SUPPORTING INFORMATION

Screening Nonionic Surfactants for Enhanced Biodegradation of Polycyclic Aromatic Hydrocarbons Remaining in Soil After Conventional Biological Treatment

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SI contains:

25 pages

4 procedures

10 tables

13 figures

7 references

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Procedure for Measuring Surfactant Soil/water Partitioning and Pyrene Solubilization

Incubations of each surfactant with bioreactor-treated soil were prepared in duplicate for ten doses, including a no-surfactant dose. Incubations were prepared by adding 1-g dry weight bioreactor-treated soil to 15-mL glass crimp-top vials with PTFE-lined septa. Surfactant stock solution in phosphate buffer (pH 7.5) was added to give the desired surfactant doses. Additional phosphate buffer was added to give a final solids content of 15% (w/w). Additionally triplicate soil-free incubations of surfactant in buffer at known concentrations were prepared as a check on surfactant quantification. Incubation vials were purged with nitrogen and then put on a wrist-action shaker in the dark to allow sorption of the surfactant to the soil with minimal aerobic biodegradation of surfactant and PAHs. Because the experiment required a mass of bioreactor-treated soil larger than that produced by the bioreactor in a single week, incubations were set up over multiple weeks using separate batches of bioreactor-treated soil. Each batch was analyzed for pyrene concentration in triplicate. After two days, which was sufficient to reach apparent equilibrium liquid-phase surfactant and pyrene concentrations based on preliminary experiments (data not shown), incubation vials were centrifuged and the liquid phase was syringe-filtered through a 0.8-µm polycarbonate membrane. The surface tension of the filtrate was analyzed with a Du Nouy tensiometer (CSC Scientific Co., Inc. Fairfax, VA) after necessary dilutions were made to obtain a final surface tension corresponding to a concentration below the CMC. The concentration of surfactant in the filtrate was calculated using calibration curves of surface tension vs. the log of surfactant concentration. In preliminary experiments, the surface tension of supernatant from incubations of bioreactor-treated soil incubated with fresh surfactant-free buffer under nitrogen for 48 hours was the same as that of fresh soil-free buffer. Recoveries of surfactants from soil-free incubations for Brij 30, Span 20, EH-3, POESH, and R-95 were 112% ± 4%, 112% ± 11%, 92% ± 3%, 114% ± 15%, and 88% ± 2% respectively. Liquid-phase pyrene concentration for each dose was determined by diluting the liquid phase into acetonitrile and analyzing by HPLC as described

elsewhere.[1] The percent total pyrene mass in the liquid phase was calculated using the mass of pyrene in the liquid phase at day 2 divided by the mass of pyrene in the bioreactor-treated soil added at the time of incubation setup.

Procedure for PAH Desorption

The experiment required a mass of bioreactor-treated soil larger than that produced by the bioreactor in a single week. Therefore, incubations were set up over multiple weeks using separate batches of bioreactor-treated soil, each analyzed for PAH concentrations in triplicate. No-surfactant controls were prepared for each batch of bioreactor-treated soil. Incubations were prepared by adding 2-g dry weight bioreactor-treated soil to 30-mL glass centrifuge tubes with polytetrafluoroethylene (PTFE)-lined septa screw caps. Surfactant stock solution in phosphate buffer (pH 7.5) was added to give the desired surfactant dose. Additional phosphate buffer was added to give a final solids content of 15% (w/w). The headspace in each incubation tube was purged with nitrogen and the tubes were put on a rotary shaker at 275 RPM for 48 hours in the dark to allow sorption of the surfactant to the soil with minimal aerobic biodegradation of surfactant and PAHs.

After 48 hours of anaerobic incubation, an additional 10 mL of phosphate buffer and 0.1 g of Tenax beads contained in dialysis tubing knotted at both ends were added to each incubation. Prior to use, Tenax beads were cleaned by Soxhlet extraction in 50:50 acetone:hexane for 12 to 16 h, rinsed with methanol, and air dried. It was necessary to contain the Tenax in dialysis tubing because Tenax did not float in surfactant-containing incubations, which is how it would be recovered in conventional desorption experiments.[2] After adding the dialysis tubing to each incubation tube, the headspaces were purged with nitrogen again. The incubation tubes were then returned to the orbital shaker for a period of seven days. In previous work we found that the total fraction of PAHs taken up by freely suspended Tenax during a 7-day period in bioreactor treated slurry (without surfactant) was similar to the total fraction taken up by the Tenax in dialysis tubing.[3] We also found that for both freely suspended Tenax

and Tenax contained in dialysis tubing, the fraction desorbed reached an apparent maximum after seven days.

After seven days, the dialysis tubing was removed from each incubation tube and rinsed with deionized water to dislodge any soil adhering to the tubing. Each Tenax-containing dialysis tube was then slit and the Tenax along with the tubing was added to 15-mL glass vials with PTFE-lined septa and screw caps. The Tenax was extracted overnight with 10 mL methanol on an orbital shaker (275 RPM). The extracts were then vacuum-filtered through 0.2-µm nylon membrane filter and volumized to 25 mL with acetonitrile. Some volumized extracts were concentrated under a gentle stream of nitrogen to decrease the quantification limit for PAHs. Tenax solvent-extracts were then analyzed by HPLC to determine the masses of desorbed PAHs. The incubation tubes containing the higher dose for each surfactant were centrifuged and residual PAH concentrations in the post-desorption soil pellet measured. The sum of the residual mass of a given PAH in the soil pellet plus the mass sorbed to the Tenax was compared to the initial mass in the bioreactor-treated soil to calculate a recovery for each PAH in the higher-dose incubations (Table S8) .

There were several conditions for which the PAH mass desorbed in some replicates was below the method LLOQ. An average and standard deviation of mass desorbed for an individual PAH with at least two replicates above the LLOQ were calculated using Cohen's maximum likelihood estimator method.[4, 5] Individual PAHs with less than two replicates above the LLOQ for a condition are listed as <LLOQ (Cohen's method is not applicable to conditions with less than two uncensored observations).

Procedure for Extraction and Analysis of Feed Soil

To measure the concentrations of the 14 EPA priority-pollutant PAHs analyzed by HPLC as denoted in the footnotes to Table S2, 28-g wet-weight feed soil was slurried in bioreactor buffer to make a 15% (w/w) slurry. The slurry was passed through a 2-mm screen (comparable to the procedure for feeding the reactor), collected, and centrifuged at 3900 RPM for 20 minutes. Aliquots (3 g wet weight)

of centrifuged soil were transferred to each of six 30-mL glass centrifuge tubes with PTFE-lined septa with screwcaps. Three tubes were spiked with a known amount of anthracene-D_{10} as a recovery surrogate. Sodium sulfate (10 g) was mixed into each tube and the soil was extracted overnight twice (each time with 10-mL acetone and 10-mL dichloromethane) and analyzed for PAH concentrations by HPLC as described elsewhere.[1]

For the remaining PAHs, feed soil was extracted using the QuEChERS method. Briefly, 5-g wet-weight feed soil was added to each of three 50-mL centrifuge tubes. The soil was spiked with 100-µL 10-µg/mL recovery surrogates (naphthalene-D_8, acenaphthene-D_{10}, chrysense-D_{12}, and perylene-D_{12}) in acetonitrile and the tubes shaken vigorously for 3 min. Then 5 mL of water was added and shaken vigorously for 3 min, followed by the addition of 12 mL acetonitrile and QuEChERS AOAC salt packet (Agilent 5982-5755) and vigorous shaking for another 3 min. Tubes were then centrifuged for 3500 RPM for 10 min. A 5-mL aliquot of the supernatant was transferred to a 15-mL dispersive SPE tube (Agilent 5982-5158) and shaken for 3 minutes, centrifuged at 3500 RPM for 10 min and filtered through a 0.45µm PVDF syringe filter.

GC-MS Analysis Procedure

QuEChERS extracts of the feed soil and solvent-extracts from the followup biodegradation experiment (after filtering through sodium sulfate to remove residual water) were analyzed by GC-MS for additional PAHs and alkyl-PAHs as described elsewhere.[6]

Table S1. Properties of surfactants tested and doses used.

Surfactant	Alternative and structural names	HLB	CMC[a] (mg/L)	Dose (mg/g-dry soil)	
				Higher	Lower
Brij 30	Brij L4, Polyethylene glycol dodecyl ether, Polyoxyethylene (4) lauryl ether.	9.7	18	12	4
Span 20	Sorbitan monolaurate.	8.7	17	15	5
EH-3	2-ethyl hexanol ethylene oxide-propylene oxide copolymer.	7.9	680	60	20
POESH	Poly(ethylene glycol) sorbitol hexaoleate, Polyoxyethylene sorbitol hexaoleate.	10	260	24	8
R-95	3-((6-deoxy-2-O-(6-deoxy-alpha-L-mannopyranosyl)-alpha-L-mannopyranosyl)oxy)-Decanoic acid 1-(carboxymethyl)octyl ester.	10	38	9	3

[a] CMC measured in phosphate buffer.

Table S2. Concentrations of Priority PAHs in feed soil.[a]

EPA Priority PAH	**Abbreviation**	**Concentration (µg/g dry soil)**
Naphthalene	NAP	22.9 ± 3.0
Acenaphthylene	ACY	11.0 ± 0.5
Acenaphthene	ACE	9.81 ± 0.71
Fluorene	FLU	4.95 ± 0.67
Phenanthrene	PHN	99.4 ± 5.1
Anthracene	ANT	9.03 ± 0.83
Fluoranthene	FLA	67.1 ± 4.1
Pyrene	PYR	108 ± 6
Benz[*a*]anthracene	**BaA**	39.2 ± 3.6
Chrysene	**CHR**	61.9 ± 3.1
Benzo[*b*]fluoranthene	**BbF**	24.4 ± 1.8
Benzo[*k*]fluoranthene	**BkF**	12.0 ± 1.0
Benzo[*a*]pyrene	**BaP**	29.8 ± 2.7
Indeno[1,2,3-*cd*]pyrene	**IDP**	15.7 ± 0.9
Dibenz[*a,h*]anthracene	**DBA**	2.01 ± 0.21
Benzo[*g,h,i*]perylene	BgP	25.5 ± 2.2
Total EPA priority PAHs [b]	Σ 16 PAH	542 ± 11
Total carcinogenic PAHs [b]	Σ cPAH	185 ± 6

[a] Values represent means and standard deviations. The 14 PAHs measured by HPLC were NAP, ACE, FLU, PHN, ANT, FLA, PYR, BaA, CHR, BbF, BkF, BaP, DBA, and BgP. All other PAHs were measured by GC-MS. For feed soil only, values measured by HPLC are from 6 replicates and others are from triplicates. Bolded compounds are EPA probable human carcinogens. [b] Standard deviation of totals calculated by propagation of error.

