

## Supplementary Information

### **A Novel Approach Coupling Ferrous Iron Bio-oxidation and Ferric Iron Chemo-reduction to Promote Biomineralization in Simulated Acidic Mine Drainage**

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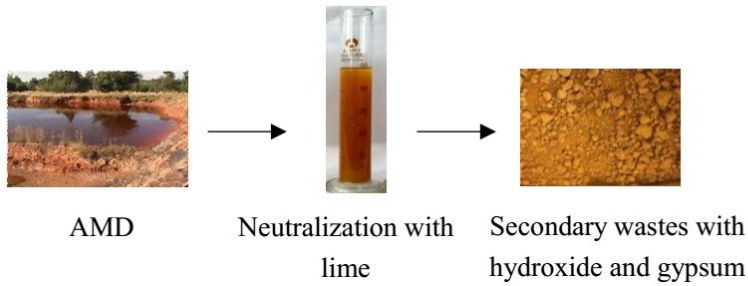
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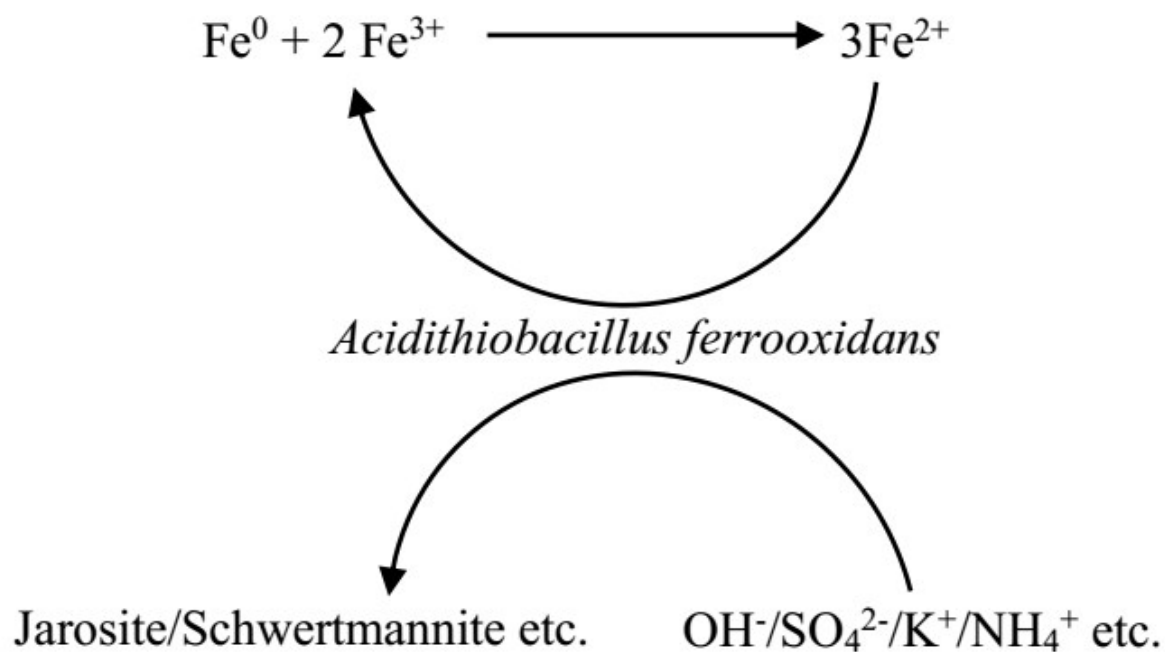
**(A) AMD treatment by neutralization with lime**



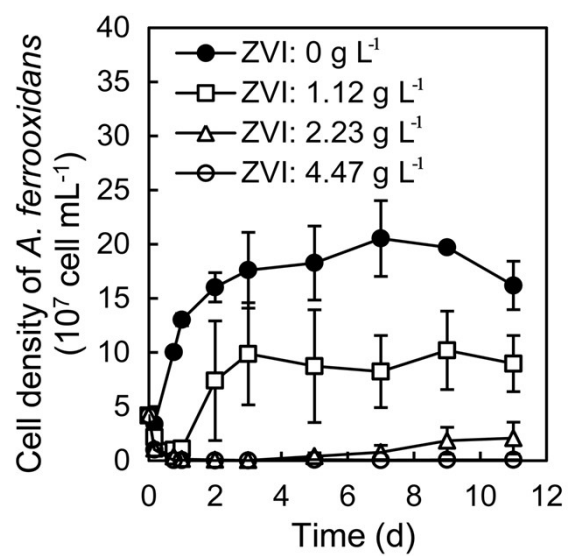
**(B) AMD treatment by ZVI coupling system before neutralization with lime**



