SUPPORTING INFORMATION

ENVIRONMENTAL SCIENCE AND TECHNOLOGY

Arsenic biotransformation in solid waste residue: comparison of contributions from bacteria with arsenate and iron reducing pathways

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LB broth (1L) 10 g tryptone, 5 g yeast extract, and 10 g NaCl were dissolved in 1 L deionized (DI) water. The pH of the medium was adjusted to 7.0 using 1 mol/L NaOH.

CDM broth (1L) The CDM medium contained the following: K_2HPO_4 (0.225), KH_2PO_4 (0.225), NaCl (0.46), $(NH_4)_2SO_4$ (0.225), $MgSO_4 \cdot 7H_2O$ (0.117), yeast extract (1.0), Na lactate (2.24), $Na_3AsO_4 \cdot 12H_2O$ (0.424), and $NaHCO_3$ (4.2) (all in grams per liter); trace element solution (1.0 mL); and vitamin solution (10 mL).[1]

Metal resistant ability The purpose of choosing different solid to liquid ratios was to compare the effect of different bacteria on arsenic mobility at the maximum level of toxic metals they could tolerate. The solid waste residue has various heavy metals with extremely high concentrations which is toxic to bacteria cell and will affect bacteria growth. Therefore, we performed series of preliminary experiments to find a maximum solid to liquid ratio where bacteria can survive and maintain their functionality. As shown in Table S4-6, different residue concentrations (0.4-15 g/L) were applied for strain IMH, OhILAs, and MR-1 in the preliminary incubation experiments. For the As(V) reducing bacteria (IMH and OhILAs), the As(III) ratio in the aqueous phase was measured. For the iron reducer (MR-1), the Fe(II) ratio was determined. The results show that the reduction capacity for As(V) or Fe(III) dramatically decreased when the solid concentration was higher than a threshold value. This critical concentration (10 g/L for strain IMH, 1 g/L for OhILAs and 2 g/L for MR-1) was then used in our incubation experiments.

SEM sample preparation The sample was first stabilized by 2.5% glutaraldehyde solution prepared in 0.2 M phosphate buffer (pH 7.4) and then incubated at 4°C overnight. After the fixation step, samples were rinsed with 0.1 M phosphate buffer (pH 7.4) three times for 20 min each time. Then,

samples were re-fixed by 1% osmic acid for 2-4 h and re-rinsed with 0.1 M phosphate buffer (pH 7.4) three times for 15 min each time. After the double fixation step, samples were dehydrated with a graded series of ethanol including 30%, 50%, 70%, 80%, 90% and 95% ethanol/water once for 15 min, and 100% ethanol twice for 20 min. The third step is replacement. Samples were immersed in isoamyl acetate twice for 15 min. The last step is drying. Samples were dried according to a procedure called critical point drying (CPD). Finally, the powder samples were mounted on aluminum stubs using double-sided tape and were ready to be viewed in the SEM (FESEM, Hitachi SU8020, Tokyo, Japan).

XRD analysis X-ray powder diffraction (XRD) patterns of the solid residual samples were recorded using an X'Pert PRO (PANalytical, The Netherlands) instrument in Bragg-Brentano geometry with iron-filtered Cu Kα radiation (40 kV, 30 mA). Samples were placed on a rotating zero-background holder made of single-crystal Si, gently pressed to obtain a sample thickness of about 0.5 mm, and scanned in the 2θ range of 10° to 90° with a step size of 0.02° and a count time of 2 s per step. The analyses of the XRPD patterns were performed using the PDF-2 reference database from the International Center for Diffraction Data (ICDD).

Fe(III) reduction by strain OhILAs Ferrihydrite was prepared according to the method suggested by Jiang and Lee, with slight modification.[2] Briefly, ferric chloride solution (0.1 M $FeCl_3$) was slowly titrated with 1.0 M NaOH to pH 7, and the resulting dark brown precipitate was washed with DI water ten times and stored after freeze-drying. The XRD analysis showed that the synthesized ferrihydrite is the 2-line product (Fig. S3).

