Appendix A

Supporting Information for

Chapter 3: A Hydrous Manganese Oxide Doped Gel Probe Sampler for Measuring *In Situ* Reductive Dissolution Rates: I. Laboratory Development

Contents: 6 tables and 8 figures

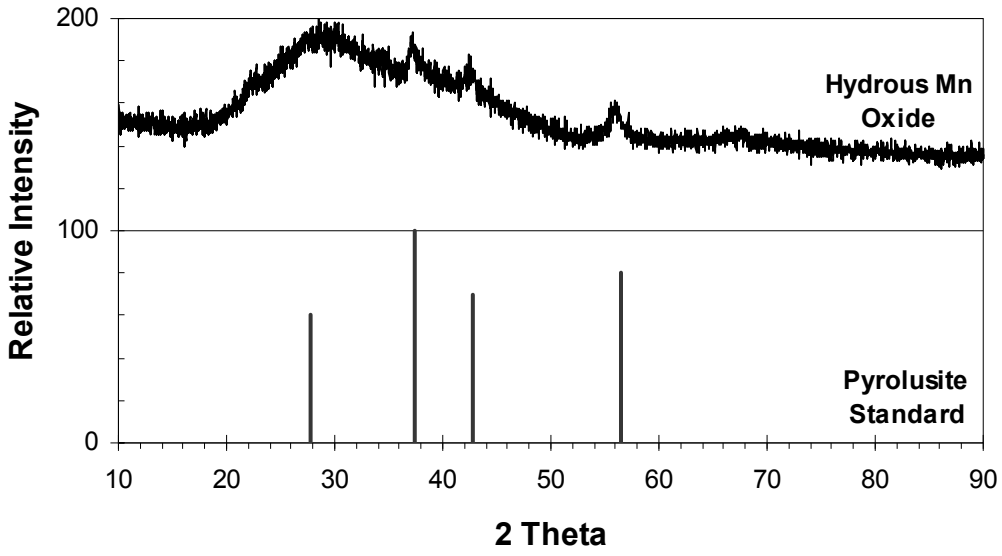


Figure A.1. X-ray diffractogram for synthesized HMO with the pyrolusite (β-MnO_2) standard, the best match for the small peaks. The broad, ill-defined peak is indicative of an amorphous solid.

Table A.1. Final mass balance in abiotic miniprobe reductive dissolution experiments

Reductant, pore size	(1) Total Lost from Gels	(2) Storage Soln. before Expt.[a]	(3) Reduced from Gels[b]	(4) Bulk Soln.	% Unaccounted[c]
Ascorbate 0.45 μm	7.39	3.68	3.71	3.55	2.2%
1.0 μm	7.50	3.68	3.81	3.70	1.5%

Listed data are μmoles of Mn.

[a] The percentage of Mn lost to the gel storage solution (50%) is relatively high in this example. Typically ≤ 20% of Mn leached into the storage solution.

[b] The amount of Mn reduced from the gels (3) is equal to the difference between the total Mn lost from the gels (1) and the Mn lost to the gel storage solution before the experiment (2).

[c] Perfect mass balance would lead to the amount of Mn reduced from the gels (3) equaling the amount of Mn measured in the bulk solution at the end of the experiment (4). The percentage is given as: $[(3) - (4)]\cdot(1)^{-1}$.

Table A.2. Basic components of MR-1 minimal medium[a]

Chemical	Concentration (mM or given)
Sodium Lactate	6
PIPES	3
Sodium Hydroxide	7.5
Ammonium Chloride	28.0
Potassium Chloride	1.34
Sodium Phosphate (monobasic)	4.35
Sodium Fumarate	30
Vitamins:	(nM)
d-biotin	81.9
folic acid	45.3
pyridoxine-HCl	486
riboflavin	133
thiamine-HCl·H_2O	141
nicotinic acid	406
d-pantothenic acid, hemicalcium salt	210
B12	0.74
p-aminobenzoic acid	365
thioctic acid	242
Amino Acids:	(mg l^{-1})
L-glutamic acid	2
L-arginine	2
DL-serine	2

[a] In addition, 10 ml of Wolfe's Mineral Solution (which comprises trace amounts of Mg, Mn, Na, Fe, Ca, Co, Zn, Cu, Al, B, and Mo salts) were added to this medium.