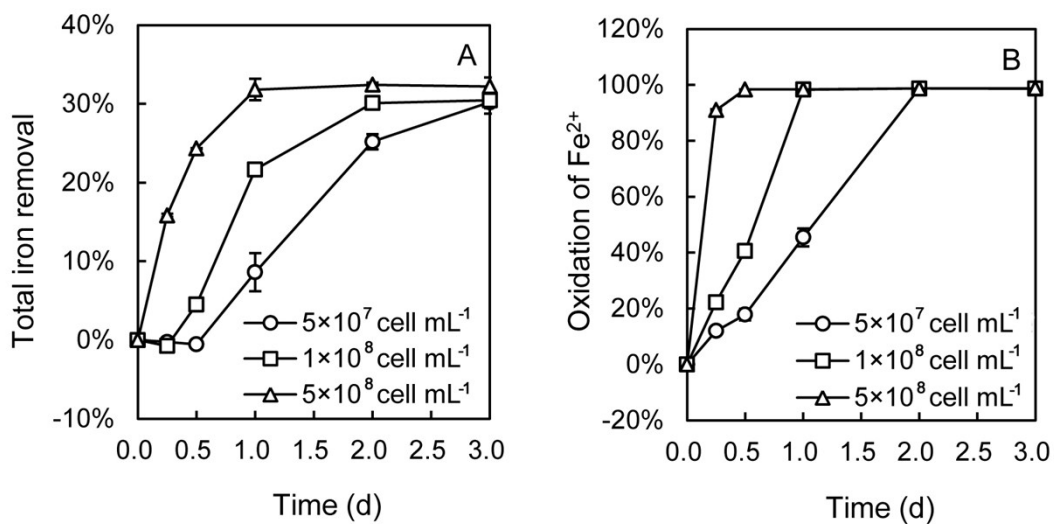
**Fig. S1** The flow chart of (A) AMD treatment by neutralization with lime and (B) AMD treatment by biological mineralization in *A. ferrooxidans* system coupling with zero-valent iron before neutralization with lime



**Fig. S2** Diagram of coupling process of *A. ferrooxidans* mediated biological mineralization and chemical reduction with zero-valent iron.



**Fig. S3** Toxicity of ZVI powder (1.12~4.47 g L<sup>-1</sup>) added initially to *A. ferrooxidans* density in bio-mineralization system.



**Fig. S4** Total iron removal (A) and oxidation of Fe<sup>2+</sup> (B) in *A. ferrooxidans*-mediated bio-mineralization system inoculated with three cell density by 5 × 10<sup>7</sup>, 1 × 10<sup>8</sup>, 5 × 10<sup>8</sup> cell mL<sup>-1</sup> initially.

**Table S1** Amount of reductive reagents addition in approach coupling with bio-oxidation and chemo-reduction

	Amount of ZVI addition (g)													
Day	3	5	7	9	11	13	15	17	19	21	23	25	27	29
Exp. #1														
ZVI	0.181	0.157	0.134	0.122	0.108	0.095	0.077	0.07	0.068	0.05	0.04	0.04	0.04	0.03
Exp. #2														
ZVC ( Cu ) *	0.224	0.139	0.088	0.063	0.041	0.028	0.019	0.017	0.009	0.008	0.005	0.002	0.002	0.002
ZVI ( Fe )	0.197	0.173	0.154	0.136	0.127	0.109	0.103	0.093	0.079	0.073	0.067	0.059	0.053	0.048
Exp. #3														
T1-2d	0.210	0.194	0.157	0.144	0.126	0.121	0.111	0.118	0.086	0.076	0.071	0.062		
T2-4d	0.210		0.170		0.146		0.145		0.121		0.104			
T3-8d	0.210				0.145				0.132					
Exp. #4														
N-ZVI	0.189	0.172	0.162	0.136	0.122	0.118	0.108	0.096	0.088	0.083	0.074	0.060	0.059	0.054
NF-ZVI	0.191	0.173	0.168	0.056	0.136	0.123	0.112	0.101	0.094	0.087	0.075	0.069	0.064	0.059
Exp. #5														
AMD-ZVI	0.310	0.295	0.260	0.224	0.193	0.165	0.141	0.118	0.092					

\*ZVC was added in system instead of ZVI.

**Table S2** ZVC and ZVI reductive efficiency

Days	ZVC			ZVI		
	Before reduction	After reduction	Ratio of reduction	Before reduction	After reduction	Ratio of reduction
	Fe <sup>3+</sup> [g L <sup>-1</sup> ]	Fe <sup>3+</sup> [g L <sup>-1</sup> ]		Fe <sup>3+</sup> [g L <sup>-1</sup> ]	Fe <sup>3+</sup> [g L <sup>-1</sup> ]	
3	28.33±0.08	10.08±0.86	0.64±0.03	28.58±0.24	8.34±2.08	0.71±0.07
5	17.57±0.42	8.02±0.40	0.54±0.02	24.78±0.75	10.01±1.17	0.59±0.06
7	10.95±0.27	7.05±0.12	0.36±0.03	21.76±0.37	10.81±1.98	0.50±0.09
9	7.91±0.15	4.81±0.14	0.39±0.01	19.51±0.20	9.96±1.53	0.49±0.08
11	5.22±0.15	3.24±0.00	0.38±0.02	18.04±0.39	9.01±0.99	0.50±0.04
13	3.49±0.10	2.84±0.23	0.19±0.05	15.66±0.62	9.73±0.36	0.38±0.02
15	2.41±0.20	2.19±0.13	0.09±0.03	14.71±0.68	6.60±1.55	0.55±0.13
17	2.11±0.08	1.64±0.23	0.22±0.08	13.35±0.62	6.31±0.35	0.53±0.05
19	1.16±0.10	1.33±0.15	-0.15±0.14	11.29±0.99	5.42±0.64	0.52±0.06
21	1.02±0.10	1.06±0.08	-0.05±0.06	10.46±1.15	5.51±0.41	0.47±0.05
23	0.61±0.16	0.82±0.22	-0.34±0.10	9.55±1.26	4.79±0.69	0.48±0.12
25	0.21±0.10	0.56±0.11	-2.35±1.57	8.41±1.09	3.98±0.46	0.52±0.10
27	0.19±0.16	0.37±0.017	1.46±3.02	7.64±0.92	3.74±0.28	0.51±0.04
29	0.27±0.10	0.25±0.03	-0.06±0.37	6.93±1.04	3.26±0.39	0.52±0.06