The capacity of iron reduction by strain OhILAs was measured under anaerobic conditions. Strain OhILAs was inoculated in the CDM medium containing 100 mg/L ferrihydrite and incubated in the

darkness without agitation at 30°C for 120 h. Suspension samples were periodically collected to determine the concentration of aqueous Fe(II).

Table S1. Basic properties of the bacteria used in the incubation experiments.

Strain	Oxygen condition	Growth medium	As(V) reduction related genes	Fe(III) reducing ability	SO_4^{2-} reducing ability	Source
Pantoea sp. IMH	Aerobic	LB	*ars*C (KM233198)	No	No	Isolated from soil[3]
Shewanella oneidensis MR-1	Anaerobic	LB	*ars*C (SO_2871)	Yes	Yes	Donated from Dr. Gao Haichun
Alkaliphilus oremlandii OhILAs	Anaerobic	CDM	*Arr*A[4]	Yes	No	Obtained from ATCC (BAA-1360™)

Table S2. Metal concentration in the solid waste residue.

Element	As	Fe	Zn	Pb	Cu	Cd	Ca
Content (mg/g)	9.5	27.5	3.8	0.5	11.7	3.1	215.7

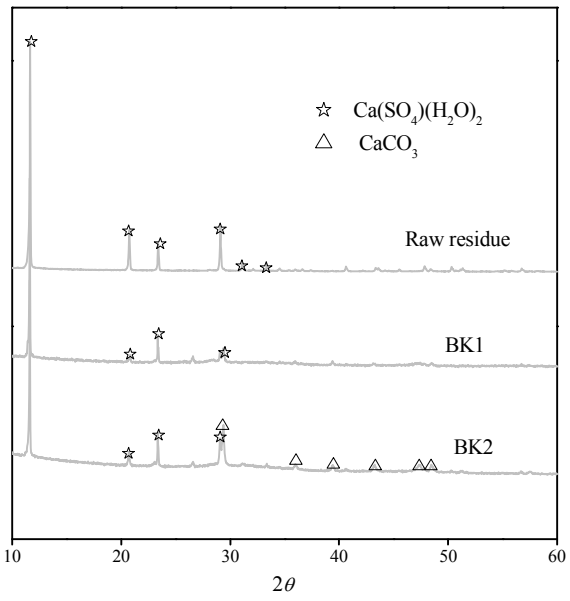

Figure S1. XRD patterns of the residue affected by culture media.

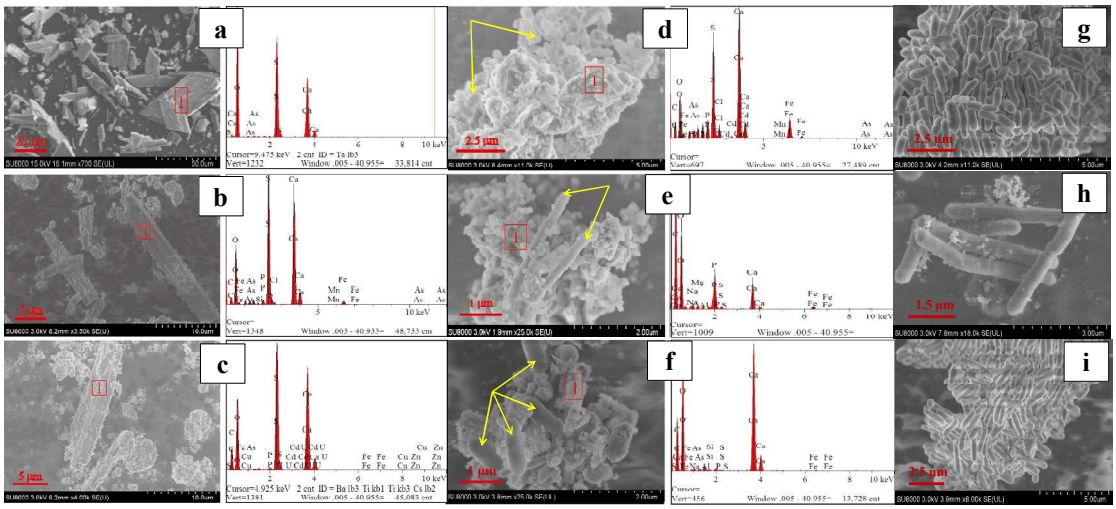

Figure S2. SEM images of the residue incubated with the three bacteria (a: raw residue; b: BK1; c: BK2; d: IMH; e: OhILAs; f: MR-1) and the original bacteria cells (g: IMH; h:OhILAs; i: MR-1)