Although direct reduction of Mn oxide by the MR-1 medium was not tested, previous studies of the individual compounds suggest this would have a negligible effect on the measured rates. Fumarate, lactate, and ammonia do not appear to reduce Mn oxide when present in excess (Stone and Morgan 1984, Burdige and Nealson 1986). Only DL-lactic acid reduced MnO_2, at an average rate of 0.47 μmol m^{-2} h^{-1} at pH 5 (Wang and Stone 2006a), which corresponds to a range of 3.4–20×10^{-11} M s^{-1} for the conditions of our study; even at the maximum estimated rate, this would account for less than 5% of our measured HMO reduction rate.

Table A.3. Parameters input to or solved by AQUASIM model

Experiment		$[C_6H_7O_6^-]_{0,\ bulk}$ or $[Red]_{0,\ bulk}$ (mM)	$[HMO]_{0,\ gel}$ (mM)[a]	k ($M^{-1}\ s^{-1}$)	k″ (s^{-1})	A ($m^2\ l^{-1}$)	D_{Mn} ($cm^2 s^{-1}$)	$D_{A,\ bulk}$ or $D_{Red,\ bulk}$ ($cm^2 s^{-1}$)	$D_{A,\ gel}$ or $D_{Red,\ gel}$ ($cm^2 s^{-1}$)	V_{bulk} (ml)	χ^2
Abiotic	HMO gels	2.0	7.77	4.2	---	33.1	6.88e-6	3.0e-6	4.28e-7[b]	10	2.3
	MP 1.0 μm	2.0	6.68	4.2	---	28.5	6.88e-6	3.0e-6	1.03e-6[b]	25	2.0
Microbially-mediated	HMO	1.182	0.360[d]	3.5e-3	7.5e-6 [b]	1.53	---	---	---	55	2.6
	HMO gels	1.182[c]	10.2	3.5e-3[b]	---	25.0	6.88e-6	3.0e-6	4.28e-7	10	1.2
	MP 1.0 μm	1.182	6.59	3.5e-3[b]	---	28.1	6.88e-6	3.0e-6	1.03e-6	25	1.4

[a] This concentration is normalized to the volume of pore space in the gel, which is approximately 92.3% × 0.225 g × 1 ml g^{-1}, or 0.208 ml.

[b] Denotes a fitted parameter.

[c] Assumed to be 5 times Mn_T for this system.

[d] In this case, the HMO was added to the bulk solution, and no gel compartment was necessary. Consequently, no diffusion coefficients were needed for this simulation.

Table A.4. AQUASIM mass balance at steady state

Experiment		t_{ss} (h)	HMO_0 (μmol)	$Mn_{T,t}$ (μmol)	% Error[a]	Red_0[b] (μmol)	Red_{red}[c] (μmol)	$Red_{T,t}$ (μmol)	% Error[a]
Abiotic	HMO gels	6	1.794	1.792	0.07%	20	1.792	18.25	0.2%
	MP 1.0 μm	25.1	1.542	1.541	0.04%	50.03	1.541	48.6	0.2%
Microbially-mediated	HMO	150	19.8	19.8	0.01%	65.01	6.91[d]	58.25	0.2%
	HMO gels	360	2.364	2.362	0.06%	11.82	2.362	9.48	0.2%
	MP 1.0 μm	420	1.521	1.520	0.09%	29.55	1.520	28.01	0.08%

[a] % Error is the absolute value of either the difference between HMO_0 and $Mn_{T,t}$ divided by HMO_0, or the difference between Red_0 and the sum of Red_{red} and $Red_{T,t}$ divided by Red_0.

[b] Red denotes the reductant, either ascorbate (abiotic) or the generic reductant (microbially mediated).

[c] The amount of Red that is reduced is equal to the amount of Mn(II) produced.

[d] The amount of Red that is reduced is equal to 35% of the amount of Mn(II) produced.

Table A.5a. [Mn(II)] release over time:
HMO-doped gels and ascorbate (184 μM Mn_T)

Time (hrs)	0.5	1	2	3	4
$[Mn]_{diss}$ (μM)	38	64	116	132	145
Std Dev (μM)	7	1	16	27	8

Table A.5b. [Mn(II)] release over time:
Miniprobes, 0.45 μm membrane, and ascorbate (52 μM Mn_T)

Time (hrs)	0.6	1.7	3.25	4.75
$[Mn]_{diss}$ (μM)	4.0	12	20	25
Std Dev (μM)	1	1	2	2

Table A.5c. [Mn(II)] release over time:
Miniprobes, 1.0 μm membrane, and ascorbate (52 μM Mn_T)

