

Supplementary

Simultaneous Sequestration of Co²⁺ and Mn²⁺ by Fungal Manganese Oxide through Asbolane Formation

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Table S1, and Figures S1-S5.

Table S1 Summary of repeated treatment experiments for newly formed and heated biogenic manganese oxides (BMOs)^a under aerobic conditions at pH 7.0^b.

Condition		Sequestration efficiency ^c (%) (added ions; mM)			Two-step extraction					
		Co ²⁺		Mn ²⁺	Total Co (mM)	Exchangeable (%)	Reducible (%)	Total Mn (mM)	Exchangeable (%)	Reducible (%)
Newly formed Aerobic Only Mn ²⁺ (n = 4)	Before				-	-	-	0.87±0.04	13.6±0.3	86.4±0.3
	1st	-	>99	(0.84±0.10)	-	-	-	1.79±0.05	15.2±0.5	84.8±0.5
	2nd	-	>99	(0.77±0.14)	-	-	-	2.70±0.06	13.3±0.2	86.7±0.2
	3rd	-	98.8±0.3	(0.78±0.03)	-	-	-	3.60±0.04	11.6±0.1	88.6±0.1
	Total	-	>99		-	-	-			
Newly formed Aerobic Only Co ²⁺ (n = 4)	Before				-	-	-	1.00±0.06	15.1±0.2	84.9±0.2
	1st	>99	(0.16±0.00)	-	0.14±0.00	49.4±1.7	50.6±1.7	0.87±0.04	6.4±0.4	93.6±0.4
	2nd	70.0±1.3	(0.16±0.00)	-	0.27±0.02	56.8±1.5	43.2±1.5	0.83±0.07	4.0±0.5	96.0±0.5
	3rd	36.3±6.2	(0.17±0.02)	-	0.32±0.01	59.1±1.5	40.9±1.5	0.79±0.02	1.9±0.0	98.0±0.0
	Total	68.4±1.5		-						
Newly formed Aerobic Only Co ²⁺ (n = 3)	Before				-	-	-	1.05±0.02	15.0±0.4	85.0±0.4
	1st	85.9±1.5	(0.42±0.00)	-	0.43±0.02	51.5±0.6	48.5±0.6	1.08±0.16	3.6±0.1	96.4±0.1
	2nd	43.4±1.7	(0.43±0.01)	-	0.72±0.02	45.4±1.9	54.6±1.9	1.28±0.14	2.7±0.1	97.3±0.1
	3rd	23.7±1.6	(0.43±0.01)	-	0.68±0.01	44.0±0.7	56.0±0.7	0.89±0.04	3.0±0.1	97.0±0.1
	Total	51.0±0.8		-						
Newly formed Aerobic Only Co ²⁺ (n = 4)	Before				-	-	-	1.04±0.05	13.2±0.2	86.8±0.2
	1st	85.4±0.8	(0.81±0.01)	-	0.75±0.00	38.4±0.1	61.6±0.1	1.01±0.02	2.8±0.1	97.2±0.1
	2nd	47.7±1.1	(0.80±0.01)	-	1.19±0.02	29.9±0.7	70.1±0.7	1.01±0.04	2.2±0.2	97.8±0.2
	3rd	24.4±1.9	(0.80±0.01)	-	1.24±0.11	29.4±3.0	70.6±3.0	0.81±0.10	2.6±0.3	97.4±0.3
	Total	52.7±0.7		-						
Newly formed Aerobic Only Co ²⁺ (n = 4)	Before				-	-	-	0.89±0.04	14.2±0.31	85.8±0.1
	1st	86.4±0.6	(1.29±0.02)	-	1.14±0.02	25.9±0.8	74.1±0.8	0.83±0.04	3.1±0.1	96.9±0.1
	2nd	42.4±1.5	(1.29±0.01)	-	1.58±0.03	21.6±0.4	78.4±0.4	0.80±0.02	2.5±0.0	97.5±0.0
	3rd	19.2±1.9	(1.26±0.02)	-	2.03±0.03	17.7±0.5	82.3±0.5	0.86±0.01	1.9±0.0	88.1±0.0
	Total	49.6±0.8		-						
Newly formed Aerobic Only Co ²⁺ (n = 4)	Before				-	-	-	0.81±0.01	14.7±0.3	85.3±0.3
	1st	81.5±3.6	(1.75±0.02)	-	1.41±0.03	22.6±0.7	77.4±0.7	0.81±0.03	1.6±0.1	98.4±0.1
	2nd	32.8±3.6	(1.72±0.02)	-	1.93±0.16	18.2±1.3	81.8±1.3	0.81±0.08	1.1±0.1	98.9±0.1
	3rd	13.2±2.6	(1.70±0.03)	-	1.80±0.09	17.1±0.4	82.9±0.4	0.76±0.06	15.6±0.8	84.4±0.8
	Total	42.9±3.1		-						
Heated Aerobic Only Co ²⁺ (n = 4)	Before				-	-	-	0.99±0.02	15.5±0.3	84.5±0.3
	1st	23.1±2.8	(0.84±0.01)	-	0.24±0.01	58.2±0.3	41.8±0.3	0.87±0.02	4.5±0.1	95.5±0.1
	2nd	10.7±6.9	(0.87±0.06)	-	0.28±0.02	56.0±1.3	44.0±1.3	0.82±0.06	3.1±0.2	96.9±0.2
	3rd	5.3±1.6	(0.84±0.01)	-	0.33±0.02	47.0±0.8	53.0±0.8	0.78±0.05	2.9±0.2	97.1±0.2
	Total	13.1±3.2		-						

