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Response of Sulfate-Reducing Bacteria and Supporting Microbial Community to Persulfate Exposure in a Continuous Flow System

Supplementary Information

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Table SI.1: Most-Probable-Number (MPN) for Sulfate-Reducing Bacteria

	Day 17	95% confidence intervals	Day 26	95% confidence intervals 2	Day 50	95% confidence intervals 3	Day 67	95% confidence intervals 4	Day 82	95% confidence intervals 5
PS 1	46	7.1	0	0	4300	700	15,000	3,000	93,000	15,000
		240		0		21,000		44,000		380,000
PS 2	46	7.1	0	0	430	70	930	150	23,000	4,000
		240		0		2,100		3,800		120,000
PS 3	28	10	0	0	4300	700	430	70	93,000	15,000
		150		0		21,000		2,100		380,000
ALK-PS 1	64	15	0	0	4300	700	15,000	3,000	43,000	7,000
		380		0		21,000		44,000		210,000
ALK-PS 2	24	3.6	10	1.5	2300	400	4,300	700	430,000	70,000
		130		38		12,000		13,000		2,100,000
ALK-PS 3	43	7	1	0.1	930	150	4,300	700	43,000	7,000
		210		2.3		3,800		13,000		210,000
ALK1	64	15	1	0.3	120000	30,000	2,300	400	21,000	3,500
		380		3.6		380,000		12,000		47,000
ALK2	64	15	2	0.3	930	150	750	140	23,000	4,000
		380		3.7		3,800		2,300		120,000
ALK3	75	14	1	0.1	2300	400	2,300	400	4,300	700
		230		3.6		12,000		12,000		21,000
CTRL 1	46	7.1	46	7.1	43	7	21	4	9,300	15,000
		240		240		210		47		38,000
CTRL 2	46	7.1	46	7.1	150	30	430	70	93	15
		240		240		440		2,100		380
CTRL 3	15	3	15	3	230	40	43	7	43,000	7,000
		44		44		1,200		210		210,000

Legend: PS= unactivated persulfate bioreactors; ALK-PS= alkaline-activated persulfate bioreactors; ALK= Alkaline-only treatment bioreactors; CTRL= Control bioreactors. Values shown represent SRB cells per milliliter. Treatment was applied on Day 23.

Table SI.2: Target Genes and Primers Used in Colony PCR and qPCR

Target Gene	Primers	5'-3' sequence	Amplicon size (bp)	Reference
dsrAB (PCR)	DSR1F DSR4R	AC[C/G]CACTGGAAGCACG GTGTAGCAGTTACCGCA	~1.9 kb	Wagner *et al.*, 1998
dsrB (qPCR/ SRB-DGGE)	DSRp2060F DSR4R	CAACATCGTYCAYACCCAGGG GTGTAGCAGTTACCGCA	~350 bp	Geets *et al.*,2006
V3 region of 16S rDNA (Universal-DGGE)	357F 518R	GC Clamp-CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	~270 bp	Ogino *et al.*, 2001

Detailed DGGE Protocol:

Both group-specific SRB and universal-based community profiles were generated using DGGE. Extracted DNA from bioreactor effluent samples was amplified using the SRB primers previously described (DSRpF2060 and DSR4R) along with an analysis using universal primers (e.g., 357F-GC and 518-R). Prior to gel separation, sample DNA was amplified using universal and SRB degenerate primers for universal DGGE and SRB-based DGGE, respectively. Universal DGGE used 50 µL reactions, SRB DGGE used 100 µL reactions. Universal DGGE required primer 357f (5'-CCTACGGGAGGCAGCAG-3), with GC-clamp at the 5' end, and 518r (5'-ATTACCGCGGCTGCTGG-3') (Sigma Aldrich; Oakville, ON, Canada). SRB-based DGGE used *dsrB* primers with the addition of a GC-clamp. The universal primer set anneals to the V3 region of 16S rDNA in bacterial genomes.[38] The PCR reaction contained 1 x Go-Taq™ Flexi buffer, 1.5 µM MgCl, 0.5 µM of forward and reverse primers, 200 µM dNTPs, 1.5 U Go-Taq™ flexi and 21.3 µL of Milli-Q (Millipore) water. Five (5) µL of template (sample) DNA was used in each reaction. Touchdown PCR was used to meet the conditions suggested by Muyzer *et al*.[39]. PCR protocol for DGGE preparation used an initial denaturation step of 94°C for 5 min, followed by 20 cycles of 94°C, 65°C, and 72°C for 1 min each and decreasing annealing temperatures (every 2 cycles) starting at 65°C and declining by 1°C to 56°C by the 20th cycle. The touchdown protocol was completed by 10 cycles of 94°C, 55°C, and 72°C for 1 min each. A final extension step was included for 7 min at 72°C. PCR products were then run on a 1.8% agarose gel and stained with ethidium bromide to ensure the proper gene segment was amplified. Once confirmed, the final PCR product(s) was used for DGGE analysis. SRB-based DGGE profile creation followed the procedure developed by Geets *et al*.[36] with slight modifications to the annealing temperature. PCR reactions contained a total volume of 100 µL. Each PCR mixture contained 1 µL template, 20 µL reaction buffer, 100 µM of dNTPs, 2.5 U of Go-Taq Flexi DNA polymerase and 1 µM of each primer. The cycling protocol included an initial denaturation step of 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53.8°C for 1 min, elongation at 72°C for 1 min and a final elongation step at 72°C for 10 min. PCR products were run as previously

described. Once the PCR products and ladder were loaded onto the denaturing gel, the protocol was run using a CBS apparatus system in accordance with the procedure as described in Green *et al.*[40]. As denaturing gels can contain different gradients depending on the size of fragment being run, universal DGGE used a 40-65% denaturing gradient run for 17 hours, and SRB-based DGGE used a 40-70% denaturing gradient with a 14-hour run time. Both profiles were generated using 8% acrylamide gels. Gels were stained for 1-2 hours in SYBR Gold and imaged. The banding pattern profiles were analyzed using a GelCompar II software system (Applied Maths Inc., Sint-Martens-Latem, Belgium). The microbial community profile was examined using SRB-based and universally-based primers for DGGE analysis. For each series of bioreactors, the results from two types of analyses are shown; specifically species diversity which uses the Shannon index (H') to measure community evenness and richness proportionally, and (2) community species richness. e.g.41

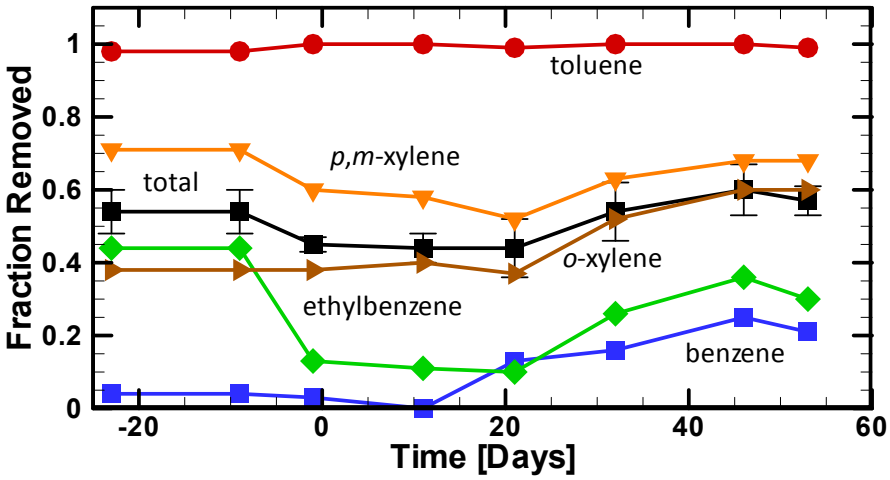

Figure SI.1. Average fraction of benzene, toluene, ethylbenzene, *p*,*m*-xylene, *o*-xylene and total BTEX removed from the three control (CTRL) bioreactors over the monitoring period.

