Supporting Information

Microbes Enhance Mobility of Arsenic in Pleistocene Aquifer Sand from Bangladesh

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Summary: Detailed results of incubation experiments and absorption experiments with post-incubated sediments are summarized in 6 pages (S1-S6) of Supporting Information. SI include four tables (Table S1-S4) and one figure (Figure S1) .

Supporting Information

Shewanella ANA-3 Culture

Preparation of Freezer Stock: Kanamycin-resistant *Shewanella* strains ANA-3 were grown on Luria Bertani (LB) media spiked with kanamycin (50 µg mL^{-1}) by incubating the strains at 28°C overnight aerobically. The microbes that grew in LB media were spun down into a pellet by centrifuging at ~ 4°C (3000 rpm for 5 minutes). The supernatant was decanted until about 4 mL of media remained with the pellet. The pellet was then re-suspended and 0.5 mL of the concentrated bacteria suspension was transferred to a new vial to which 0.5 mL of autoclave-sterilized glycerol was also added. Three such vials of freezer stock were prepared and stored in the -80°C freezer.

Aerobic Growth: The strains were grown aerobically in autoclave-sterilized TME media with kanamycin (50 µg mL^{-1}). A 100 µL volume of freezer stock was transferred into 20 mL of TME media solution and grown at 28-30°C in an incubator shaker (~250 rpm) for 24-48 hours. The culture was spun down (3000 rpm for 5 minutes, at ~4°C) and the media decanted until about 4 mL was left with the pellet, which was then re-suspended for anaerobic growth.

Anaerobic Growth: Aerobically-grown strains were added to autoclave-sterilized TME media with an anaerobic electron acceptor ($Na_2HAsO_4.7H_2O$) and with 50 µg mL^{-1} of kanamycin. The bottles were filled to the top to eliminate headspace and sealed with parafilm immediately after adding the strain. The microbes were grown at 28-30°C in incubator shaker (~250 rpm) for 3-5 days.

Transfer of Bacteria to AGW: The anaerobic grown cultures were spun down into the pellets (5000 rpm for 15 minutes at ~4°C) and most of the media was decanted. An equal volume of artificial groundwater (AGW) was added to the pellet and re-suspend using a vortex mixer. AGW was prepared by adding salts in Milli Q water of composition similar to that of groundwater from similar depth associated with the Pleistocene orange sands was used (Table S1). The suspension was again centrifuged at 5000 rpm for 15 minutes at 4°C and spun down into a pellet. The cells were re-suspended into an equal volume of fresh AGW by carefully pouring off the most of AGW.

Potential Transfer of As to Incubations No significant amount of As contained in the original TME media transferred into the culture tubes. Concentrations of As in the culture tubes with only artificial groundwater and the same aliquot of microbes as the culture tubes containing orange sands were not detectable (i.e. < 0.1 µg L^{-1}).

Isotherms

The partitioning of As spikes added to groundwater and Pleistocene sediments was determined at the end of the incubations. Post-incubated sediments in 2 replicates without (C_0) and with lactate (C_{lac}),incubated for 90 days were spiked with As(III) immediately after incubation. Three such As (III) spikes were conducted in 88 days and partitioning of spiked As(III) was monitored. Each time 10 µL of 1000 mg L^{-1} As(III) was spiked to each incubation tube with 10 mL de-oxygenated AGW to add ~1 mg L^{-1} solution concentration. Experiments were done in anaerobic chamber and pH was measured prior to analyses of As. As(III) was determined in non-acidified supernatant immediately after filtration (0.23 µm) by DPCSV with a detection limit of 0.2 µg L^{-1} after a 1 to 10 dilution. Total As in filtered and acidified supernatant was analyzed by HR ICP-MS. Results are summarized in Table- S4. Sorptive capacity of the sediments was indicated by S_{max}, calculated using the Langmuir equation and was expressed as maximum sorption of As (III) by Fe(III) oxyhydroxide surface which is dominant in orange colored Pleistocene aquifer sands (Fig. S1).