Time (hrs)	0.6	1.7	3.25	4.75
$[Mn]_{diss}$ (μM)	5.7	14	21	25
Std Dev (μM)	1	1	2	2

Table A.5d. [Mn(II)] release over time:
HMO and *S. oneidensis* MR-1 (360 μM Mn_T)

Time (hrs)	0.25	0.5	0.75	1	1.5	2	3	4
$[Mn]_{diss}$ (μM)	0.5	8.1	4.8	3.7	24	34	39	60
Std Dev (μM)	4	13	11	10	4	35	21	39

Table A.5e. [Mn(II)] release over time:
HMO-doped gels and *S. oneidensis* MR-1 (295 μM Mn_T)

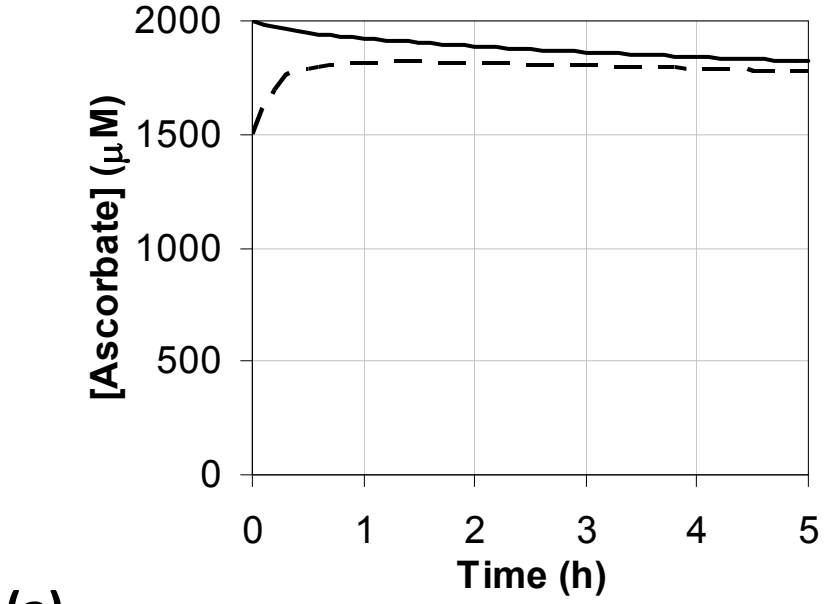
Time (hrs)	4.5	8	16	25	36
$[Mn]_{diss}$ (μM)	13	23	43	65	86
Std Dev (μM)	2	4	7	6	12

Table A.5f. [Mn(II)] release over time:
Miniprobes, 1.0 μm membrane, and *S. oneidensis* MR-1 (62 μM Mn_T)

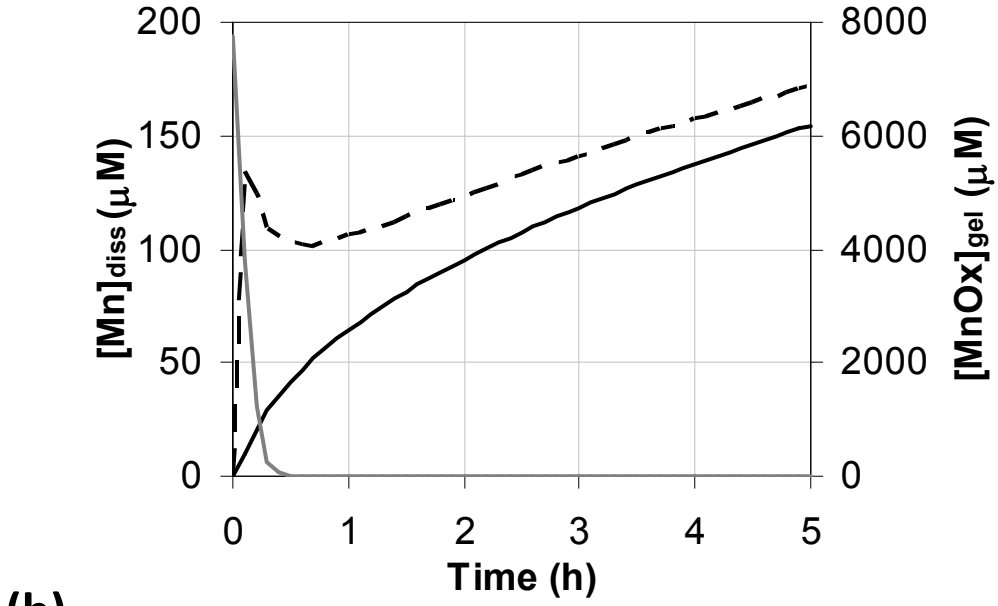
Time (hrs)	7.9	14	20	26	38.8	63
$[Mn]_{diss}$ (μM)	-0.4	2.2	5.5	8.1	14	27
Std Dev (μM)	1	2	1	3	4	6