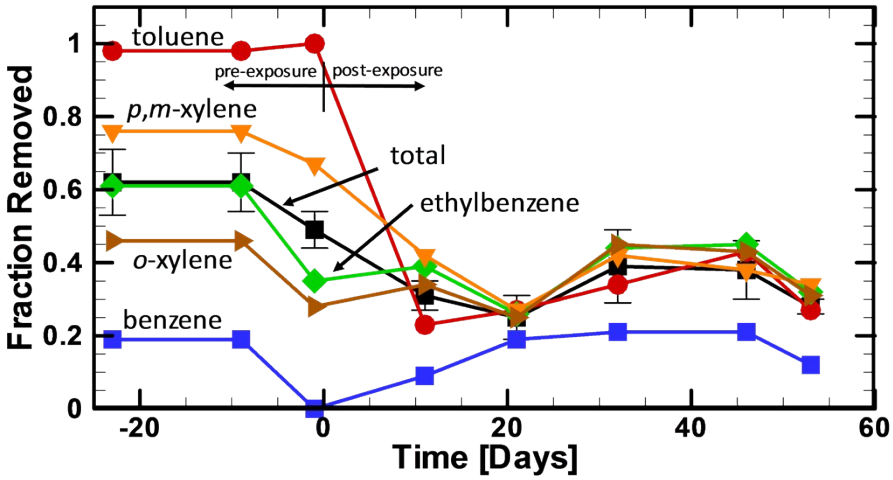

Figure SI.2. Average fraction of benzene, toluene, ethylbenzene, *p*,*m*-xylene, *o*-xylene and total BTEX removed from the three persulfate (PS) bioreactors over the monitoring period. The bioreactors where exposed to the persulfate solution (20 g/L) on Day 0.

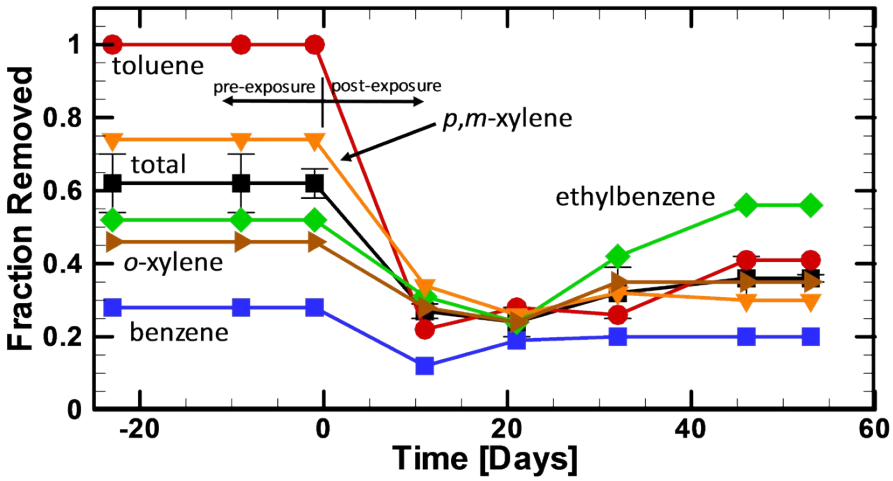

Figure SI.3. Average fraction of benzene, toluene, ethylbenzene, *p*,*m*-xylene, *o*-xylene and total BTEX removed from the three alkaline activated persulfate (ALK-PS) bioreactors over the monitoring period. The bioreactors were exposed to the alkaline activated persulfate solution (20 g/L; pH 12) on Day 0.