Table S1. Chemical composition of Artificial groundwater(AGW) and Dari deep water assoicated with orange sands (n=41)

Dissolved parameters		**AGW**	**Dari Deep water**
pH		7.89	6.52 ± 0.06
As	(µg l^{-1})	-	<1
Fe	(mg l^{-1})	-	0.56 ± 0.28
Mn	(mg l^{-1})	-	0.10 ± 0.04
Na^+	(mmole l^{-1})	2.9	2.6 ± 0.24
K^+	(mmole l^{-1})	0.05	0.05 ± 0.01
Ca^{2+}	(mmole l^{-1})	0.06	0.40 ± 0.02
Mg^{2+}	(mmole l^{-1})	0.29	0.22 ± 0.02
Cl^-	(mmole l^{-1})	0.53	0.77 ± 0.02
HCO_3^-	(mmole l^{-1})	3	2.98 ± 0.09
PO_4^{3-}	(mmole l^{-1})	0.005	0.004 ± 0.0003
SO_4^{2-}	(mmole l^{-1})	0.01	0.014 ± 0.01

Table S2. Aqueous properties and As, Fe, Mn Concentrations

Liquid conc.(Solid eqv.) of Analytes	Control			*Shewanella* sp. ANA-3	
	C_0	C_{lac}	$C_{lac+kan}$	S_{kan}	$S_{lac+kan}$
23 days of incubation					
As_µg l^{-1}(mg kg^{-1})	nd*	nd*	nd*	2.6 ± 0.01 (0.005)	17.3 ± 3.7 (0.035)
As(III)_µg l^{-1}(mg kg^{-1})	nd*	nd*	nd*	nd*	16.1 ± 1.9 (0.032)
Fe_mg l^{-1}(mg kg^{-1})	nd*	7.6 ± 0.6 (17)	4.4 ± 0.4 (10)	0.4 ± 0.04 (1)	7.3 ± 1.1 (16)
Fe (II)_mg l^{-1}(mg kg^{-1})	nd*	7.6 ± 0.7 (17)	4.3 ± 0.4 (9)	0.4 ± 0.06 (1)	7.2 ± 1.1 (16)
Mn_mg l^{-1}(mg kg^{-1})	nd*	7.4 ± 0.9 (17)	8.1 ± 0.8 (18)	0.8 ± 0.08 (2)	7.9 ± 0.2 (18)
pH	8.13 ± 0.02	7.41 ± 0.02	7.11 ± 0.02	7.89 ± 0.02	7.13 ± 0.01
42 days of incubation					
As_µg l^{-1}(mg kg^{-1})	nd*	0.9 ± 0.1 (0.002)	0.8 ± 0.1 (0.002)	2.1 ± 0.03 (0.004)	17.0 ± 2.4 (0.034)
As(III)_µg l^{-1}(mg kg^{-1})	nd*	nd*	nd*	nd*	12.1 ± 1.8 (0.024)
Fe_mg l^{-1}(mg kg^{-1})	nd*	15.0 ± 0.7 (33)	13.3 ± 0.2 (30)	0.05 ± 0.02 (0.1)	14.2 ± 0.8 (32)
Fe (II)_mg l^{-1}(mg kg^{-1})	nd*	15.0 ± 0.6 (33)	12.9 ± 0.2 (29)	nd*	14.2 ± 0.8 (32)
Mn_mg l^{-1}(mg kg^{-1})	nd*	8.1 ± 0.03(18)	7.8 ± 0.2 (17)	0.5 ± 0.02 (1)	7.5 ± 0.2 (17)
pH	7.87 ± 0.01	6.90 ± 0.02	6.86 ± 0.02	7.71± 0.01	6.90 ± 0.01
92 days of incubation					
As_µg l^{-1}(mg kg^{-1})	nd*	1.7 ± 0.1 (0.003)	1.3 ± 0.01 (0.002)	2.0 ± 0.03 (0.004)	16.1 ± 1.2 (0.032)
As(III)_µg l^{-1}(mg kg^{-1})	nd*	nd*	nd*	nd*	11.9 ± 0.5 (0.024)
Fe_mg l^{-1}(mg kg^{-1})	nd*	15.7 ± 0.03 (35)	13.9 ± 0.3 (31)	0.05 ± 0.01 (0.1)	21.8 ± 5.0 (48)
Fe (II)_mg l^{-1}(mg kg^{-1})	nd*	15.6 ± 0.03 (35)	14.0 ± 0.3 (31)	nd*	21.7 ± 5.0 (48)
Mn_mg l^{-1}(mg kg^{-1})	nd*	15.6 ± 0.2 (35)	14.4 ± 0.2 (32)	0.6 ± 0.02 (1)	16.1 ± 1.7 (36)
pH	8.17 ± 0.01	7.45 ± 0.01	7.45 ± 0.01	7.42 ± 0.02	7.36 ± 0.02

