

## **Supporting Information**

### **Enhanced bioremediation of aged polycyclic aromatic hydrocarbons in soil using immobilized microbial consortia combined with strengthening remediation strategies**

Haixuan Zhou<sup>1†</sup>, Xiurong Gao<sup>1,2†</sup>, Suhang Wang<sup>1,2</sup>, Youchi Zhang<sup>1</sup>, Frédéric Coulon<sup>3</sup>,  
Chao Cai<sup>1\*</sup>

<sup>1</sup> Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

<sup>2</sup> University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup> School of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, UK

<sup>†</sup> These authors contributed equally to this work.

\* Correspondence should be addressed to: ccai@iue.ac.cn (Chao Cai)

## **Supporting Methodology**

**Chemicals:** Phenanthrene (PHE), pyrene (PYR), benzo (b) fluoranthene (BbF) and (BaP) benzo[a]pyrene (>97% purity), the surrogate standard mix contained naphthalene-d<sup>10</sup>, acenaphthene-d<sup>10</sup>, phenanthrene-d<sup>10</sup>, chrysene-d<sup>12</sup> and perylene-d<sup>12</sup>, a standard solution of 16 US EPA PAHs, were purchased from AccuStandard (CT, USA). N-hexane, methanol, acetone, and dichloromethane (all HPLC-spectrograde) were purchased from Tedia (TC, USA). Internal standard hexamethylbenzene (99% purity) was acquired from Dr. Ehrenstorfer (Augsburg, Germany). All the inorganic reagents were of analytical grade (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China).

**Medium:** Minimal salt medium (MSM) contained (g/L) KH<sub>2</sub>PO<sub>4</sub> 3.4 g, Na<sub>2</sub>HPO<sub>4</sub> 19.6 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1 g, MgSO<sub>4</sub> 97.4 mg and trace element, such as CuSO<sub>4</sub> 1 g, ZnSO<sub>4</sub> 1 g, FeSO<sub>4</sub> 1 g, CaCl<sub>2</sub> 1 g, H<sub>3</sub>BO<sub>4</sub> 1 g.

LB medium contained (g/L) Tryptone 10g, Yeast extract 5g, NaCl 10g.

LB solid medium was made by adding 20% agar.

**The determination of physical and chemical properties of soil** were according to the methods described in Lu (1999), and the specific procedures are as follows.

pH was measured in a suspension of soil/biochar with soil or biochar /water ratio of 1:2.5 (W/V). The content of soil organic matter was determined by potassium dichromate oxidation-volumetric method.

The contents of C, N, S, H and O elements in the soil/biochar were determined by Vario

Max elemental analyzer (VarioMax CNS, Germany).

The specific surface area and pore size of biochar were measured using 99.99% N<sub>2</sub> as adsorbent and at 1.036 bar saturated vapor pressure by automatic specific surface area, micropore and chemical adsorption instrument (ASAP 2020M+C).

**PAH analysis in CP soil:** A surrogate standard mix containing naphthalene-d<sup>10</sup>, acenaphthene-d<sup>10</sup>, phenanthrene-d<sup>10</sup>, chrysene-d<sup>12</sup> and perylene-d<sup>12</sup> (25 µL) at concentration of 400 µg/mL was added to the soil phase prior to extraction. Following the US EPA Method 3630C, the extracts were concentrated to 1 mL by rotary vacuum evaporation at 36°C. Then 15 mL of hexane were added and further concentrated to 1 mL. An internal standard hexamethylbenzene 15 µL (10 µg/mL in acetone) was added to the final solution added before analysis by GC-MS.

**Table S1 The total concentrations of 16 US EPA PAHs in CP soil**

PAHs	Abbreviation	Ring number	Concentration(mg/kg)
Naphthalene	NAP	2	$1.89 \pm 0.27$
Acenaphthylene	ANY	2	$0.44 \pm 0.15$
Acenaphthene	ANA	3	$2.74 \pm 0.37$
Fluorene	FLU	3	$1.58 \pm 0.29$
Phenanthrene	PHE	3	$8.99 \pm 1.73$
Anthracene	ANT	3	$1.88 \pm 0.26$
Fluoranthene	FLT	4	$21.91 \pm 3.87$
Pyrene	PYR	4	$22.46 \pm 2.61$
Benzo(a)anthracene	BaA	4	$20.78 \pm 2.67$
Chrysene	CHR	4	$23.52 \pm 3.35$
Benzo(b)fluorene	BbF	5	$31.87 \pm 4.43$
Benzo (k)fluorene	BkF	5	$14.91 \pm 2.05$
Benzo(a)pyrene	BaP	5	$32.05 \pm 3.67$
Indeno(1,2,3-c,d)pyrene	ICP	6	$7.36 \pm 0.76$
Dibenzo(a,h)anthracen	DBA	6	$36.03 \pm 3.11$
Benzo(g,h,i)perylene	BPE	6	$27.07 \pm 2.03$
Total PAHs			$255.47 \pm 22.46$

**The physico-chemical properties of the biochar** were as follows: pH 10.0, total N 1.47 %, total C 47.6 %, total O 16.1 %, total H 2.25 %, specific surface area 2.98 m<sup>2</sup>/g.