a: BMOs were formed by cultivating *Acremonium strictum* KR21-2 in HAY liquid media with 1 mM MnSO₄ for 72h. b: HEPES buffer (100 mM) was used to maintain solution pH at pH7.0.

c: Sequestration efficiency was calculated from the concentration difference before and after treatments.

Table S1 (continued)

Condition	Sequestration efficiency (%) (added ions; mM)						Two-step extraction				
		Co ²⁺		Mn ²⁺		Total Co (mM)	Exchangeable (%)	Reducible (%)	Total Mn (mM)	Exchangeable (%)	Reducible (%)
Newly formed	1st	>99	(0.16±0.01)	>99	(0.79±0.07)	0.18±0.00	19.7±0.5	80.3±0.5	0.90±0.07	14.9±0.2	85.1±0.2
Aerobic	2nd	>99	(0.16±0.00)	>99	(0.76±0.03)	0.38±0.01	21.6±0.3	78.4±0.3	1.85±0.07	11.2±0.2	88.8±0.2
Co ²⁺ /Mn ²⁺	3rd	>99	(0.17±0.00)	>99	(0.82±0.05)	0.56±0.01	20.6±0.6	79.4±0.6	2.79±0.05	8.3±0.3	91.7±0.3
(n = 4)	Total	>99		>99					3.75±0.10	8.1±0.2	91.9±0.2
Newly formed	1st	97.5±0.7	(0.42±0.01)	97.9±0.1	(0.77±0.01)	0.49±0.00	38.5±0.9	61.5±0.9	0.99±0.01	17.0±0.5	83.0±0.5
Aerobic	2nd	86.8±2.8	(0.43±0.01)	97.9±0.3	(0.83±0.08)	0.92±0.01	29.2±0.3	70.8±0.3	1.93±0.07	5.8±0.4	94.2±0.4
Co ²⁺ /Mn ²⁺	3rd	63.3±7.3	(0.42±0.00)	91.9±3.6	(0.82±0.05)	1.18±0.04	24.3±1.1	75.7±1.1	2.84±0.05	4.3±0.0	95.7±0.0
(n = 3)	Total	82.5±3.6		95.9±1.4					3.53±0.10	5.1±0.3	94.9±0.3
Newly formed ^d	1st	92.7±1.0	(0.79±0.01)	>99	(0.88±0.03)	0.82±0.02	32.4±0.6	67.6±0.2	1.05±0.00	15.2±0.5	84.8±0.5
Aerobic	2nd	71.6±3.5	(0.80±0.02)	98.5±0.2	(0.84±0.02)	1.52±0.04	26.2±0.8	73.8±0.8	1.97±0.07	3.7±0.1	96.3±0.1
Co ²⁺ /Mn ²⁺	3rd	48.4±7.8	(0.81±0.01)	87.3±6.6	(0.85±0.01)	1.93±0.04	21.5±1.9	78.5±1.9	3.08±0.07	4.7±0.6	95.3±0.6
(n = 4)	Total	70.8±4.0		95.0±2.2					3.85±0.18	5.5±0.3	94.5±0.3
Newly formed	1st	76.7±2.4	(1.41±0.02)	97.8±0.8	(0.75±0.01)	0.61±0.03	22.5±0.3	77.5±0.3	0.81±0.03	19.4±0.2	80.6±0.2
Aerobic	2nd	34.4±5.3	(1.44±0.04)	82.8±4.3	(0.77±0.03)	1.09±0.07	17.5±0.6	82.5±0.6	1.39±0.04	7.3±0.1	92.7±0.1
Co ²⁺ /Mn ²⁺	3rd	20.6±4.4	(1.49±0.05)	44.1±5.9	(0.76±0.04)	1.75±0.06	20.1±1.0	79.9±1.0	2.06±0.17	6.2±0.6	93.8±0.6
(n = 4)	Total	43.5±3.2		74.9±3.0					2.91±0.14	5.8±0.3	94.2±0.3
Newly formed	1st	66.8±1.4	(1.74±0.04)	99.1±0.1	(0.80±0.01)	1.32±0.03	26.5±0.6	73.4±0.6	0.81±0.01	14.7±0.3	85.3±0.3
Aerobic	2nd	24.9±4.6	(1.74±0.01)	74.5±6.4	(0.81±0.01)	1.85±0.10	21.9±1.3	78.1±1.3	1.68±0.04	2.9±0.1	97.1±0.1
Co ²⁺ /Mn ²⁺	3rd	8.6±3.8	(1.69±0.01)	38.6±2.9	(0.79±0.01)	1.81±0.04	19.8±1.1	80.2±1.1	2.35±0.15	5.4±0.5	94.6±0.5
(n = 4)	Total	33.7±1.7		70.9±2.1					2.38±0.06	0.4±0.0	99.6±0.0
Heated	1st	26.2±2.4	(0.87±0.01)	6.0±9.8	(0.89±0.01)	0.22±0.01	46.6±0.4	53.4±0.4	0.99±0.02	15.5±0.2	84.5±0.2
Aerobic	2nd	12.2±2.4	(0.89±0.02)	14.2±2.1	(0.91±0.01)	0.31±0.01	39.9±0.5	60.1±0.5	0.89±0.06	10.6±0.2	89.4±0.2
Co ²⁺ /Mn ²⁺	3rd	12.1±4.4	(0.93±0.03)	12.9±2.5	(0.93±0.02)	0.39±0.04	37.4±0.9	62.6±0.9	0.89±0.04	9.7±0.2	90.3±0.2
(n = 4)	Total	16.7±2.8		11.1±4.0					1.16±0.09	13.8±0.2	86.2±0.2