*** : not detectable. Detection limit for As and Mn by HR ICPMS are 0.10 µgl^{-1} (0.0002 mgkg^{-1}) and 1 µgl^{-1} (0.002 mgkg^{-1}) respectively. Detection limit for AsIII is 2 µgl^{-1} (0.004 mgkg^{-1}) by DPCSV and for Fe is 0.03 mgl^{-1} (0.06 mgkg^{-1}) by Ferrozine method.**

Table S3. Sediment properties and As, Fe, Mn Concentrations

Solid Phase parameters	Pre-incubated	Control			*Shewanella* sp. ANA-3	
		C_0	C_{lac}	$C_{lac+kan}$	S_{kan}	$S_{lac+kan}$
		a. Post-incubated sediments after 23 days				
Color	Orange	Orange	Orange	Orange	Orange	Grey
$[As_{solid}]/[As_{liquid}]$ (L/kg)		>320[#]	>320[#]	>320[#]	218	33
P-extractable As (mg kg^{-1})	0.14 ± 0.03	0.15 ± 0.01	0.17 ± 0.05	0.15 ± 0.03	0.58 ± 0.04	0.56 ± 0.01
P-extractable AsIII (mg kg^{-1})	nd[*]	nd[*]	nd[*]	nd[*]	nd[*]	nd[*]
24 hrs HCl leachable Fe (g kg^{-1})	10.6 ± 1.2	12 ± 0.6	12 ± 0.8	11 ± 0.7	11 ± 1.6	12 ± 0.3
1 hr HCl leachable Fe (g kg^{-1})	0.79 ± 0.15	3.43 ± 0.69	5.63 ± 0.63	5.11 ± 0.83	2.94 ± 0.12	5.4 ± 0.04
1 hr HCl leachable FeII (g kg^{-1})	0.04 ± 0.002	0.2 ± 0.02	0.41 ± 0.01	0.33 ± 0.02	0.2 ± 0.03	3.2 ± 0.02
HCl leachable FeII/Fe ratio	0.051	0.061	0.073	0.065	0.068	0.593
HCl leachable Mn (g kg^{-1})	0.2 ± 0.05	0.2 ± 0.09	0.3 ± 0.03	0.2 ± 0.04	0.2 ± 0.04	0.2 ± 0.02
		b. Post-incubated sediments after 92 days				
Color	Orange	Orange	Grey	Grey	Orange	Grey
$[As_{solid}]/[As_{liquid}]$ (L/kg)		>320[#]	129	129	318	31
P-extractable As (mg kg^{-1})	0.14 ± 0.03	0.14 ± 0.03	0.22 ± 0.05	0.19 ± 0.04	0.62 ± 0.01	0.51 ± 0.04
P-extractable AsIII (mg kg^{-1})	nd[*]	nd[*]	nd[*]	nd[*]	0.09 ± 0.01	0.29 ± 0.01
24 hrs HCl leachable Fe (g kg^{-1})	10.6 ± 1.2	12 ± 0.1	16 ± 1.0	18 ± 0.7	12 ± 0.5	23 ± 0.3
1 hr HCl leachable Fe (g kg^{-1})	0.79 ± 0.15	2.43 ± 0.23	3.62 ± 0.05	3.46 ± 0.39	3.88 ± 0.08	4.12 ± 0.30
1 hr HCl leachable FeII (g kg^{-1})	0.04 ± 0.002	0.24 ± 0.03	2.55 ± 0.04	1.52 ± 0.02	0.28 ± 0.02	3.16 ± 0.16
HCl leachable FeII/Fe ratio	0.051	0.099	0.702	0.439	0.072	0.767
HCl leachable Mn (g kg^{-1})	0.2 ± 0.05	0.2 ± 0.10	0.3 ± 0.04	0.2 ± 0.03	0.2 ± 0.07	0.3 ± 0.10