Table S3. Concentrations of other PAHs in feed soil.[a]

Other PAH	Abbreviation	Concentration (μg/g dry soil)
2-Methylnaphthalene	2MNAP	7.30 ± 0.98
1-Methylnaphthalene	1MNAP	6.22 ± 0.79
Biphenyl	BIP	0.77 ± 0.08
2,6-Dimethylnaphthalene	DMNAP	7.29 ± 0.35
Dibenzofuran	DBF	0.64 ± 0.07
2,3,5-Trimethylnaphthalene	TMNAP	BDL
C1-Naphthalenes	C1-NAP	13.7 ± 1.8
C2-Naphthalenes	C2-NAP	33.5 ± 1.9
C3-Naphthalenes	C3-NAP	43.4 ± 1.4
C4-Naphthalenes	C4-NAP	16.9 ± 0.1
1-Methylfluorene	1MFLU	3.34 ± 0.19
C1-Fluorenes	C1-FLU	10.8 ± 1.2
C2-Fluorenes	C2-FLU	13.2 ± 0.9
Dibenzothiophene	DBT	0.45 ± 0.06
C1-Dibenzothiophene	C1-DBT	1.76 ± 0.04
C2-Dibenzothiophene	C2-DBT	2.30 ± 0.09
C3-Dibenzothiophene	C3-DBT	1.70 ± 0.17
1-Methylphenanthrene	1MPHN	122 ± 3
C1-Phenanthrenes/Anthracenes	C1-PHN/ANT	264 ± 1
C2-Phenanthrenes/Anthracenes	C2-PHN/ANT	181 ± 4
C3-Phenanthrenes/Anthracenes	C3-PHN/ANT	83.3 ± 2.2
C4-Phenanthrenes/Anthracenes	C4-PHN/ANT	11.2 ± 0.5
C1-Fluoranthenes/Pyrene	C1-FLA/PYR	53.5 ± 2.0
C2-Fluroanthrene/Pyrene	C2-FLA/PYR	21.4 ± 1.0
C3-Fluoranthrene/Pyrene	C3-FLA/PYR	12.1 ± 0.3
Retene	RET	148 ± 4
C1-Chrysenes	C1-CHR	33.1 ± 0.9
C2-Chrysenes	C2-CHR	11.0 ± 0.6
C3-Chrysenes	C3-CHR	3.27 ± 0.17
C4-Chrysenes	C4-CHR	0.98 ± 0.04
Benzo[*e*]pyrene	BeP	18.2 ± 0.9
Perylene	PER	4.99 ± 0.33
Coronene	COR	3.18 ± 0.14

[a] "BDL" indicates below detection limit. Other notes as in Table S2.

Table S4. Concentrations of PAHs in the bioreactor-treated soil samples used in the preliminary biodegradation and followup (geno)toxicity experiments.[a]

PAH	Preliminary biodegradation experiments (µg/g)					Follow-up (geno)toxicity experiments (µg/g)
	Brij 30	Span 20	EH-3	POESH	R-95	
NAP	22.3 ± 2.1	18.7 ± 2.1	22.7 ± 1.0	19.8 ± 2.9	19.2 ± 0.4	22.4 ± 0.9
ACE	3.4 ± 0.3	1.5 ± 0.2	2.9 ± 0.3	1.4 ± 0.4	1.9 ± 0.4	2.1 ± 0.3
FLU	2.0 ± 0.2	1.5 ± 0.1	2.3 ± 0.2	1.9 ± 0.3	1.7 ± 0.2	2.3 ± 0.7
PHN	30.5 ± 3.5	24.1 ± 2.2	35.0 ± 3.5	27.2 ± 6.0	27.6 ± 2.7	33.5 ± 5.0
ANT	3.1 ± 0.2	2.6 ± 0.2	3.6 ± 0.3	3.0 ± 0.4	2.8 ± 0.2	3.6 ± 0.9
FLA	18.3 ± 1.3	15.9 ± 1.1	21.3 ± 1.6	16.5 ± 2.1	17.1 ± 0.8	23.1 ± 3.0
PYR	31.7 ± 2.1	26.4 ± 2.3	34.9 ± 2.1	29.7 ± 4.1	29.3 ± 0.9	35.7 ± 4.2
BaA	15.6 ± 1.3	15.1 ± 1.5	18.1 ± 1.4	14.1 ± 1.4	15.7 ± 0.6	18.4 ± 2.0
CHR	24.5 ± 1.6	15.9 ± 2.3	28.9 ± 1.3	22.3 ± 2.7	25.8 ± 0.9	20.4 ± 2.3
BbF	13.4 ± 0.6	11.6 ± 0.8	14.8 ± 0.8	12.0 ± 1.5	13.2 ± 0.5	13.8 ± 0.7
BkF	6.7 ± 0.4	6.1 ± 0.3	6.6 ± 0.1	5.6 ± 0.3	6.3 ± 0.2	7.1 ± 0.3
BaP	18.7 ± 1.4	15.7 ± 1.1	20.7 ± 1.4	15.4 ± 1.7	17.5 ± 0.8	18.6 ± 1.4
DBA	1.4 ± 0.1	1.1 ± 0.2	1.4 ± 0.3	1.2 ± 0.2	1.2 ± 0.1	1.3 ± 0.2
BgP	24.7 ± 1.8	24.0 ± 3.6	26.4 ± 2.7	21.3 ± 2.8	24.0 ± 1.3	24.2 ± 1.7
Total PAH	216 ± 13	180 ± 12	239 ± 13	191 ± 24	203 ± 6	227 ± 19

[a] Data represent means and standard deviations of six replicates. Abbreviations are defined in Table S2.

Table S5. PAH mass desorbed for incubations with Brij 30 and initial concentration of PAHs in bioreactor-treated soil used to prepare desorption incubations.

PAH	Bioreactor-treated soil (µg/g)	Mass Desorbed (%)		
		No Surfactant	Brij 30 lower	Brij 30 higher
NAP	20.6 ± 0.1	0.27 ± 0.27[a]	2.57 ± 0.61*	2.95 ± 0.40*
ACE	1.9 ± 0.3	1.38 ± 1.06[a]	34.5 ± 6.1*	52.3 ± 9.8*
FLU	1.8 ± 0.2	0.25 ± 0.17[a]	13.0 ± 1.5*	19.2 ± 7.9*
PHN	37.3 ± 0.3	0.11 ± 0.03	3.27 ± 0.31*	9.60 ± 6.47*
ANT	3.7 ± 0.2	0.09 ± 0.04[a]	5.46 ± 0.44*	8.08 ± 1.84*
FLA	22.1 ± 0.3	0.15 ± 0.09	4.54 ± 0.12*	5.24 ± 0.43*
PYR	37.5 ± 0.8	0.15 ± 0.08	3.81 ± 0.18*	4.41 ± 0.42*
BaA	12.7 ± 0.2	0.17 ± 0.13	1.48 ± 0.11*	1.79 ± 0.37*
CHR	16.9 ± 0.8	0.13 ± 0.10	1.15 ± 0.13*	1.30 ± 0.17*
BbF	12.0 ± 0.1	0.09 ± 0.08[a]	0.26 ± 0.14	0.37 ± 0.16*
BkF	7.5 ± 0.1	0.11 ± 0.10	0.22 ± 0.12	0.30 ± 0.14
BaP	23.0 ± 0.5	0.10 ± 0.09	0.19 ± 0.07	0.25 ± 0.13
DBA	2.2 ± 0.1	<0.20	<0.40	<0.40
BgP	25.3 ± 1.0	0.07 ± 0.13[a]	0.05 ± 0.05[a]	0.10 ± 0.07
Total PAH[b]	225 ± 2	0.14 ± 0.04	2.56 ± 0.09*	4.11 ± 1.08*

[a] Cohen's Maximum Likelihood Estimate was used to account for one or more replicates below the lower limit of quantification (LLOQ). "Less than" values are below LLOQ. [b] Total PAH percent mass desorbed does not include mass of individual PAH below the LLOQ. "Lower" and "higher" refer to surfactant doses shown in Table S1. An asterisk indicates a significant difference (α=0.05) in mass desorbed (µg) between a treatment and no-surfactant control. Abbreviations are defined in Table S2.

Table S6. PAH mass desorbed for incubations with Span 20 and POESH and concentration of PAHs in bioreactor-treated soil used to prepare desorption incubations.[a]

PAH	Bioreactor-treated soil (µg/g)	Mass Desorbed (%)				
		No surfactant	Span 20 lower	Span 20 higher	POESH lower	POESH higher
NAP	22.4 ± 0.9	0.32 ± 0.06	1.21 ± 0.42*	1.36 ± 0.14*	0.90 ± 0.42*	1.26 ± 0.11*
ACE	3.2 ± 0.2	1.05 ± 0.23	4.28 ± 1.91*	9.68 ± 1.60*	3.01 ± 2.14[a]	8.49 ± 1.66*
FLU	2.5 ± 0.1	0.21 ± 0.08	1.63 ± 0.71*	3.54 ± 0.51*	0.89 ± 0.82[a]	2.71 ± 0.35*
PHN	40.2 ± 1.7	0.10 ± 0.03	0.45 ± 0.37	0.67 ± 0.09*	0.41 ± 0.33	0.85 ± 0.17*
ANT	4.0 ± 0.3	0.08 ± 0.02	0.30 ± 0.33[a]	0.62 ± 0.13*	0.31 ± 0.31[a]	0.83 ± 0.18*
FLA	24.0 ± 1.1	0.12 ± 0.03	0.88 ± 0.41*	2.43 ± 0.34*	0.51 ± 0.31*	1.77 ± 0.31*
PYR	40.0 ± 2.4	0.12 ± 0.02	0.68 ± 0.34*	1.50 ± 0.12*	0.31 ± 0.19	1.10 ± 0.31*
BaA	19.5 ± 1.3	0.06 ± 0.01	0.27 ± 0.08*	0.58 ± 0.09*	0.18 ± 0.05*	0.46 ± 0.08*
CHR	23.6 ± 0.9	0.07 ± 0.06	0.18 ± 0.12	0.47 ± 0.09*	0.10 ± 0.06[a]	0.36 ± 0.06*
BbF	14.6 ± 1.5	0.04 ± 0.00	<0.12	<0.12	<0.12	0.11 ± 0.02*
BkF	7.9 ± 0.4	0.03 ± 0.00	0.09 ± 0.02*	0.14 ± 0.03*	0.05 ± 0.01*	0.08 ± 0.01*
BaP	20.1 ± 1.4	0.03 ± 0.01	0.10 ± 0.02*	0.14 ± 0.02*	0.07 ± 0.03*	0.09 ± 0.01*
DBA	1.5 ± 0.2	<0.15	<0.60	<0.60	<0.60	<0.60
BgP	24.6 ± 1.7	0.02 ± 0.00	<0.04	<0.04	<0.04	<0.04
Total PAH[b]	248 ± 8	0.10 ± 0.01	0.46 ± 0.09*	0.90 ± 0.05*	0.33 ± 0.08*	0.84 ± 0.07*

[a] Notes as in Table S5.