Table A.5g. [Mn(II)] release over time:
Miniprobes, 5.0 μm membrane, and *S. oneidensis* MR-1 (60 μM Mn_T)

Time (hrs)	7.9	14	20	26	38.8	63
$[Mn]_{diss}$ (μM)	0.6	4.1	7.4	10	17	29
Std Dev (μM)	1	1	2	3	4	7

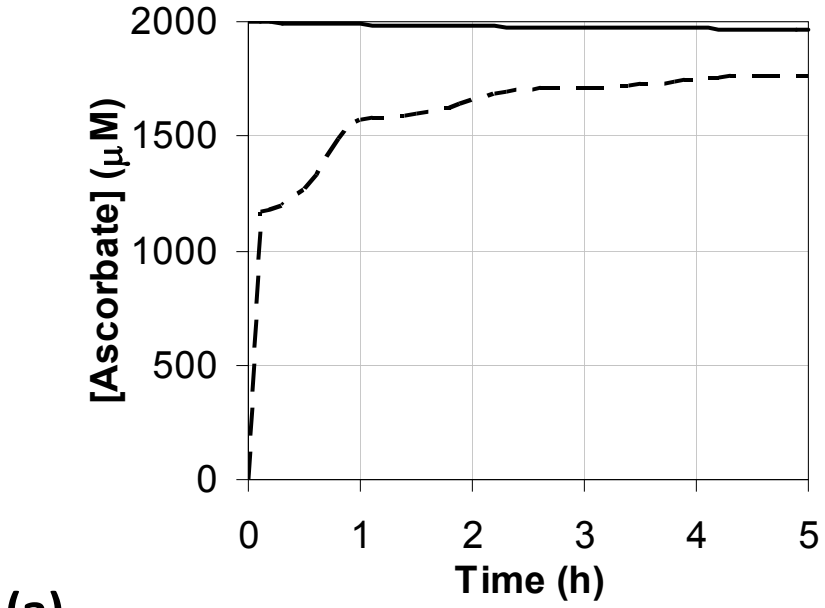


(a)

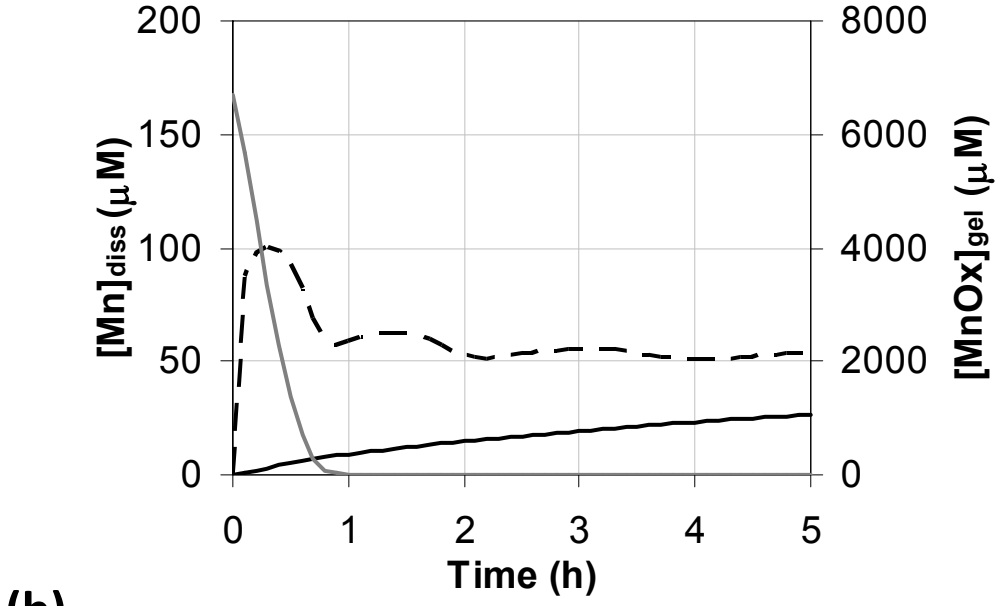


(b)

Figure A.2. AQUASIM model output for the reaction of ascorbate and HMO-doped gels in solution (modeled data is in Table A.5a). Shown are ascorbate (a) and dissolved Mn (b) in the gel (dashed line) and in the bulk (solid black line); the concentration of Mn oxide in the gel is represented with a solid gray line in (b).

