 Y. Sekiguchi, Y. Kamagata, K. Nakamura, A. Ohashi and H. Harada, 1999, **65**, 1280–1288.

2 H. Daims, A. Brühl, R. Amann, K.-H. Schleifer and M. Wagner, *Syst. Appl. Microbiol.*, 1999, **22**, 434–444.

3 H. Lee, Y. Park, E. Choi and J. Lee, *J. Microbiol. Biotechnol.*, 2008, **18**, 1826–1835.

4 R. I. Amann, B. J. Binder, R. J. Olson, S. W. Chisholm, R. Devereux and D. A. Stahl, *Appl Env. Microbiol*, 1990, **56**, 1919–1925.