Table S7. PAH mass desorbed for incubations with R-95 and EH-3 and concentration of PAHs in bioreactor-treated soil used to prepare desorption incubations.[a]

PAH	Bioreactor-treated soil (µg/g)	Mass Desorbed (%)				
		No Surfactant	R-95 lower	R-95 higher	EH-3 lower	EH-3 higher
NAP	28.4 ± 3.3	0.17 ± 0.03[a]	0.26 ± 0.10	0.55 ± 0.22*	1.25 ± 0.16*	2.40 ± 1.10*
ACE	2.7 ± 0.4	<0.83	1.69 ± 1.08	4.33 ± 1.38	30.0 ± 9.3	21.6 ± 5.1
FLU	2.7 ± 0.2	<0.16	<0.16	1.05 ± 0.54	6.66 ± 2.76	11.5 ± 2.9
PHN	47.9 ± 1.3	0.04 ± 0.02	0.07 ± 0.00	0.15 ± 0.11	1.32 ± 0.34*	3.40 ± 1.92*
ANT	4.8 ± 0.5	<0.05	0.07 ± 0.01	0.20 ± 0.09	5.97 ± 0.76	4.81 ± 0.98
FLA	29.6 ± 2.6	0.04 ± 0.01	0.13 ± 0.08	0.48 ± 0.25*	3.69 ± 1.80*	1.82 ± 0.24*
PYR	54.9 ± 5.0	0.04 ± 0.01	0.10 ± 0.05	0.29 ± 0.14*	2.93 ± 1.39*	1.28 ± 0.23*
BaA	24.7 ± 2.2	0.03 ± 0.01	0.04 ± 0.02	0.15 ± 0.06*	2.06 ± 2.48	0.43 ± 0.04*
CHR	38.1 ± 4.6	<0.02	<0.02	0.10 ± 0.03	1.55 ± 1.91	0.33 ± 0.05
BbF	18.6 ± 2.6	<0.05	<0.02	0.06 ± 0.04[a]	0.89 ± 1.37	0.11 ± 0.05[a]
BkF	9.1 ± 0.4	0.02 ± 0.01[a]	0.02 ± 0.01	0.06 ± 0.02*	0.78 ± 1.23	0.07 ± 0.01*
BaP	27.4 ± 2.0	0.02 ± 0.01[a]	0.03 ± 0.00	0.05 ± 0.02	0.69 ± 1.08	0.07 ± 0.01*
DBA	1.8 ± 0.2	<0.25	<0.25	<0.25	<0.50	<0.50
BgP	32.1 ± 2.7	<0.01	<0.01	<0.01	<0.02	<0.02
Total PAH[b]	323 ± 26	0.03 ± 0.00	0.08 ± 0.02	0.24 ± 0.05*	2.01 ± 0.46*	1.53 ± 0.33*

[a] Notes as in Table S5.

Table S8. PAH mass recovered from higher-dose surfactant desorption incubations.

	Mass Recovered (%)[a]				
PAH	Brij 30	Span 20	EH-3	POESH	R-95
NAP	89.8 ± 2.4	83.5 ± 7.7	85.0 ± 12.9	84.1 ± 12.7	84.1 ± 12.7
ACE	107 ± 21	77.7 ± 12.6	84.5 ± 15.5	83.3 ± 12.1	72.1 ± 22.9
FLU	98.3 ± 16.2	76.9 ± 13.8	104 ± 17	82.0 ± 9.2	83.0 ± 10.7
PHN	95.1 ± 12.2	68.1 ± 11.7	68.3 ± 18.0	73.9 ± 11.8	67.9 ± 10.3
ANT	97.0 ± 6.2	64.9 ± 9.9	103 ± 20	69.6 ± 7.5	67.6 ± 11.8
FLA	105 ± 2	74.2 ± 9.4	106 ± 18	79.6 ± 6.3	62.5 ± 11.7
PYR	97.9 ± 2.6	65.7 ± 9.1	90.8 ± 9.6	69.3 ± 5.1	60.3 ± 11.8
BaA	107 ± 4	80.2 ± 10.8	101 ± 19	90.1 ± 6.1	66.5 ± 12.5
CHR	99.0 ± 6.3	70.6 ± 6.8	98.9 ± 15.0	73.3 ± 6.1	77.0 ± 18.1
BbF	102 ± 4	79.7 ± 13.4	104 ± 19	93.1 ± 9.7	88.2 ± 17.2
BkF	102 ± 3	80.2 ± 10.5	101 ± 7	90.4 ± 7.1	86.6 ± 9.2
BaP	98.9 ± 3.4	80.6 ± 11.0	96.5 ± 11.2	90.1 ± 9.6	83.4 ± 10.5
DBA	96.7 ± 8.1	84.9 ± 20.8	94.4 ± 13.2	91.8 ± 12.2	82.3 ± 17.7
BgP	98.4 ± 4.7	77.6 ± 12.9	94.5 ± 10.7	91.5 ± 14.3	84.1 ± 10.5

[a] Values represent means and standard deviations of triplicates.

Table S9. Concentrations of PAHs in followup biodegradation experiment.[a]

PAH	Concentration (µg/g)						
	Bioreactor treated	No surfactant	Brij 30 lower	Brij 30 higher	POESH lower	POESH higher	Span 20 higher
NAP	20.8 ± 1.3	17.3 ± 1.3	17.1 ± 1.3	17.2 ± 0.8	19.3 ± 0.8	18.1 ± 1.3	19.3 ± 1.2
ACY	12.3 ± 0.4	10.9 ± 1.0	10.4 ± 2.8	-	-	12.6 ± 0.5	12.7 ± 0.9
ACE	2.08 ± 0.31	1.48 ± 0.44	1.21 ± 0.23	1.30 ± 0.19	1.63 ± 0.34	1.25 ± 0.28	1.74 ± 0.36
FLU	1.64 ± 0.21	1.41 ± 0.22	1.14 ± 0.08	1.10 ± 0.12	1.52 ± 0.26	1.20 ± 0.14	1.54 ± 0.22
PHN	27.1 ± 2.4	20.5 ± 2.6	16.5 ± 2.1	14.1 ± 0.6	20.1 ± 1.9	16.4 ± 1.1	19.9 ± 2.1
ANT	2.97 ± 0.23	2.34 ± 0.24	1.93 ± 0.18	1.87 ± 0.09	2.33 ± 0.21	2.06 ± 0.35	2.45 ± 0.42
FLA	17.8 ± 0.8	13.1 ± 1.3	5.47 ± 0.86	9.46 ± 0.83	8.05 ± 0.81	4.97 ± 0.47	8.35 ± 1.87
PYR	30.2 ± 1.5	22.7 ± 2.0	9.12 ± 0.80	25.2 ± 1.9	13.4 ± 1.3	7.52 ± 0.61	12.7 ± 2.0
BaA	15.4 ± 0.9	12.1 ± 1.1	4.93 ± 0.43	13.3 ± 0.8	6.85 ± 0.77	3.84 ± 0.37	6.61 ± 1.18
CHR	24.4 ± 1.1	18.8 ± 1.8	7.02 ± 0.39	15.4 ± 0.7	9.76 ± 1.18	4.62 ± 0.52	8.59 ± 1.09
BbF	13.7 ± 1.0	11.4 ± 0.6	8.23 ± 0.29	12.8 ± 0.6	6.73 ± 0.59	5.65 ± 0.52	6.89 ± 0.80
BkF	6.63 ± 0.33	5.53 ± 0.33	3.63 ± 0.10	6.29 ± 0.25	3.32 ± 0.26	2.00 ± 0.23	3.15 ± 0.50
BaP	16.7 ± 0.9	13.9 ± 0.9	11.7 ± 0.1	15.4 ± 0.5	9.27 ± 0.90	8.21 ± 1.03	9.47 ± 1.24
IDP	15.0 ± 1.2	13.9 ± 0.7	13.0 ± 3.9	-	-	15.2 ± 1.1	15.5 ± 2.0
DBA	0.99 ± 0.12	0.81 ± 0.08	0.90 ± 0.11	1.05 ± 0.10	0.64 ± 0.22	0.72 ± 0.18	0.59 ± 0.13
BgP	23.0 ± 0.6	21.4 ± 1.7	20.9 ± 0.5	20.8 ± 1.1	22.1 ± 1.1	21.1 ± 1.2	23.2 ± 1.2
Σ 16 PAH[b]	231 ± 4	188 ± 5	133 ± 6	-	-	125 ± 3	153 ± 5
Σ cPAH[b]	92.8 ± 2.3	76.6 ± 2.5	49.4 ± 3.9	-	-	40.2 ± 1.7	50.8 ± 3.0
2MNAP	7.04 ± 0.50	6.10 ± 0.92	5.21 ± 1.39	-	-	6.41 ± 0.68	6.33 ± 0.54
1MNAP	4.17 ± 0.17	3.48 ± 0.52	2.83 ± 0.80	-	-	3.31 ± 0.31	3.51 ± 0.25
BIP	0.83 ± 0.03	0.70 ± 0.09	0.62 ± 0.16	-	-	0.72 ± 0.07	0.84 ± 0.06
DMNAP	2.61 ± 0.11	1.96 ± 0.17	1.56 ± 0.36	-	-	1.94 ± 0.13	4.38 ± 4.79
DBF	0.52 ± 0.04	0.43 ± 0.04	0.38 ± 0.11	-	-	0.46 ± 0.05	0.55 ± 0.06
TMNAP	1.38 ± 0.11	1.01 ± 0.08	0.54 ± 0.08	-	-	0.68 ± 0.01	0.97 ± 0.19
C1-NAP	12.5 ± 0.6	10.4 ± 1.3	8.89 ± 2.44	-	-	10.6 ± 0.9	11.1 ± 0.6