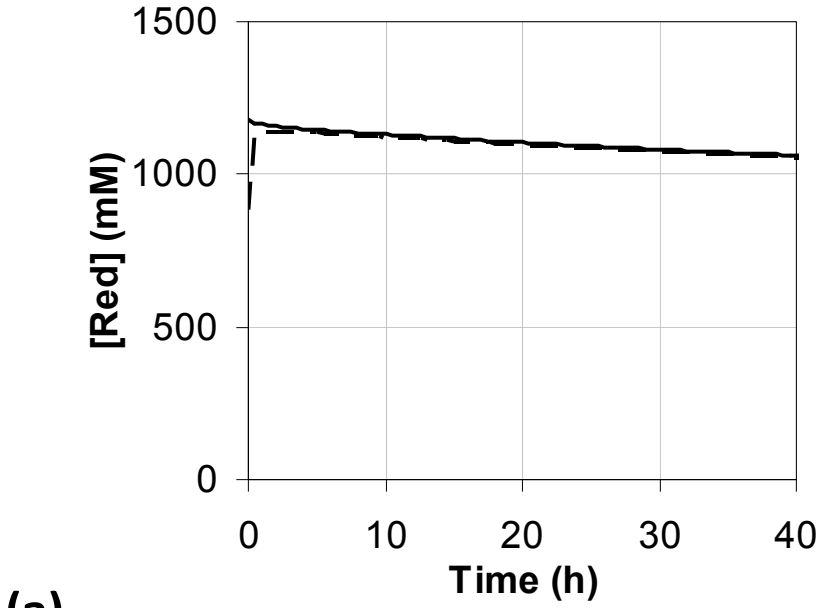


(a)

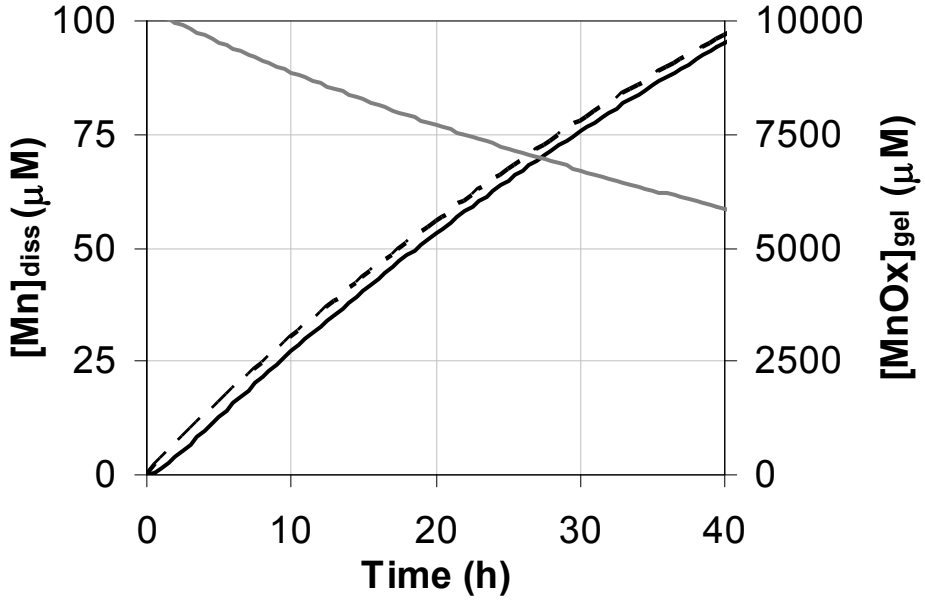


(b)

Figure A.3. AQUASIM model output for the reaction of ascorbate and HMO-doped gels in a miniprobe (1.0 μm membrane; modeled data is in Table A.5c). Shown are ascorbate (a) and dissolved Mn (b) in the gel (dashed line) and in the bulk (solid black line); the concentration of Mn oxide in the gel is represented with a solid gray line in (b).