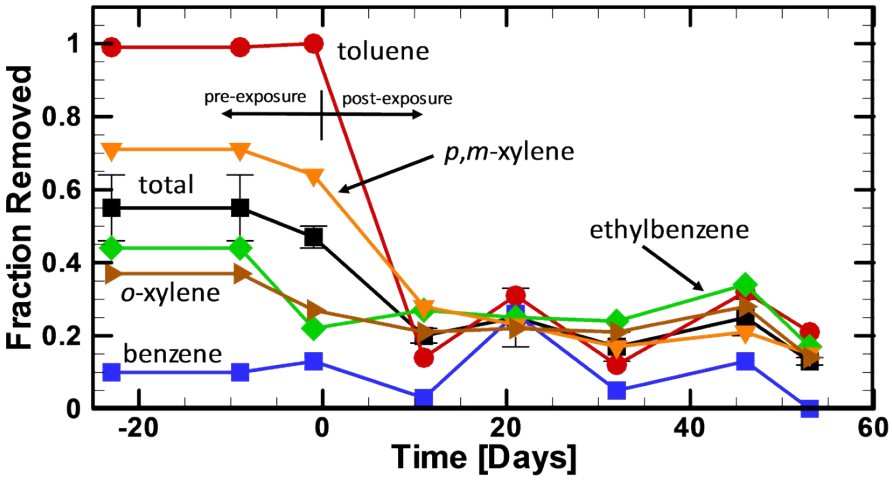

Figure SI.4. Average fraction of benzene, toluene, ethylbenzene, *p*,*m*-xylene, *o*-xylene and total BTEX removed from the three alkaline activated (ALK) bioreactors over the monitoring period. The bioreactors were exposed to the alkaline solution (pH 12) on Day 0.

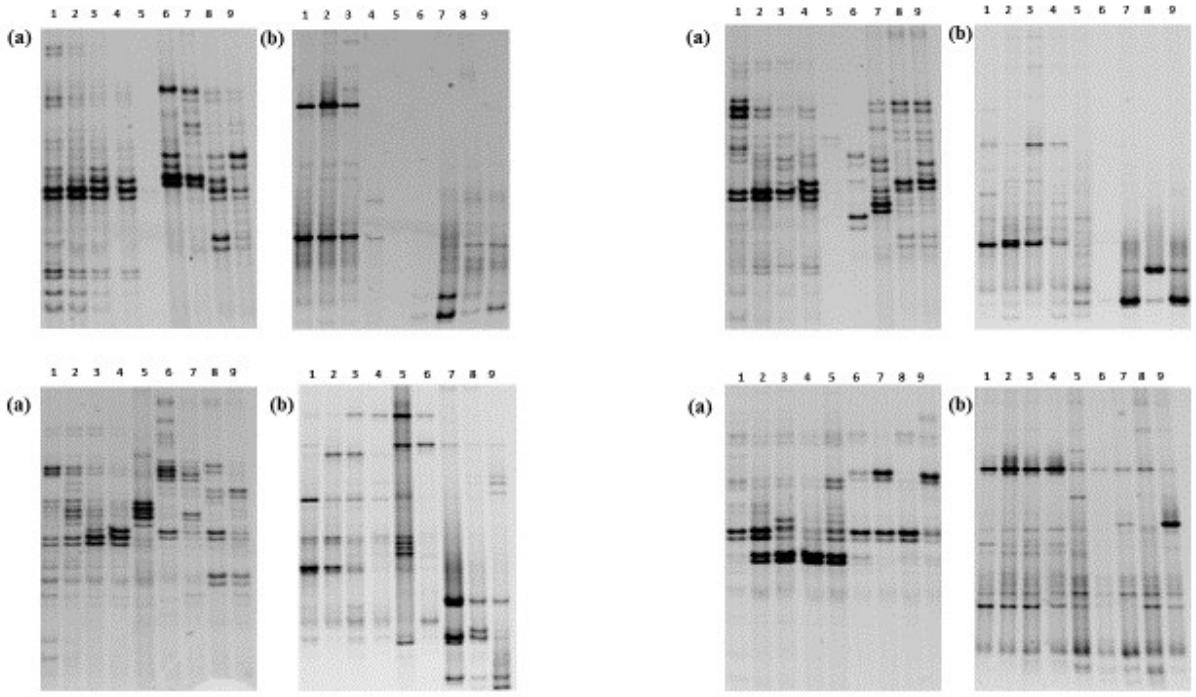

Figure SI.5. DGGE profiles observed before and after exposure (Top left: PS; Top Right: ALK-PS; Bottom Left: ALK; Bottom Right: CTRL) (a) Universal-based DGGE and (b) SRB-based DGGE. Lanes 1-3 represent pre-exposure in triplicate of the microbial community, and Lanes 4-9 represent triplicate samples collected on Day 11 and Day 21.