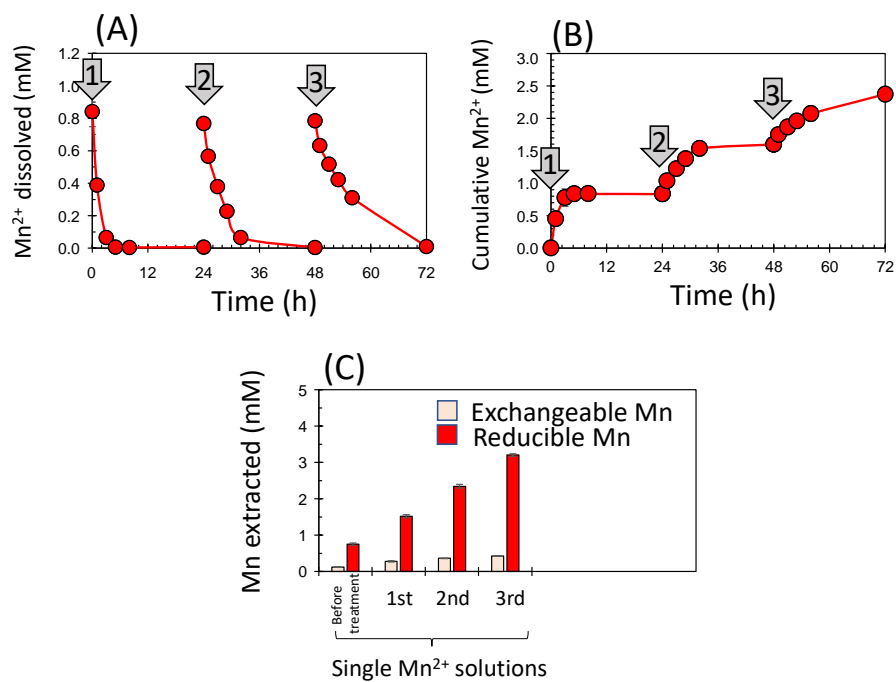


Figure S1. Repeated treatment experiments of newly formed biogenic manganese oxides (BMOs; 1 mM as Mn) under aerobic conditions at pH 7.0 (100 mM HEPES buffer) in 0.8 mM exogenous Mn^{2+} . (A) Changes in concentrations of dissolved Mn^{2+} , and (B) Cumulative concentrations of Mn sequestered. (C) Mn concentrations in exchangeable and reducible phases for BMOs repeatedly treated in 0.8 mM single Mn^{2+} solutions. The bathing solutions were renewed every 24 h. Data are shown as mean \pm standard deviation ($n = 4$).