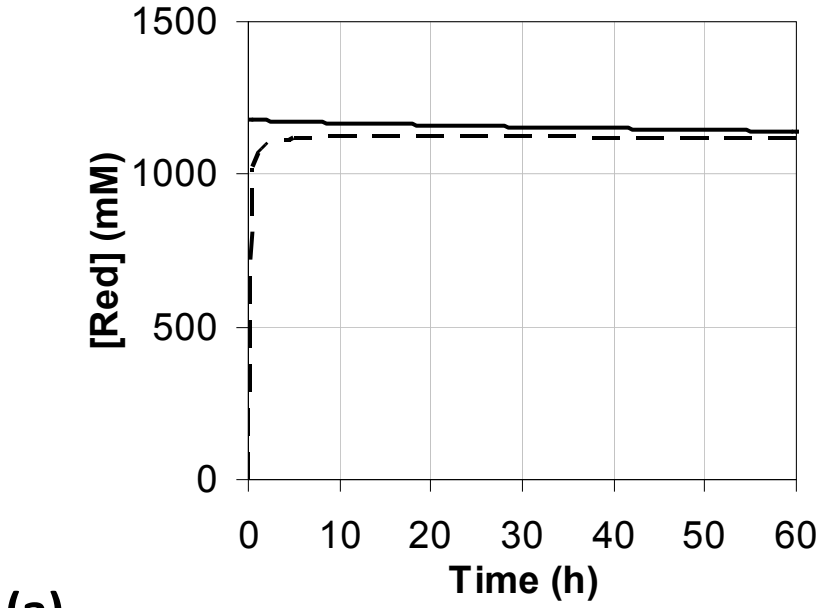


(a)

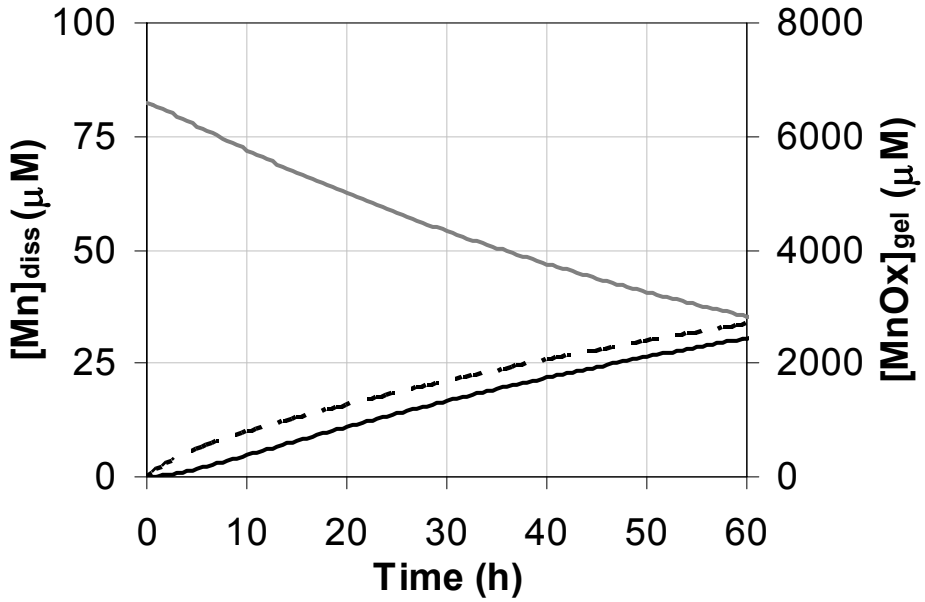


(b)

Figure A.4. AQUASIM model output for the reaction of *S. oneidensis* MR-1 and HMO-doped gels in solution (modeled data is in Table A.5e). Shown are the reductant "Red" (a) and dissolved Mn (b) in the gel (dashed line) and in the bulk (solid black line); the concentration of Mn oxide in the gel is represented with a solid gray line in (b).



(a)



(b)

Figure A.5. AQUASIM model output for the reaction of *S. oneidensis* MR-1 and HMO-doped gels in a miniprobe (1.0 μm membrane; modeled data is in Table A.5f). Shown are the reductant "Red" (a) and dissolved Mn (b) in the gel (dashed line) and in the bulk (solid black line); the concentration of Mn oxide in the gel is represented with a solid gray line in (b).