Table S9. (continued)	Bioreactor treated	No surfactant	Brij 30 lower	Brij 30 higher	POESH lower	POESH higher	Span 20 higher
C2-NAP	13.5 ± 0.7	10.4 ± 1.4	8.10 ± 2.00	-	-	9.68 ± 0.47	11.4 ± 1.4
C3-NAP	10.7 ± 0.6	7.47 ± 0.93	5.08 ± 1.09	-	-	5.79 ± 0.29	7.39 ± 1.34
C4-NAP	4.57 ± 0.22	3.18 ± 0.40	1.57 ± 0.34	-	-	1.72 ± 0.12	2.33 ± 0.38
1MFLU	1.05 ± 0.04	0.75 ± 0.11	0.53 ± 0.11	-	-	0.62 ± 0.03	0.77 ± 0.09
C1-FLU	4.48 ± 0.28	2.71 ± 1.26	2.96 ± 0.71	-	-	3.35 ± 0.20	3.91 ± 0.36
C2-FLU	5.77 ± 0.44	5.01 ± 0.49	3.10 ± 0.81	-	-	3.79 ± 0.27	4.12 ± 0.34
DBT	BDL	BDL	BDL	-	-	BDL	BDL
C1-DBT	0.70 ± 0.04	0.44 ± 0.26	0.18 ± 0.18	-	-	0.16 ± 0.21	0.49 ± 0.04
C2-DBT	BDL	BDL	BDL	-	-	BDL	BDL
C3-DBT	BDL	BDL	BDL	-	-	BDL	BDL
1MPHN	34.4 ± 1.6	22.4 ± 3.0	10.5 ± 1.8	-	-	11.5 ± 0.6	16.2 ± 2.5
C1-PHN/ANT	78.3 ± 4.9	53.7 ± 6.7	29.1 ± 6.3	-	-	32.5 ± 1.1	41.1 ± 5.3
C2-PHN/ANT	67.6 ± 3.9	47.1 ± 4.7	20.4 ± 4.5	-	-	20.7 ± 1.1	27.7 ± 3.2
C3-PHN/ANT	38.4 ± 1.5	28.5 ± 3.7	10.3 ± 2.4	-	-	9.13 ± 0.48	13.9 ± 1.7
C4-PHN/ANT	BDL	BDL	BDL	-	-	BDL	BDL
C1-FLA/PYR	28.4 ± 1.3	21.4 ± 2.1	10.4 ± 2.6	-	-	9.17 ± 0.75	13.0 ± 1.9
C2-FLA/PYR	14.6 ± 0.5	11.9 ± 1.5	8.10 ± 2.12	-	-	7.63 ± 0.47	8.57 ± 0.66
C3-FLA/PYR	8.77 ± 0.42	7.28 ± 0.85	5.24 ± 1.29	-	-	4.83 ± 0.17	5.31 ± 0.31
RET	74.8 ± 3.1	54.8 ± 5.9	16.5 ± 4.0	-	-	10.9 ± 0.7	20.5 ± 2.8
C1-CHR	16.5 ± 0.9	13.5 ± 1.1	5.44 ± 1.34	-	-	3.61 ± 0.44	6.56 ± 0.51
C2-CHR	8.94 ± 0.54	7.36 ± 0.57	4.56 ± 1.23	-	-	2.87 ± 0.37	3.84 ± 0.32
C3-CHR	5.27 ± 0.22	4.46 ± 0.49	3.58 ± 1.00	-	-	3.37 ± 0.36	3.14 ± 0.27
C4-CHR	BDL	BDL	BDL	-	-	BDL	BDL
BeP	10.7 ± 0.7	9.25 ± 0.66	5.09 ± 1.30	-	-	2.57 ± 0.31	4.82 ± 0.76
PER	4.35 ± 0.41	3.97 ± 0.31	3.51 ± 0.97	-	-	3.99 ± 0.31	4.07 ± 0.35
COR	3.99 ± 0.14	3.57 ± 0.22	3.22 ± 0.91	-	-	3.77 ± 0.29	3.79 ± 0.68

[a] Values represent means and standard deviations of five replicates. “BDL” indicates below detection limit. “-“ indicates not determined. Abbreviations defined in Tables S2-S3. Other notes as in Table S2.

Table S10. Overall removals of PAHs in treatments relative to feed soil.[a]

PAH	Removal relative to untreated feed soil (%)						
	Bioreactor alone	No Surfactant	Brij 30 lower	Brij 30 higher	POESH lower	POESH higher	Span 20 higher
NAP	9.26 ± 13.2	24.6 ± 11.3	25.3 ± 11.2	24.8 ± 10.4	15.4 ± 11.5	20.9 ± 11.7	15.6 ± 12.2
ACY	-11.2 ± 6.1	1.19 ± 9.72	5.50 ± 25.3	-	-	-13.9 ± 6.6	-15.3 ± 9.7
ACE	78.8 ± 3.6	84.9 ± 4.7	87.7 ± 2.5	86.8 ± 2.2	83.4 ± 3.7	87.3 ± 3.0	82.3 ± 3.8
FLU	66.9 ± 6.2	71.6 ± 5.9	76.9 ± 3.5	77.7 ± 3.9	69.3 ± 6.7	75.7 ± 4.3	68.9 ± 6.2
PHN	72.7 ± 2.8	79.4 ± 2.8	83.4 ± 2.2	85.8 ± 1.0	79.8 ± 2.2	83.5 ± 1.4	80.0 ± 2.3
ANT	67.1 ± 3.9	74.1 ± 3.6	78.6 ± 2.8	79.4 ± 2.1	74.3 ± 3.3	77.1 ± 4.4	72.9 ± 5.3
FLA	73.5 ± 2.0	80.5 ± 2.2	91.8 ± 1.4	85.9 ± 1.5	88.0 ± 1.4	92.6 ± 0.8	87.6 ± 2.9
PYR	71.9 ± 2.0	78.9 ± 2.1	91.5 ± 0.9	76.6 ± 2.2	87.5 ± 1.3	93.0 ± 0.7	88.2 ± 2.0
BaA	60.8 ± 4.3	69.0 ± 4.0	87.4 ± 1.6	66.1 ± 3.7	82.5 ± 2.5	90.2 ± 1.3	83.1 ± 3.4
CHR	60.6 ± 2.7	69.6 ± 3.3	88.7 ± 0.9	75.1 ± 1.7	84.2 ± 2.1	92.5 ± 0.9	86.1 ± 1.9
BbF	43.8 ± 5.8	53.2 ± 4.4	66.3 ± 2.8	47.5 ± 4.7	72.4 ± 3.2	76.8 ± 2.7	71.7 ± 3.9
BkF	44.7 ± 5.3	53.9 ± 4.7	69.7 ± 2.6	47.5 ± 4.8	72.3 ± 3.1	83.3 ± 2.3	73.7 ± 4.7
BaP	43.9 ± 5.9	53.3 ± 5.1	60.7 ± 3.5	48.2 ± 4.9	68.9 ± 4.1	72.4 ± 4.3	68.2 ± 5.0
IDP	4.54 ± 9.44	11.4 ± 6.8	17.5 ± 25.0	-	-	3.47 ± 8.82	1.84 ± 13.99
DBA	50.7 ± 7.8	59.9 ± 6.0	55.2 ± 7.3	47.8 ± 7.3	68.3 ± 11.3	64.2 ± 9.6	70.8 ± 7.1
BgP	9.83 ± 8.03	16.2 ± 9.9	17.9 ± 7.2	18.2 ± 8.2	13.4 ± 8.5	17.2 ± 8.5	8.92 ± 9.11
Σ 16 PAH	57.5 ± 1.1	65.4 ± 1.2	75.4 ± 1.1	-	-	76.9 ± 0.7	71.8 ± 1.1
Σ cPAH	49.8 ± 2.0	58.6 ± 1.9	73.3 ± 2.3	-	-	78.2 ± 1.2	72.6 ± 1.9
2MNAP	3.54 ± 14.6	16.5 ± 16.9	28.6 ± 21.3	-	-	12.2 ± 15.0	13.3 ± 13.8
1MNAP	32.9 ± 8.9	44.1 ± 11.0	54.5 ± 14.1	-	-	46.9 ± 8.4	43.6 ± 8.2
BIP	-8.12 ± 11.4	8.95 ± 14.82	18.7 ± 22.3	-	-	5.70 ± 12.9	-8.94 ± 13.4
DMNAP	64.2 ± 2.3	73.2 ± 2.7	78.6 ± 5.1	-	-	73.3 ± 2.2	39.9 ± 65.7
DBF	18.4 ± 10.4	32.2 ± 9.7	40.0 ± 19.0	-	-	27.2 ± 11.2	13.5 ± 13.3
TMNAP	NC	NC	NC	-	-	NC	NC
C1-NAP	9.30 ± 12.7	24.4 ± 13.6	35.3 ± 19.7	-	-	22.9 ± 12.1	19.2 ± 11.4