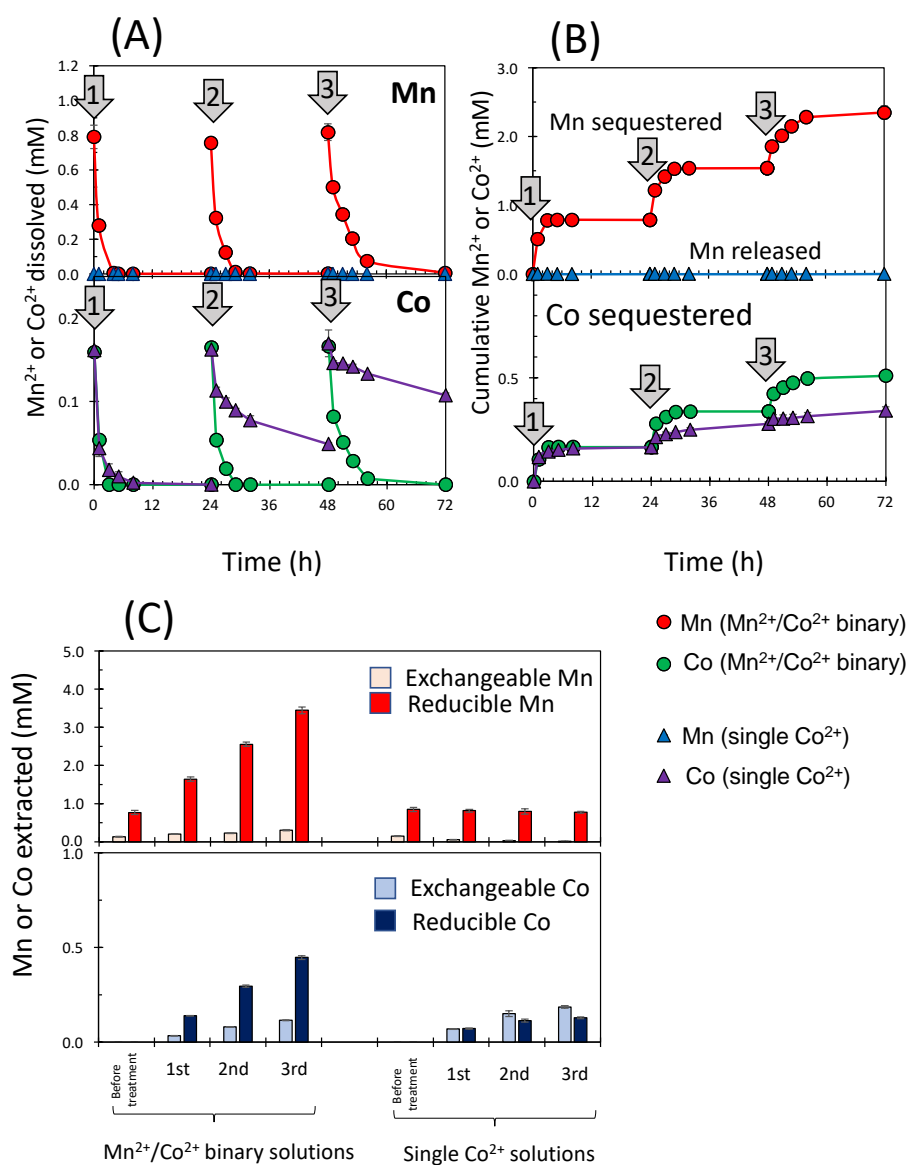


Figure S2. Repeated treatment experiments of newly formed biogenic manganese oxides (BMOs; 1 mM as Mn) under aerobic conditions at pH 7.0 (100 mM HEPES buffer) in 0.16 mM Co²⁺ solutions with and without 0.8 mM exogenous Mn²⁺. (A) Changes in concentrations of dissolved Mn²⁺ and Co²⁺. (B) Cumulative concentrations of sequestered or released Mn, and sequestered Co, where circles represent Mn²⁺/Co²⁺ binary systems and triangles represent single Co²⁺ systems. (C) Mn and Co concentrations in exchangeable and reducible phases for BMOs repeatedly treated in Mn²⁺/Co²⁺ binary and single Co²⁺ solutions. Bathing solutions were renewed every 24 h. Data are mean \pm standard deviation ($n = 4$).