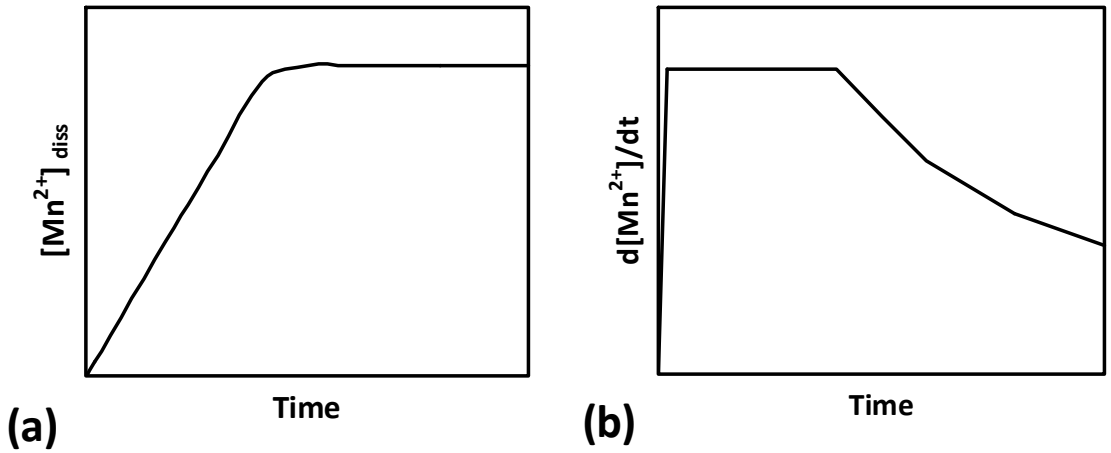


Figure A.6. Theoretical behavior of Mn reduction in an HMO-doped gel, in the absence of diffusion. Initially Mn^{2+} is produced at a constant rate (a), but when the HMO has been completely reduced, dissolved Mn^{2+} reaches a plateau. The reduction rate measured by the gel, which averages Mn loss over the cumulative time of deployment (b), would rapidly approach a plateau under steady-state conditions, but would decline after complete reduction of HMO; the gel method cannot determine when complete exhaustion has occurred during deployment.

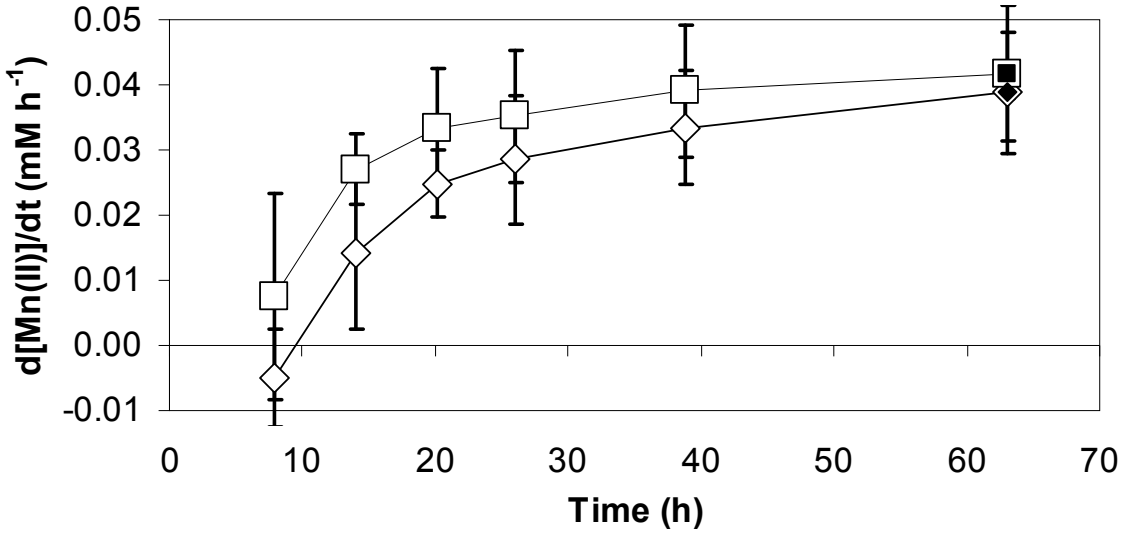


Figure A.7. Rate estimates as a function of time for the reaction of *S. oneidensis* MR-1 and HMO-doped gels in miniprobes.