**The physico-chemical properties of soil** were as follows: pH 8.6, total N 0.24 %, total C 16.6 %, total S 0.56 %, total Organic matter 8.20 %.

**Table S2 Biochemical properties of bacterial strains isolated from PAH contaminated soil**

Item	ZL7	MI	Q3
Colony color	Pink	Light-yellow	Orange
Colony surface	Dry smooth	Dry smooth	Dry smooth
Shape of cells	Short rod	Short rod	Globular
Gram staining <sup>a</sup>	+	+	+
Spore staining <sup>a</sup>	-	-	-
Degradable PAHs <sup>b</sup>	PHE, PYR, BaA, BbF, CHR and BkF	NAP, FLU, PHE, ANT, FLT, BbF and PYR	PHE, PYR, BaP, BbF, BaA, BkF, DBA, BPE and CHR

<sup>a</sup> +, Positive; -, Negative.

<sup>b</sup> Naphthalene (NAP), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), dibenzo[a,h]anthracene (DBA), benzo[g,h,i]perylene (BPE) and chrysene (CHR).

**Table S3 Total concentrations of 16 US EPA PAHs in test biochar**

PAHs	Abbreviation	Ring number	Concentration (mg/kg)
Naphthalene	NAP	2	$0.08 \pm 0.003$
Acenaphthylene	ANY	2	$0.15 \pm 0.007$
Acenaphthene	ANA	3	$0.01 \pm 0.0001$
Fluorene	FLU	3	$0.05 \pm 0.0002$
Phenanthrene	PHE	3	$0.09 \pm 0$
Anthracene	ANT	3	$0.17 \pm 0.02$
Fluoranthene	FLT	4	$0.14 \pm 0$
Pyrene	PYR	4	$0.15 \pm 0.007$
Benzo(a)anthracene	BaA	4	$0.28 \pm 0.009$
Chrysene	CHR	4	$0.34 \pm 0.01$
Benzo(b)fluorene	BbF	5	$0.24 \pm 0.007$
Benzo (k)fluorene	BkF	5	$0.37 \pm 0.02$
Benzo(a)pyrene	BaP	5	$0.31 \pm 0.002$
Indeno(1,2,3-c,d)pyrene	ICP	6	$0.47 \pm 0.02$
Dibenzo(a,h)anthracen	DBA	6	$0.53 \pm 0.003$
Benzo(g,h,i)perylene	BPE	6	$0.35 \pm 0.01$
Total PAHs			$3.72 \pm 0.04$

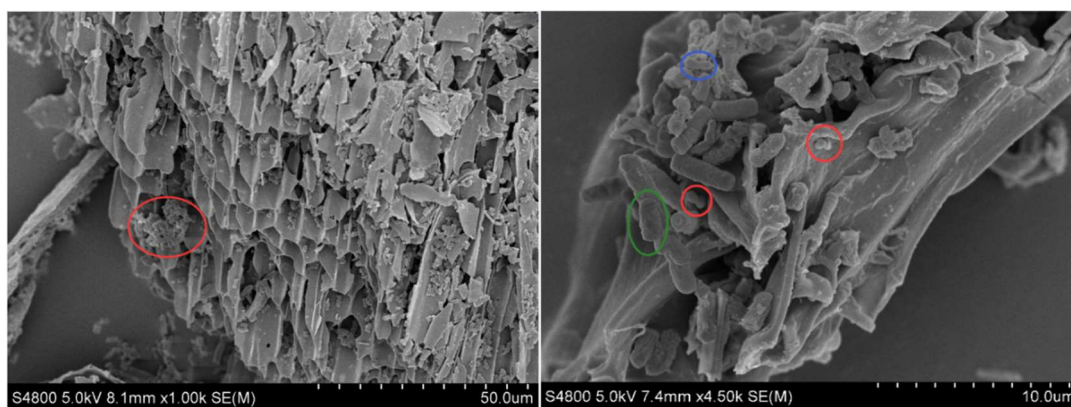
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**Table S4 Residual PAH in CP soil with different treatments for 24 days**