Table S10. (continued)	Bioreactor alone	No Surfactant	Brij 30 lower	Brij 30 higher	POESH lower	POESH higher	Span 20 higher
C2-NAP	59.7 ± 3.1	68.9 ± 4.5	75.8 ± 6.1	-	-	71.1 ± 2.2	66.0 ± 4.6
C3-NAP	75.4 ± 1.6	82.8 ± 2.2	88.3 ± 2.5	-	-	86.6 ± 0.8	83.0 ± 3.1
C4-NAP	72.9 ± 1.3	81.2 ± 2.4	90.7 ± 2.0	-	-	89.8 ± 0.7	86.2 ± 2.2
1MFLU	68.5 ± 2.2	77.5 ± 3.4	84.1 ± 3.3	-	-	81.5 ± 1.5	76.8 ± 3.1
C1-FLU	58.4 ± 5.2	74.8 ± 12.0	72.5 ± 7.2	-	-	68.9 ± 3.8	63.8 ± 5.1
C2-FLU	56.3 ± 4.4	62.1 ± 4.5	76.6 ± 6.3	-	-	71.4 ± 2.8	68.9 ± 3.3
DBT	NC	NC	NC	-	-	NC	NC
C1-DBT	60.3 ± 2.6	75.2 ± 14.9	89.7 ± 10.2	-	-	91.1 ± 12.1	72.4 ± 2.1
C2-DBT	NC	NC	NC	-	-	NC	NC
C3-DBT	NC	NC	NC	-	-	NC	NC
1MPHN	71.8 ± 1.5	81.7 ± 2.5	91.4 ± 1.5	-	-	90.6 ± 0.5	86.7 ± 2.1
C1-PHN/ANT	70.3 ± 1.9	79.7 ± 2.5	89.0 ± 2.4	-	-	87.7 ± 0.4	84.4 ± 2.0
C2-PHN/ANT	62.6 ± 2.3	73.9 ± 2.7	88.7 ± 2.5	-	-	88.5 ± 0.7	84.6 ± 1.8
C3-PHN/ANT	53.9 ± 2.2	65.8 ± 4.5	87.7 ± 2.9	-	-	89.0 ± 0.6	83.3 ± 2.0
C4-PHN/ANT	NC	NC	NC	-	-	NC	NC
C1-FLA/PYR	47.0 ± 3.1	60.0 ± 4.2	80.6 ± 4.8	-	-	82.9 ± 1.5	75.8 ± 3.6
C2-FLA/PYR	31.6 ± 4.1	44.5 ± 7.5	62.1 ± 10.1	-	-	64.3 ± 2.8	59.9 ± 3.6
C3-FLA/PYR	27.7 ± 4.0	40.0 ± 7.1	56.8 ± 10.7	-	-	60.2 ± 1.7	56.2 ± 2.8
RET	49.6 ± 2.4	63.0 ± 4.1	88.9 ± 2.7	-	-	92.7 ± 0.5	86.1 ± 1.9
C1-CHR	50.2 ± 3.0	59.4 ± 3.6	83.6 ± 4.1	-	-	89.1 ± 1.4	80.2 ± 1.6
C2-CHR	18.7 ± 6.6	33.0 ± 6.3	58.5 ± 11.4	-	-	73.8 ± 3.7	65.1 ± 3.5
C3-CHR	-61.0 ± 10.7	-36.4 ± 16.7	-9.43 ± 31.0	-	-	-3.12 ± 12.2	4.13 ± 9.55
C4-CHR	NC	NC	NC	-	-	NC	NC
BeP	41.4 ± 4.9	49.2 ± 4.4	72.0 ± 7.3	-	-	85.9 ± 1.9	73.5 ± 4.4
PER	12.8 ± 10.0	20.3 ± 8.2	29.6 ± 20.1	-	-	20.0 ± 8.1	18.4 ± 8.8
COR	-25.5 ± 7.1	-12.3 ± 8.7	-1.33 ± 29.0	-	-	-18.6 ± 10.6	-19.4 ± 22.0

[a] Values represent means and standard deviations calculated through propagation of error. "-" indicates not determined. "NC" indicates removal not calculated because concentration in feed soil or concentration in treated soil was below the detection limit. Abbreviations are defined in Tables S2-S3. Other notes as in Table S2.

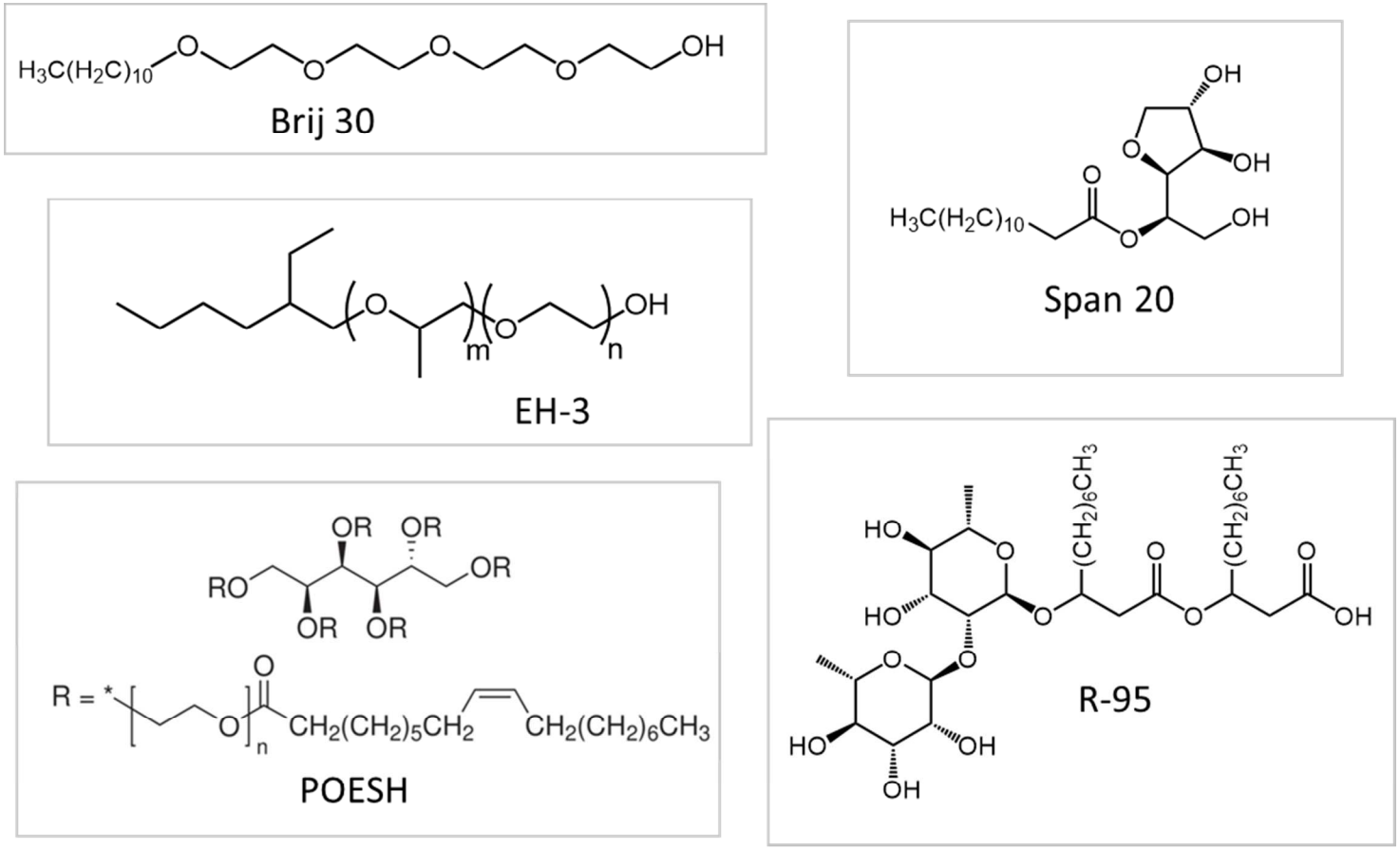

Figure S1. Structures of tested surfactants. Structure of EH-3 adapted from Li et al.[7]

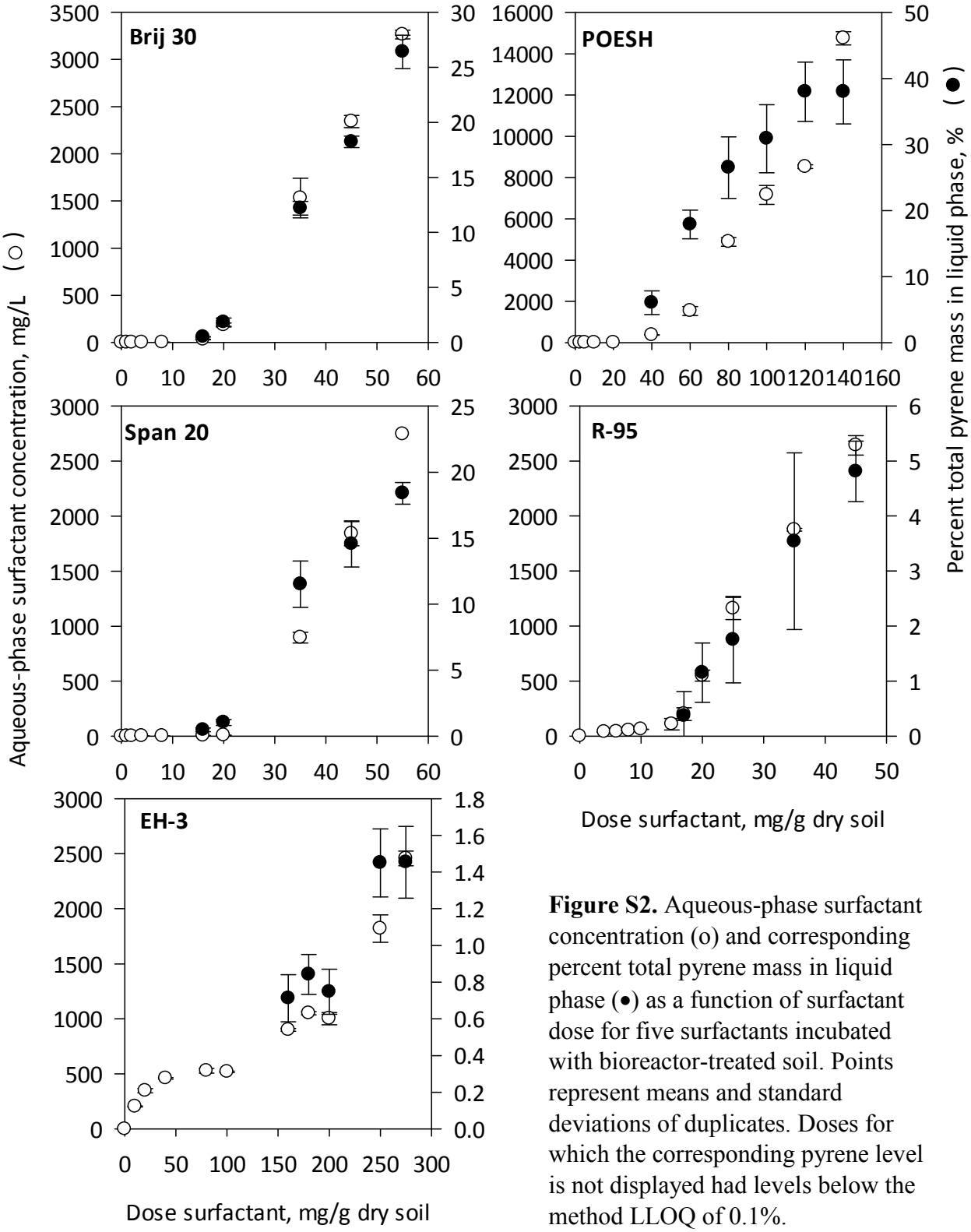

Figure S2. Aqueous-phase surfactant concentration (o) and corresponding percent total pyrene mass in liquid phase (•) as a function of surfactant dose for five surfactants incubated with bioreactor-treated soil. Points represent means and standard deviations of duplicates. Doses for which the corresponding pyrene level is not displayed had levels below the method LLOQ of 0.1%.