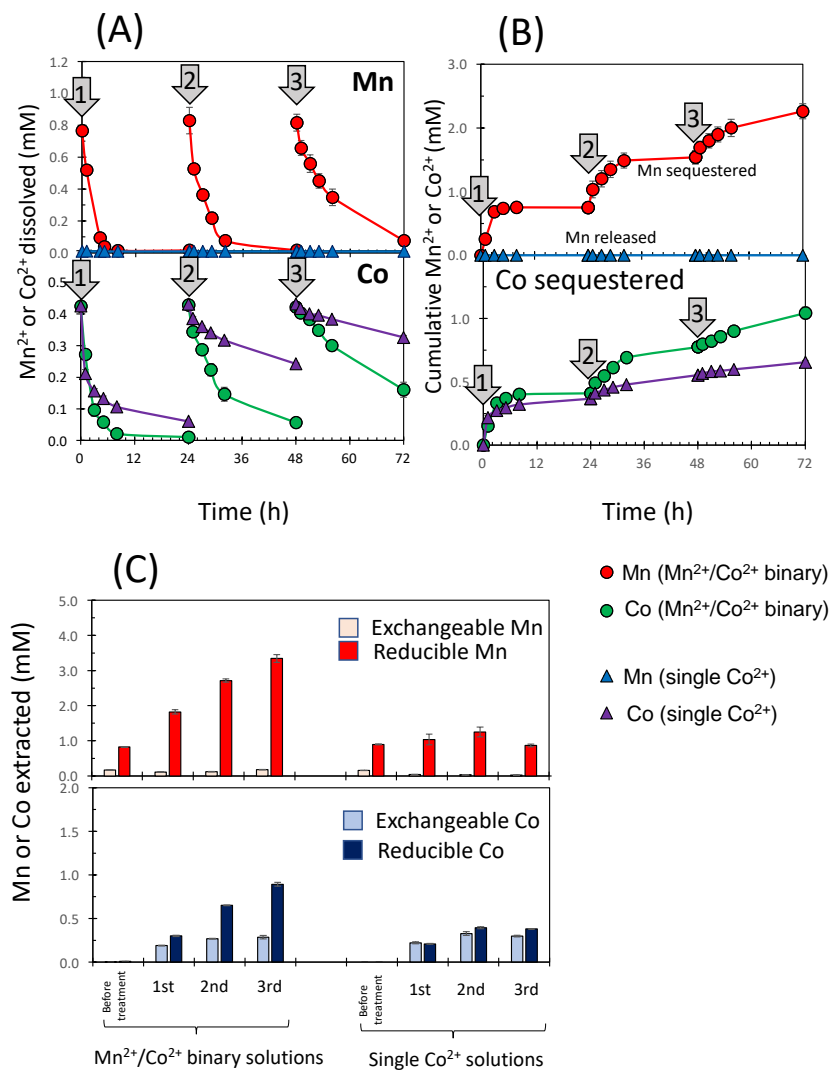


Figure S3. Repeated treatment experiments of newly formed biogenic manganese oxides (BMOs; 1 mM as Mn) under aerobic conditions at pH 7.0 (100 mM HEPES buffer) in 0.4 mM Co^{2+} solutions with and without 0.8 mM exogenous Mn^{2+} . (A) Changes in concentrations of dissolved Mn^{2+} and Co^{2+} . (B) Cumulative concentrations of sequestered or released Mn, and sequestered Co, where circles represent $\text{Mn}^{2+}/\text{Co}^{2+}$ binary systems and triangles represent single Co^{2+} systems. (C) Mn and Co concentrations in exchangeable and reducible phases for BMOs repeatedly treated in $\text{Mn}^{2+}/\text{Co}^{2+}$ binary and single Co^{2+} solutions. Bathing solutions were renewed every 24 h. Data are mean \pm standard deviation ($n = 4$).