Table S3. Metal release percent in residue affected by the three bacteria (%)

Metal	IMH	OhILAs	MR-1
As	15.1	16.8	40.0
Fe	0.6	3.7	39.9
Ca	41.3	8.9	46.4
Cu	21.3	0.2	21.0
Zn	7.7	0.4	22.1
Cd	53.1	0.0	97.4
Pb	0.7	0.5	9.8

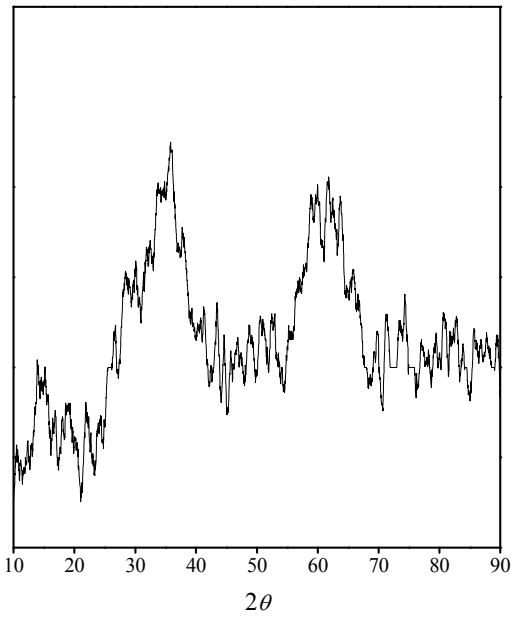

Figure S3. XRD pattern of ferrihydrite synthesized in this work.

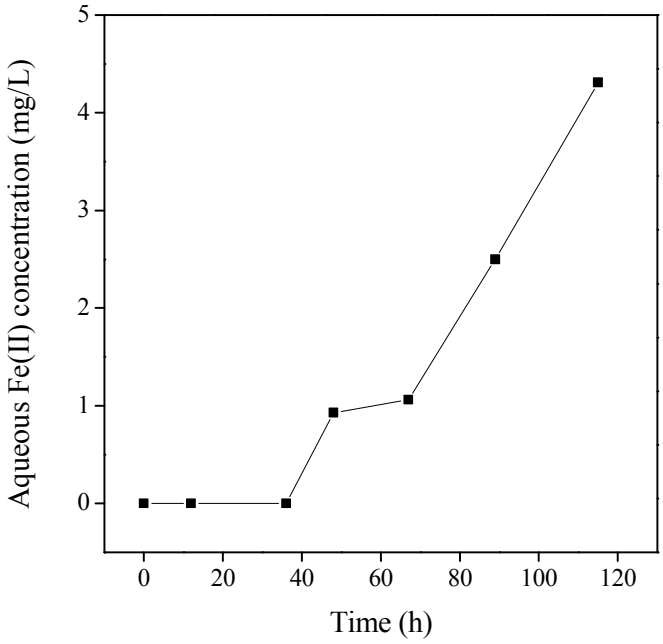

Figure S4. Reduction of ferrihydrite by strain OhILAs.

Table S4. As(V) reduction ratio by IMH in different solid waste concentrations

Items	IMH			
Solid waste concentration (g/L)	4	8	10	15
As(III) ratio* (%)	100	100	100	18

*: As(III) ratio was determined after 36 h incubation.

Table S5. As(V) reduction ratio by OhILAs in different solid waste concentrations

Items	OhILAs			
Solid waste concentration (g/L)	0.1	0.5	1	2
As(III) ratio* (%)	100	87	83	20

*: As(III) ratio was determined after 72 h incubation.

Table S6. Fe(III) reduction ratio by MR-1 in different solid waste concentrations

Items	MR-1				
Solid waste concentration (g/L)	0.4	0.8	2	4	6
Fe(II) ratio* (%)	100	100	96	30	10

*: Fe(II) ratio was determined after 72 h incubation.

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