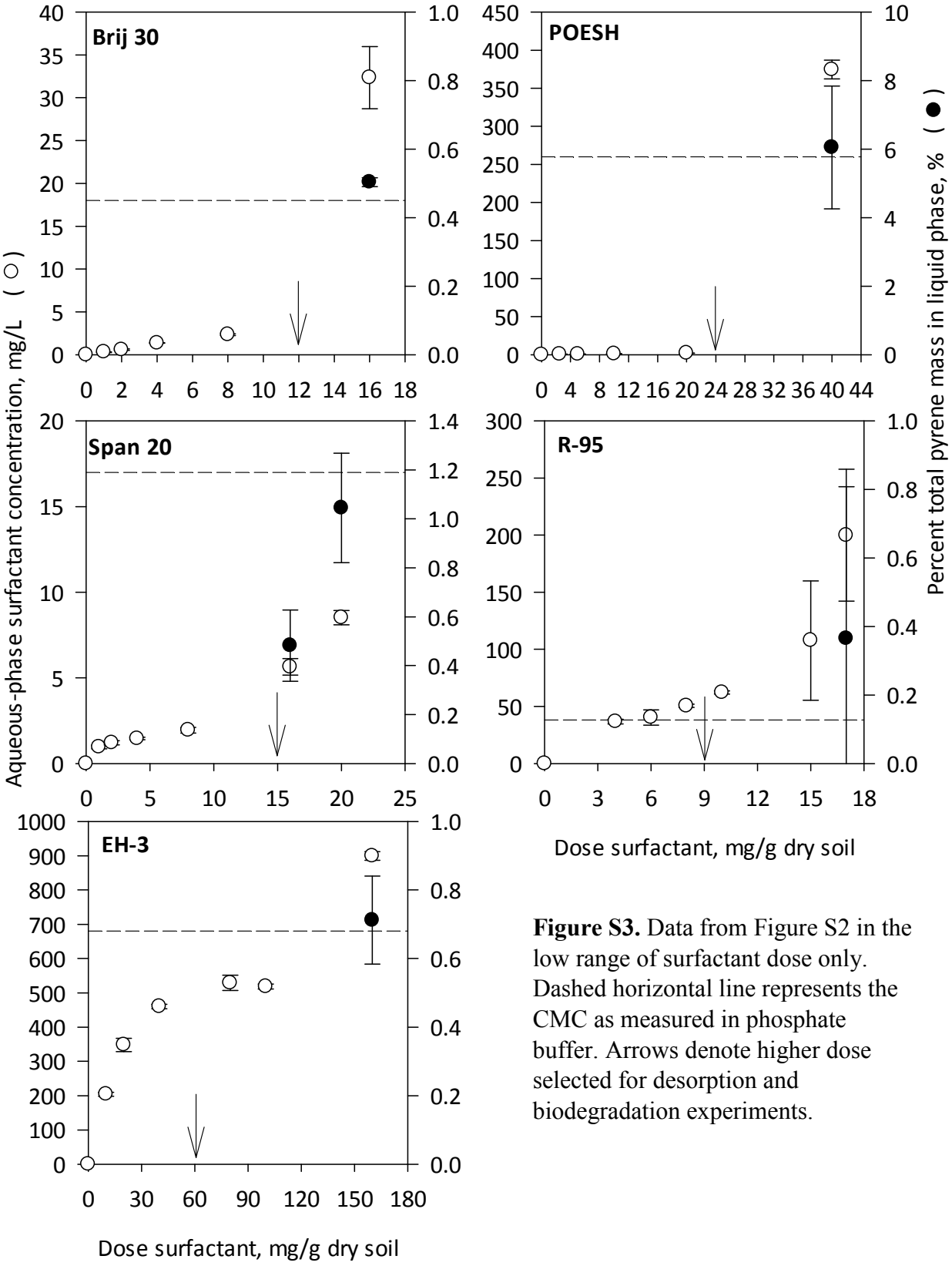

Figure S3. Data from Figure S2 in the low range of surfactant dose only. Dashed horizontal line represents the CMC as measured in phosphate buffer. Arrows denote higher dose selected for desorption and biodegradation experiments.

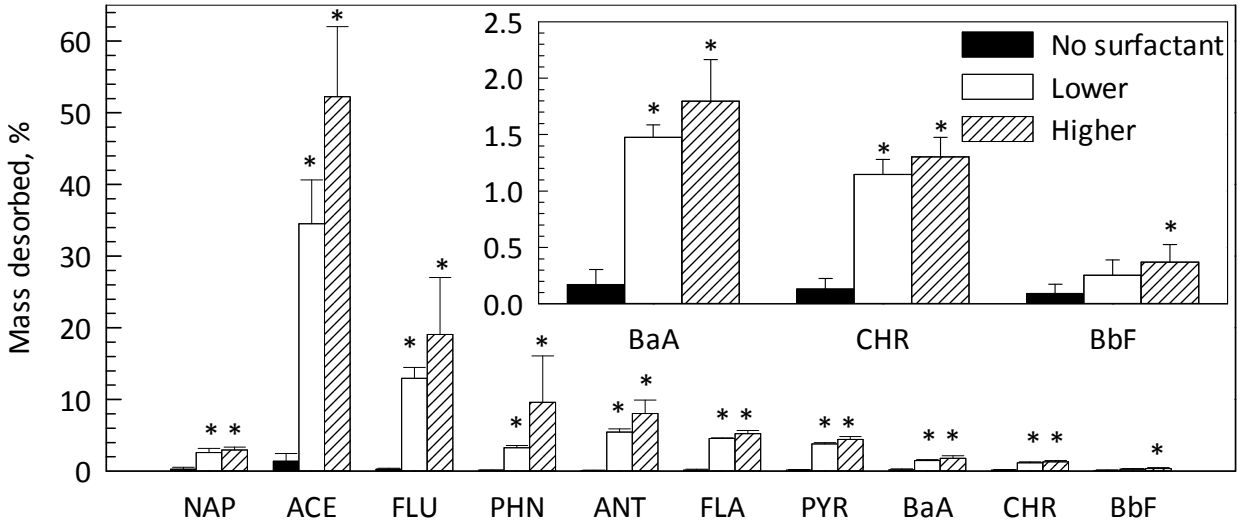

Figure S4. Cumulative desorption of PAHs from bioreactor-treated soil after seven days in the absence of surfactant or in the presence Brij 30; the inset shows the data for the three indicated compounds on a finer scale. "Lower" and "higher" refer to surfactant doses shown in Table S1. Bars represent means and standard deviations of triplicates for incubations with surfactant and four replicates for incubations without surfactant. An asterisk indicates a significant difference (α=0.05) in mass desorbed between a treatment and no-surfactant control. PAHs for which there were no significant differences between no-surfactant both doses are not shown. Abbreviations are defined in Table S2.

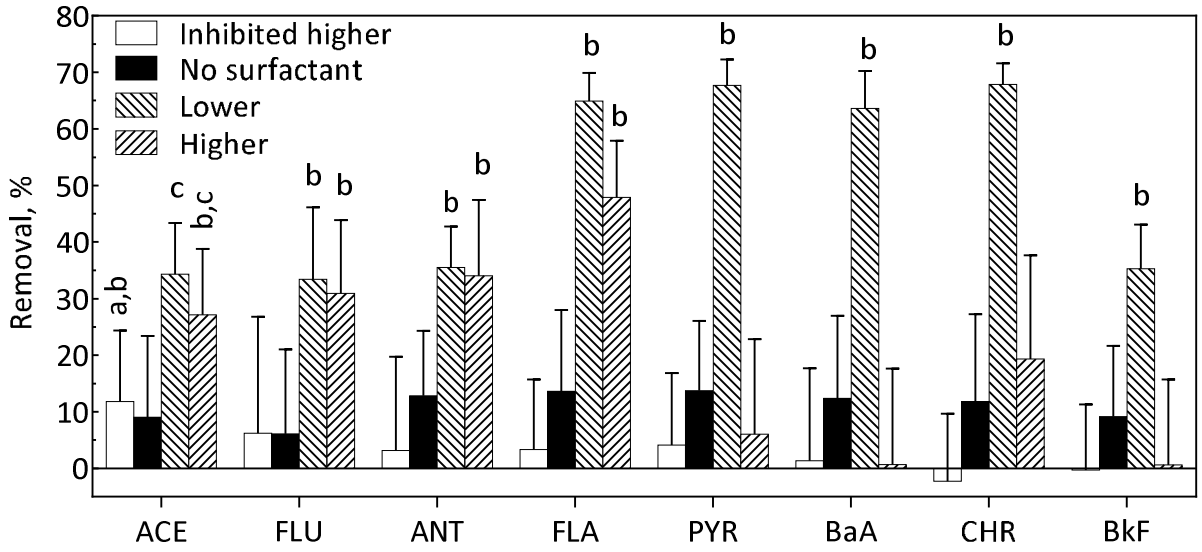

Figure S5. Effect of Brij 30 on biodegradation of residual PAHs from bioreactor-treated soil after 16 days. "Lower" and "higher" refer to surfactant doses shown in Table S1. "Inhibited" refers to controls to which sodium azide was added. Bars represent means and standard deviations of five replicates. Conditions for which there was not a significant difference (α=0.05) in final PAH soil-concentration detected by Tukey's method are assigned the same letter. Bars for which no letters are shown are implicitly designated "a". Abbreviations are defined in Table S2. PAHs for which there were no significant differences between no-surfactant controls and either surfactant are not shown.