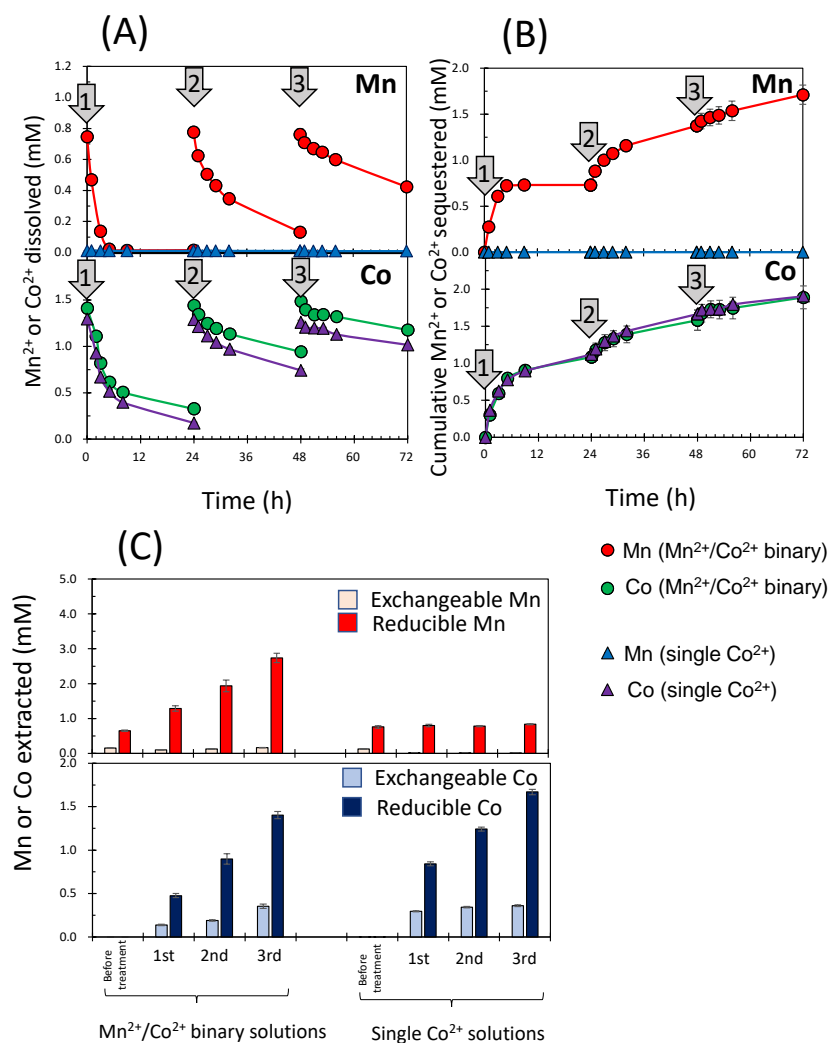


Figure S4. Repeated treatment experiments of newly formed biogenic manganese oxides (BMOs; 1 mM as Mn) under aerobic conditions at pH 7.0 (100 mM HEPES buffer) in 1.4 mM Co^{2+} solutions with and without 0.8 mM exogenous Mn^{2+} . (A) Changes in concentrations of dissolved Mn^{2+} and Co^{2+} . (B) Cumulative concentrations of sequestered or released Mn, and sequestered Co, where circles represent $\text{Mn}^{2+}/\text{Co}^{2+}$ binary systems and triangles represent single Co^{2+} systems. (C) Mn