The build-up of Mn(II) in the bulk solution over time was used to calculate the Mn loss from the gels as a function of time. For each time point, the cumulative Mn loss was divided by the time of reaction, and the resultant rates are plotted with unfilled symbols (squares for 1.0 μm membrane and diamonds for 5.0 μm membrane; raw data is in Table A.5f and g, respectively). The actual Mn recovered from the gels at the end of the experiment was used to calculate the points with filled symbols (standard deviations smaller than symbols). The two methods of rate calculation, from the solution phase and from the recovered gels, show remarkable agreement (this is possible because of the robust mass accounting in the experiment, as shown in Table A.1). This figure also shows that HMO-reduction rates with *S. oneidensis* MR-1 could be reasonably estimated by the gels after 39 h, even with an 8 h lag phase. Note that in contrast with Figure A.6b, diffusion has the affect of smoothing the approach of the plateau in d[Mn(II)]/dt vs. time. At the observed rate plateau, complete exhaustion of HMO would occur within 140 h.

Table A.6. Bias of clear gels co-located in the same miniprobe as HMO-doped gels (same conditions as experiment in Table A.5b, but Mn_T = 23.7 μM as 3 HMO-doped gels were replaced with clear gels). Concentrations were measured after 4 h.

[Mn] measured in solution (n=3 batches)	[Mn] measured in clear gels (n=3 batches×3 gels)
12.1±1.1 μM	194±105 μM

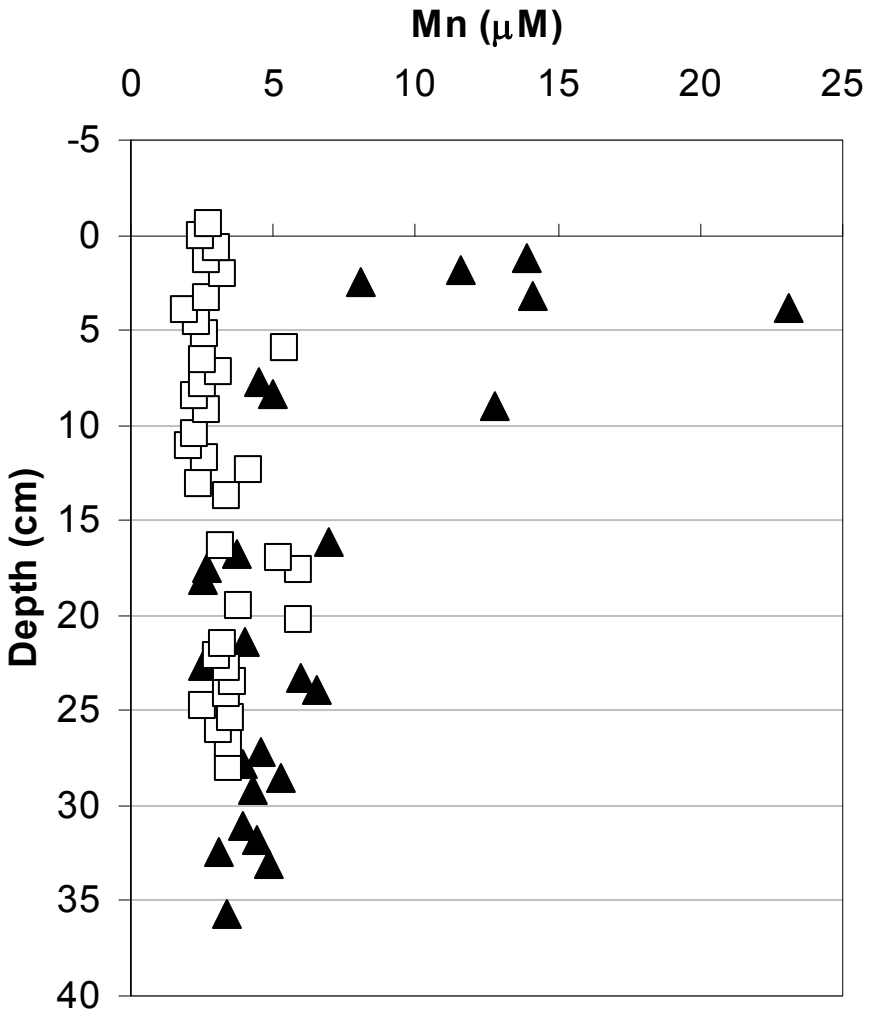


Figure A.8. Comparison of Mn concentrations measured in clear gels deployed in the same probe as HMO-doped gels (▲) and in a separate gel probe 85 cm away (□). The sediments were in a sabkha environment at Laguna Figueroa, Baja California, Mexico.