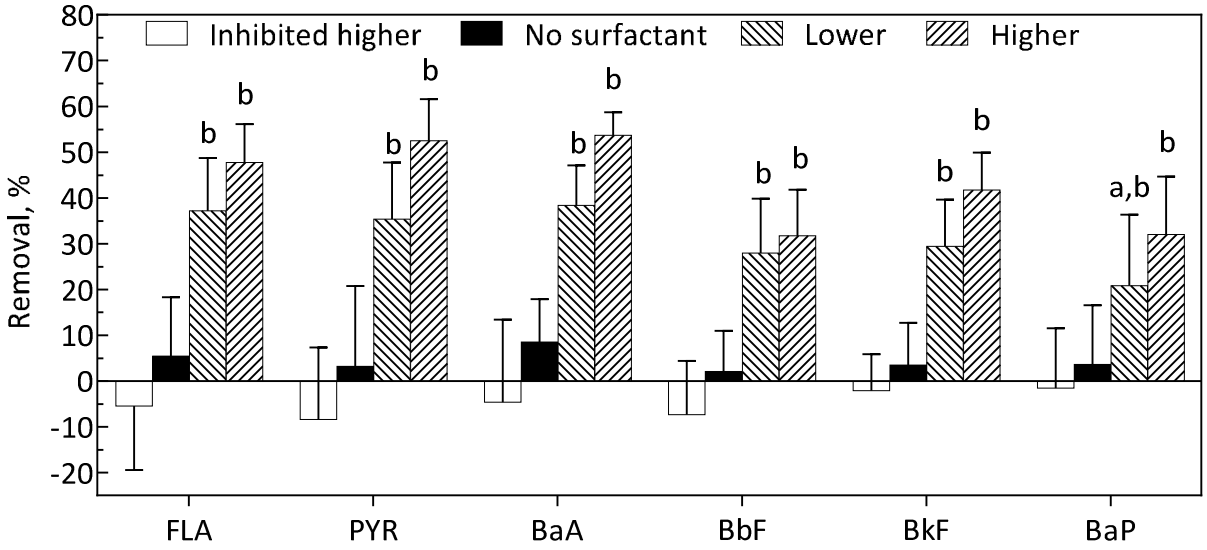

Figure S6. Effect of Span 20 on biodegradation of residual PAHs from bioreactor-treated soil after 16 days. Notes as in Figure S5.

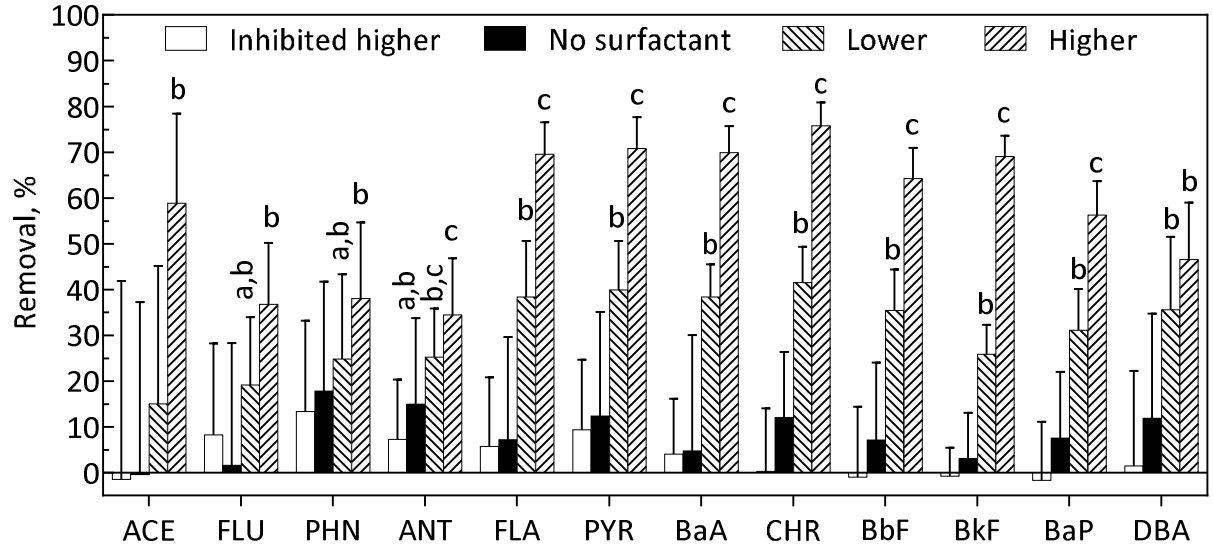

Figure S7. Effect of POESH on biodegradation of residual PAHs from bioreactor-treated soil after 16 days. Notes as in Figure S5.

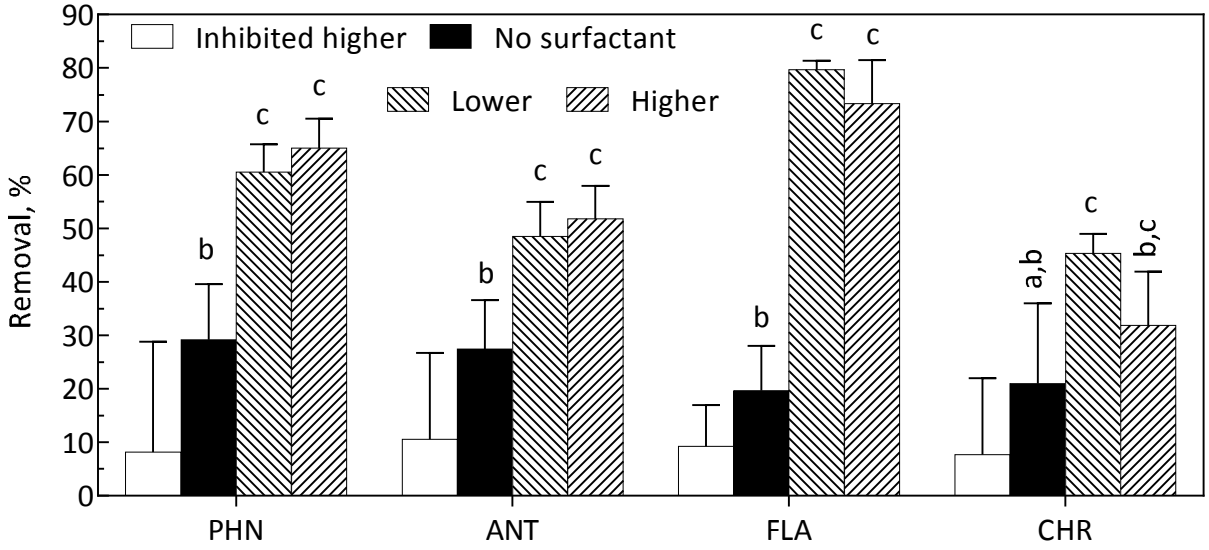

Figure S8. Effect of EH-3 on biodegradation of residual PAHs from bioreactor-treated soil after 16 days. Notes as in Figure S5.

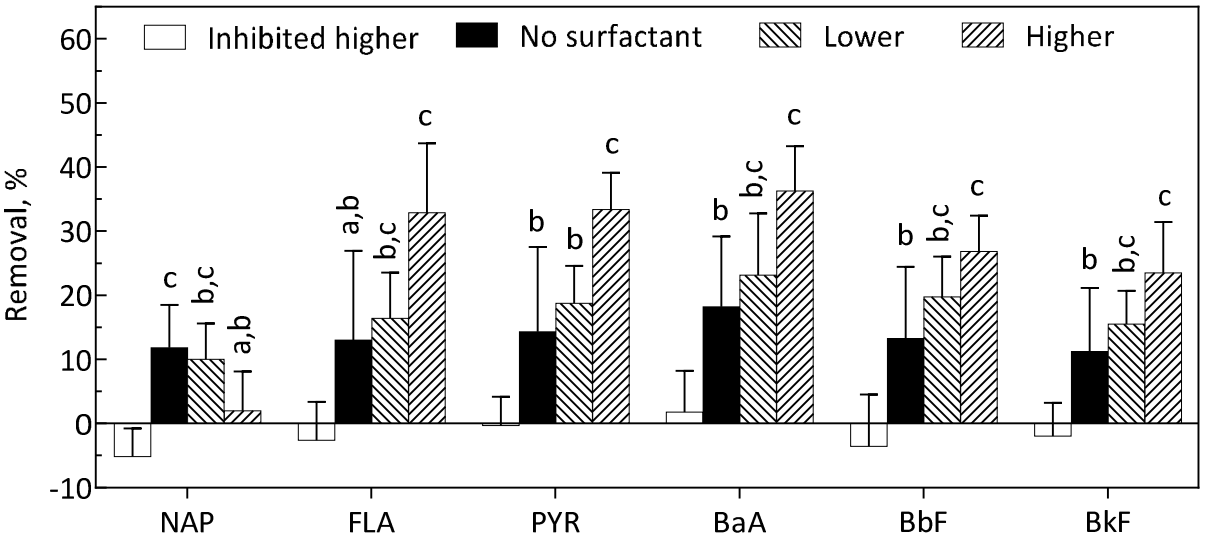

Figure S9. Effect of R-95 on biodegradation of residual PAHs from bioreactor-treated soil after 16 days. Notes as in Figure S5.