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PAHs	residual PAH (mg/kg)												
	CK	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
NAP	1.09±0.35	1.42±0.21	1.48±0.25	0.73±0.16	1.32±0.29	0.47±0.16	0.72±0.25	1.30±0.45	1.86±0.64	1.04±0.02	1.06±0.95	0.79±0.29	1.05±0.49
ANY	0.46±0.12	0.36±0.06	0.46±0.08	0.34±0.07	0.36±0.06	0.28±0.07	0.43±0.05	0.34±0.03	0.65±0.16	0.41±0.14	0.35±0.30	0.29±0.29	0.23±0.05
ANA	2.60±0.54	2.47±0.68	2.49±0.33	1.79±0.36	2.19±0.52	1.26±0.52	2.31±0.38	1.87±0.15	3.16±1.43	2.29±0.32	1.90±0.93	1.50±0.17	1.33±0.20
FLU	1.91±0.34	1.74±0.52	1.84±0.31	1.63±0.15	1.68±0.35	1.15±0.28	1.70±0.12	1.38±0.10	2.56±0.32	1.72±0.23	1.32±0.98	1.12±0.11	0.99±0.18
PHE	8.08±0.93	6.83±1.48	5.66±0.92	6.92±1.08	4.91±0.76	4.43±1.15	6.29±1.40	5.27±0.62	5.64±0.39	4.87±1.31	3.70±0.59	4.55±0.50	3.27±0.31
ANT	1.85±0.30	1.44±0.43	1.11±0.17	1.48±0.28	1.10±0.18	0.96±0.27	1.28±0.16	1.14±0.10	1.07±0.15	1.13±0.25	0.74±0.11	0.91±0.07	0.65±0.10
FLT	18.02±2.26	12.71±1.86	14.76±1.86	13.02±1.82	13.27±0.64	9.86±1.81	12.08±1.92	10.72±0.93	23.10±2.47	13.74±2.49	11.17±2.49	9.00±0.67	7.45±1.62
PYR	21.19±1.49	18.08±1.83	13.15±1.45	14.99±1.76	11.96±0.09	11.08±1.79	15.25±2.66	15.10±2.25	13.60±2.10	10.83±3.07	9.62±1.90	12.49±1.29	8.50±1.37
BaA	14.42±2.11	11.47±0.99	9.17±0.60	10.89±1.70	8.28±0.47	8.28±0.88	9.38±1.41	8.80±0.72	10.14±0.99	6.57±0.72	5.73±0.88	7.45±0.58	4.58±0.59
CHR	17.36±2.00	13.42±1.89	10.68±0.91	12.56±1.95	9.48±0.16	9.96±0.88	11.83±1.40	10.93±0.68	12.17±1.59	6.99±2.91	7.26±0.82	9.06±0.63	3.93±2.13
BbF	19.56±2.92	15.26±1.80	12.98±0.47	14.44±2.25	11.95±0.84	11.44±1.12	13.18±1.12	11.43±0.08	13.18±0.75	8.91±1.02	7.53±1.82	9.27±0.68	6.62±0.80
BkF	7.23±0.92	5.48±0.30	4.76±0.06	5.27±0.64	4.27±0.38	4.20±0.43	4.87±0.67	5.26±2.22	4.89±0.26	3.99±1.25	4.40±1.26	5.30±1.43	3.68±1.02
BaP	22.57±3.43	17.16±1.43	13.69±0.80	13.75±1.66	13.74±1.57	11.58±0.61	13.82±0.78	11.00±1.68	12.25±0.37	8.37±1.31	7.76±0.84	9.36±0.60	7.41±0.73
ICP	23.36±3.72	12.50±2.80	14.49±0.65	12.12±3.14	16.28±2.15	13.15±2.35	15.57±0.71	6.36±1.68	10.86±1.32	5.79±2.34	4.87±1.39	6.56±1.65	6.99±1.07
DBA	25.35±2.05	16.94±1.58	16.33±0.49	16.02±2.55	16.30±1.24	13.85±2.35	16.55±0.30	13.34±0.47	12.64±1.06	10.07±1.08	5.03±1.72	7.59±1.53	7.55±0.68
BPE	2.24±0.33	1.41±1.58	1.58±0.38	1.68±0.52	1.64±0.15	1.45±0.43	1.67±0.28	1.16±0.25	1.30±0.08	0.85±0.06	0.87±0.32	0.79±0.03	0.78±0.28
Total PAHs	187.29±7.14	138.70±15.48	124.61±4.73	127.60±18.17	118.74±4.64	103.40±10.74	126.93±11.47	105.39±6.80	129.13±7.33	87.59±9.42	73.33±13.03	86.01±1.36	65.01±4.71

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**Figure S1 SEM images of biochar immobilized microbial composites**



## **References**

Lu, R.K. Soil argrochemistry analysis protocols. Beijing: China Agriculture Science Press: 1999.