and Co concentrations in exchangeable and reducible phases for BMOs repeatedly treated in $\text{Mn}^{2+}/\text{Co}^{2+}$ binary and single Co^{2+} solutions. Bathing solutions were renewed every 24 h. Data are mean \pm standard deviation ($n = 4$).

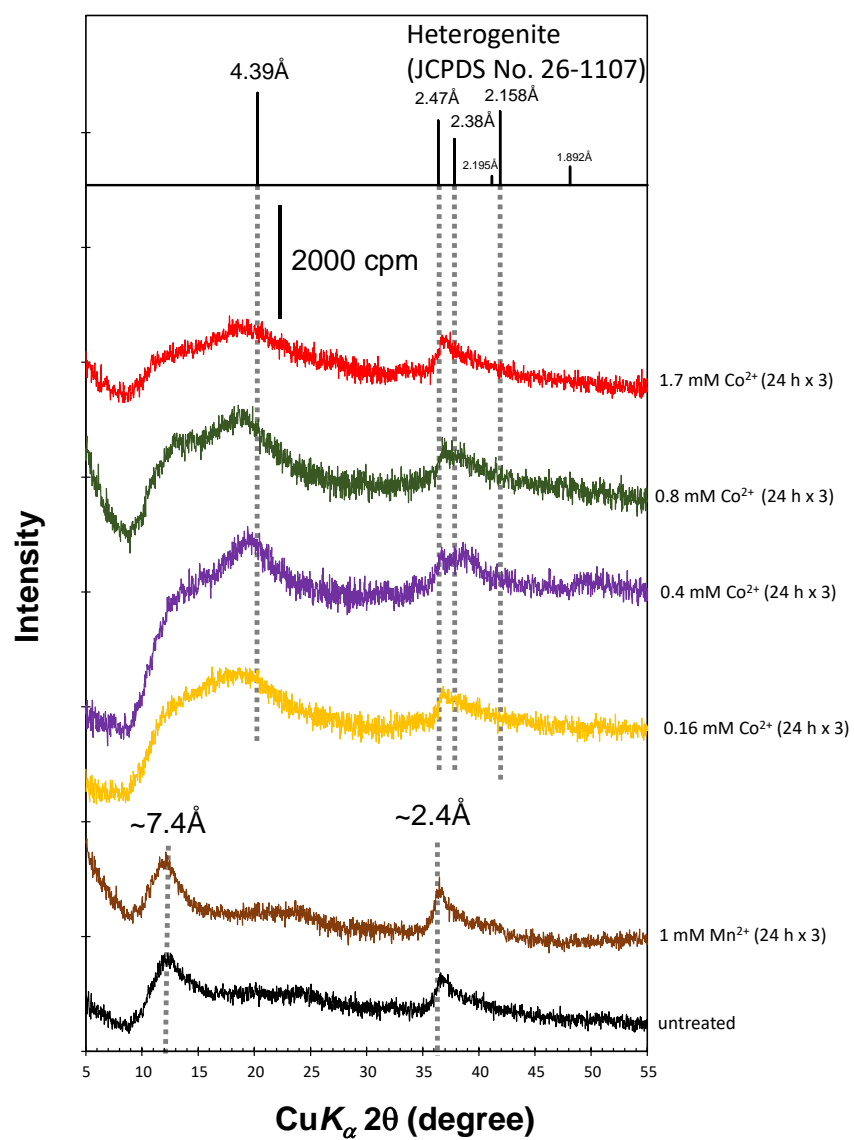


Figure S5. Powder X-ray diffraction patterns of newly formed biogenic manganese oxides (1 mM as Mn) before and after repeated treatment experiments in single Co^{2+} (0–1.7 mM) solutions under aerobic conditions at pH 7.0 (100 mM HEPES buffer).