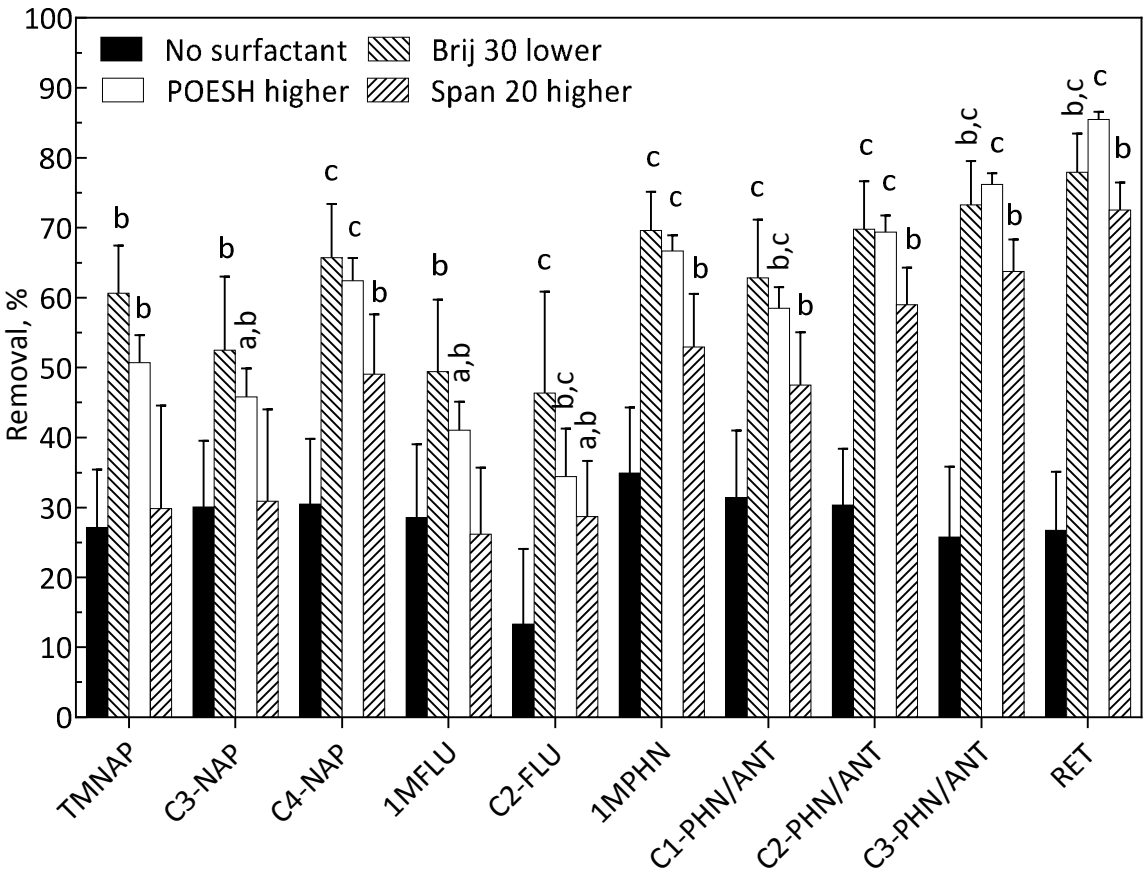

Figure S10. Effect of Brij 30, Span 20, and POESH in followup biodegradation experiment on residual alkylated 2- and 3-ring PAHs from bioreactor-treated soil after 16 days. "Lower" and "higher" refer to doses shown in Table S1. Bars represent means and standard deviations of five replicates. Conditions for which there was not a significant difference (α=0.05) in final PAH soil-concentration detected by Tukey's method are assigned the same letter. Bars for which no letter is shown are implicitly designated "a". Abbreviations are defined in Table S3. PAHs for which there were no significant differences between no-surfactant controls and all surfactant conditions are not shown.

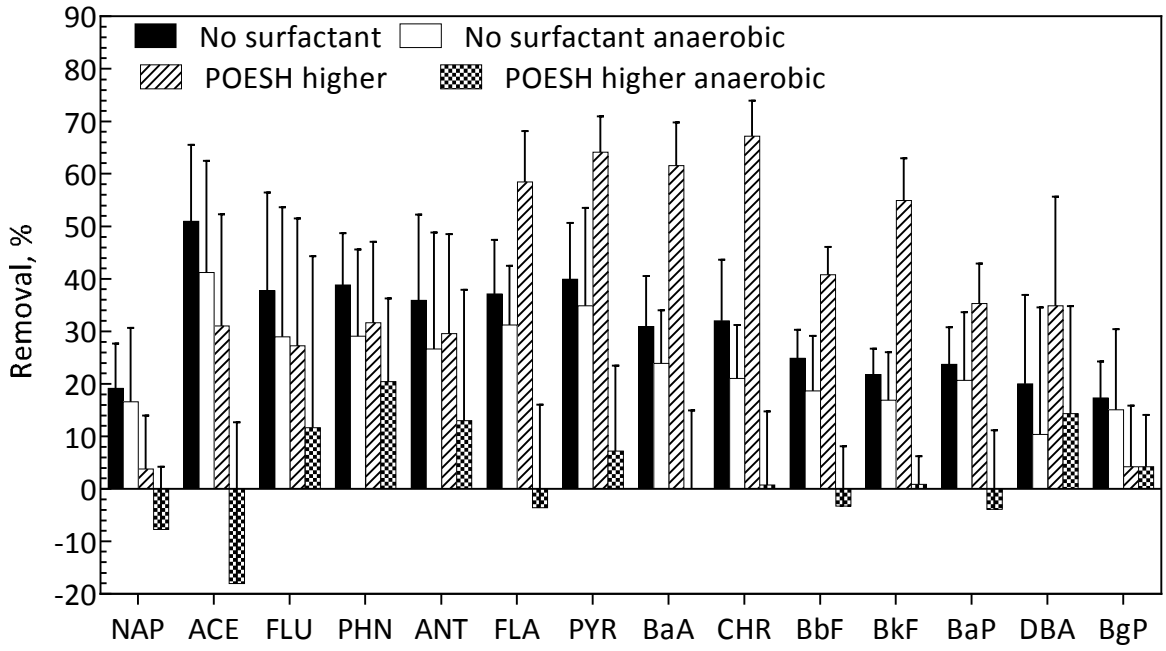

Figure S11. Effect of anaerobic incubation with POESH on the biodegradation of residual PAHs from bioreactor-treated soil after 16 days. "Higher" refers to the higher dose shown in Table S1. Bars represent means and standard deviations of four replicates. Abbreviations are defined in Table S2.

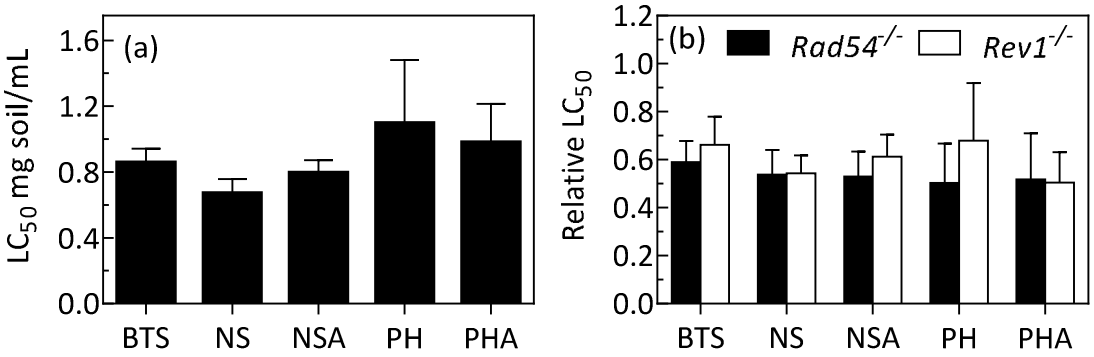

Figure S12. Effect of anaerobic incubation with POESH on (geno)toxicity of bioreactor treated soil after 16 days (referred to in the text as the followup (geno)toxicity experiment). **(a)** LC_{50} of soil for parental DT40 cell line. **(b)** Relative LC_{50}'s of soil for *Rad54*$^{-/-}$ and *Rev1*$^{-/-}$ mutants. Bars represent means and standard deviations of three experiments. In pairwise t-tests, no condition is significantly different (α=0.05) than the bioreactor-treated soil (BTS), NS, no-surfactant; NSA, no-surfactant anaerobic; PH, POESH higher; PHA, POESH higher anaerobic.

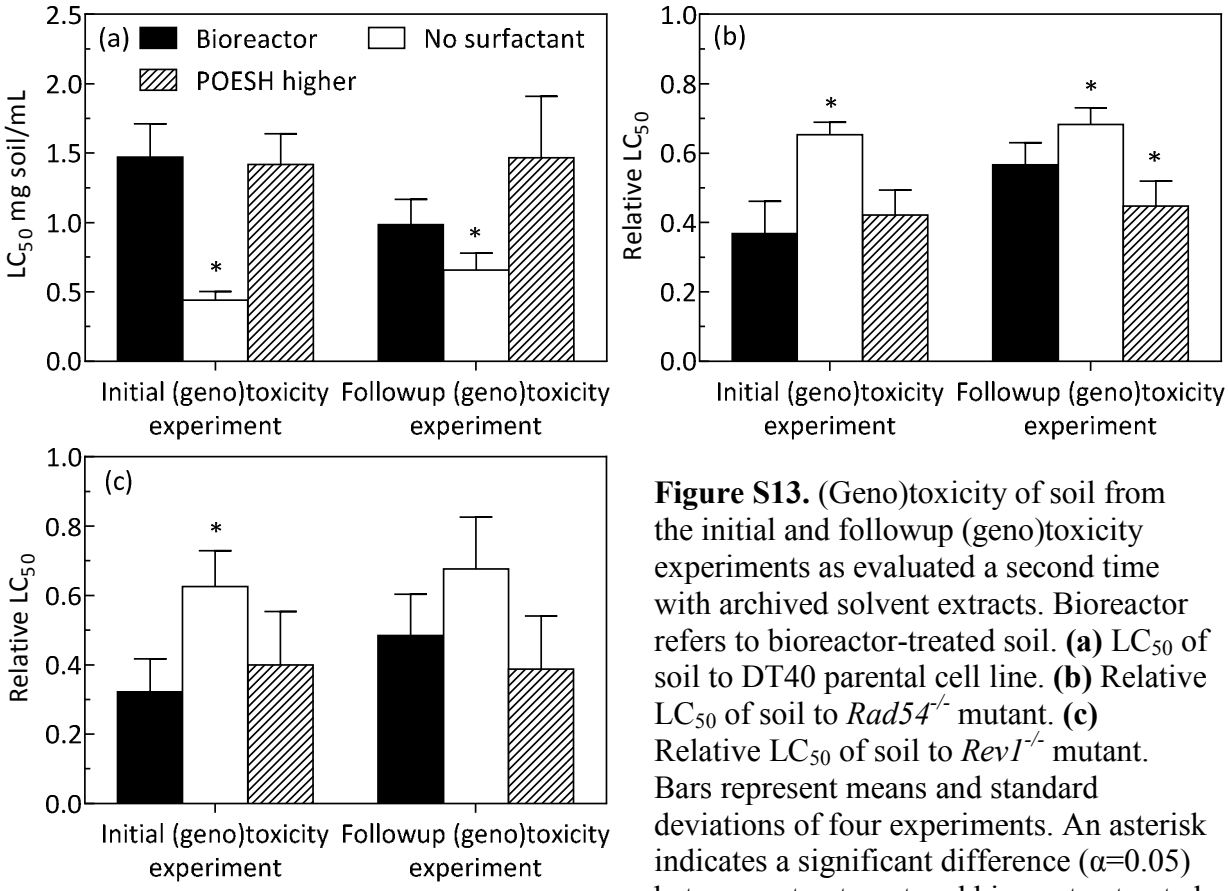

Figure S13. (Geno)toxicity of soil from the initial and followup (geno)toxicity experiments as evaluated a second time with archived solvent extracts. Bioreactor refers to bioreactor-treated soil. **(a)** LC_{50} of soil to DT40 parental cell line. **(b)** Relative LC_{50} of soil to *Rad54*$^{-/-}$ mutant. **(c)** Relative LC_{50} of soil to *Rev1*$^{-/-}$ mutant. Bars represent means and standard deviations of four experiments. An asterisk indicates a significant difference (α=0.05) between a treatment and bioreactor-treated

Supporting Information References

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