***: not detectable. # : liquid phase As was not detectable**

Table S4. Sorption of As on post-incubated Pleistocene aquifer sands

Incubation Types	C-1			C-2			C_{lac}-1			C_{lac}-2		
Parameters	pH	*As	#As(III)	pH	*As	#As(III)	pH	*As	#As(III)	pH	*As	#As(III)
		μg l^{-1}			μg l^{-1}			μg l^{-1}			μg l^{-1}	
AGW	7.89	<0.1	<2	7.89	<0.1	<2	7.89	<0.1	<2	7.89	<0.1	<2
Sampling Interval (days)	Spiked 10 μl of 1000 mg l^{-1} As(III) to 10 ml of AGW											
0	7.99	994	996	8.00	994	996	7.38	994	996	7.39	994	996
1	7.53	159	137	7.85	132	136	7.42	192	191	7.38	160	159
2	7.76	122	116	8.14	130	127	7.50	190	134	7.50	145	103
3	8.00	117	118	8.19	126	121	7.54	138	103	7.53	154	146
19	7.85	74	71	7.84	118	121	7.42	85	102	7.31	125	115
51	7.90	86	79	7.89	102	85	7.40	108	102	7.27	89	77
Sampling Interval (days)	Re-spiked 10 μl of 1000 mg l^{-1} As(III) to AGW (volume made to 10 ml)											
0	7.90	1076	1075	7.89	1092	1081	7.40	1098	1098	7.27	1079	1073
2	7.83	328	344	7.88	389	386	7.41	318	290	7.37	312	309
16	7.87	286	244	7.90	332	326	7.49	312	296	7.38	283	279
37	7.79	256	246	7.92	299	286	7.42	289	294	7.37	264	259
Sampling Interval (days)	Re-spiked 10 μl of 1000 mg l^{-1} As(III) to AGW (volume made to 10 ml)											
0	7.79	1318	1340	7.92	1379	1382	7.42	1279	1286	7.37	1254	1305
2	7.82	775	761	7.89	873	897	7.45	632	629	7.39	568	564
4	7.85	768	751	7.88	859	877	7.43	622	618	7.37	571	574

* Detection limit for As by HR ICPMS is 0.10 μg l^{-1}. # Detection limit for AsIII is 2 μg l^{-1} by DPCSV

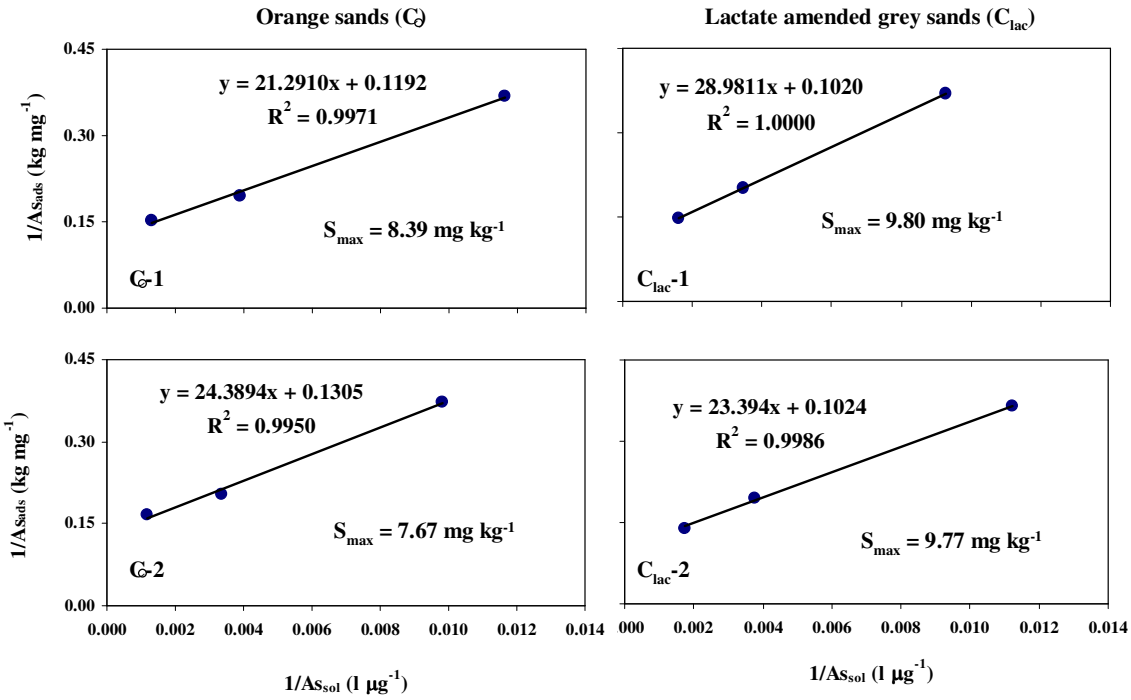

Figure S1: Isotherm and Partitioning of As in post incubated deep Pleistocene orange sediments of Araihazar, Bangladesh. Sediments without lactate amendment were shown in left panel (C_0-1 & C_0-2) and lactate amended sediments (C_{lac}-1 & C_{lac}-2) were shown in right panel. Sorptive capacity of the sediments was indicated by Smax, calculated using the Langmuir equation and was expressed as maximum sorption of As (III) by the dominant Fe(III) oxihydroxide surfaces in the Pleistocene aquifer sediments.