Supporting Information for
Kinetics of Oxytetracycline Reaction with a Hydrous Manganese Oxide

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Text S1. Oxytetracycline Application Rates in Agriculture.

Recommended OT dosing rates in the United States are up to 2 g·day⁻¹ per head of cattle, 500 g·ton⁻¹ over 5 days for poultry, 0.083 g·day⁻¹·kg_{stock}⁻¹ for salmonids (1) and 280 g·ha⁻¹ in pomoculture (2). Typical treatment of fish involves broadcasting medicated pelleted feed into ponds and raceways. Cravedi et al. (3) report that only 7-9% of OT ingested by rainbow trout is metabolized. In pomoculture, OT is applied by aerial spraying of a concentrated aqueous solution onto stems and foliage, and overspray may inadvertently contaminate surrounding ecosystems.

Text S2. Chemicals.

Double deionized water (ddH₂O) was prepared using a Barnstead NANOpure Ultrapure Water System (Dubuque, Iowa). OT, TC and CT were obtained from Acrōs Organics; meclocycline (MC) and rolitetracycline (RT) were obtained from Sigma-Aldrich; all were analytical grade or better. All other reagents and solvents (HPLC grade) were purchased through Fisher Scientific. Glassware was washed with 10% HNO₃ and silanized before use. Fluoropolymeric (i.e., Teflon[®]) materials were washed with 10% HNO₃.

Text S3. Manganese Oxide Preparation and Characterization

Hydrous manganese oxide (MnO₂) was prepared following the method of Murray (4). Briefly, 16 mL of 0.1 M NaMnO₄ was added to 328 mL of N₂-sparged ddH₂O, and the solution was made basic by addition of 32 mL of 0.1 M NaOH. Dropwise addition of 24 mL of 0.1 M MnCl₂ to the basic permanganate solution under constant stirring resulted in precipitation of the manganese oxide. The precipitate was recovered by 15-min centrifugation at 6500g, then washed three times with ~300 mL aliquots of ddH₂O recovering the MnO₂ each time by 15-min centrifugations at 6500g. Specific conductance of the supernatant following the third sedimentation was measured (typically 0.05 – 0.5 μS·cm⁻¹; always < 2 μS·cm⁻¹). Additional washes were performed if necessary until the specific conductance was < 2 μS·cm⁻¹. The product was stored as a suspension at 4°C for no longer than 4 d. The

MnO₂ concentration in the suspension was determined by drying and weighing three or four 1-mL aliquots of the suspension.

Oxide aggregate size was measured by dynamic light scattering on a Malvern ZetaSizer 3000HS. Light scattering intensity distributions indicated an aggregate size range of 0.1 to 1.75 μm with ~3.5% falling below 0.2 μm , while the estimated volume distribution ranged from 0.1 to 2.15 μm with ~0.7% falling below 0.2 μm . The Brunauer-Emmett-Teller (BET) surface area was measured by N₂ adsorption at -196°C on a Micrometrics ASAP 2010 multi-gas volumetric adsorption analyzer. The specific surface area was 190 m²·g⁻¹ (BET isotherm fit $r = 0.99997$) for a dried and degassed (120°C) sample of the oxide. X-ray diffractometry of an air-dried smear mount of the oxide indicated that the MnO₂ was poorly crystalline (data not shown). Scanning electron microscopy revealed the morphology to be similar to that of the acid birnessite examined by Villalobos et al. (5) with MnO₂ aggregates composed of particles with diameters < 200 nm.

Manganese oxide prepared following the method of Murray (4) has the formula MnO_{1.92}·0.88H₂O (6) corresponding to a formula weight of 101.5 g·mol⁻¹. The MnO₂(s) contained ~1% matrix chloride by mass, determined using an Accumet[®] chloride selective electrode after dissolution of the oxide with oxalic acid; the electrode was calibrated with MnCl₂. Godfredsen and Stone (7) reported an average oxidation state of +3.86 for MnO₂ prepared according to Murray (4).

Text S4. Reactor Setup.

Three types of reactor configurations were used in this study depending on whether the objective was to measure initial rates of antibiotic loss, trends of antibiotic loss and MnO₂ dissolution concurrently or antibiotic losses over long time periods. Each is described below. In all cases, temperature was controlled to $\pm 1^\circ\text{C}$ using an air incubator. Reactions were conducted in 10 mM buffer (sodium acetate for pH 4 to 6; 3-*N*-morpholinopropansulfonic acid (MOPS) for pH > 6) in absence of light. Stock solutions were stored in amber glassware.

Determination of initial rates. Initial rates (r_{init}) were measured for reactions conducted in triplicate 100-mL batches in tetrafluorethylene-perfluoropropylene (FEP) bottles. We determined that OT did not sorb to reactor vessels. Reactions were initiated by adding a small volume of OT solution (10% methanol in 10 mM buffer) to MnO_2 suspensions (previously equilibrated in buffer for 10-13 h) under constant stirring at the desired temperature. Aliquots (1 mL) were removed from the reactor at desired intervals, added to 2-mL amber glass HPLC vials containing 50 μ L of 80 $mg \cdot mL^{-1}$ oxalic acid solution and shaken to quench the reaction.

Oxytetracycline sorption to $MnO_2(s)$. Dissolved OT and Mn^{II} were monitored concurrently during reaction of OT with MnO_2 in duplicate continuously-stirred 100-mL FEP batch reactors, and total (dissolved + sorbed) OT was measured independently under identical conditions and for the same time intervals in two separate batch reactors. Prior to initiation of the experiment, MnO_2 (10/9 of working concentration) was equilibrated for 10-14 h in 10 mM sodium acetate buffer at 25°C in an amber glass bottle under constant stirring. The pH was adjusted with 1 M NaOH as needed. Two aliquots (5 or 10 mL) of this solution were removed, filtered, and analyzed for background dissolved Mn; and four aliquots (90-mL) were transferred to separate FEP reactors. Reactions were initiated by adding 10 mL of a 250 μ M OT solution to the 90-mL MnO_2 suspensions under continuous stirring. Two of the four reactors were sampled for dissolved OT and Mn^{II} , and two were sampled for total OT.

Samples (5 or 10 mL) for dissolved OT and Mn^{II} analysis were removed from the reactor with a silanized glass syringe and quenched by passing through a FEP syringe barrel filter (0.2- μ m $\times$ 25 mm) into a silanized glass test tube containing oxalic acid to produce a final oxalic acid concentration of 0.8 $mg \cdot mL^{-1}$ and mixed by shaking vigorously. OT was measured in 1-mL subsamples by high performance liquid chromatography with UV detection (HPLC-UV) as described in Text S7. Dissolved Mn^{II} was measured by ICP-OES (Text S7) in subsamples prepared by 1:10 dilution (1% HNO_3 as diluent) in conical polystyrene Falcon[®] tubes. At the end of the time course, the pH of the reaction solution was

measured and found to vary by < 0.1 unit. The initial concentration of dissolved Mn was multiplied by 9/10 to correct for dilution during initiation of reactions.

Longer-term OT transformation kinetics. Parameters for the retarded rate equation (k_{app} , α) were measured from quadruplicate reactions conducted in aluminum foil-wrapped 20-mL glass test tubes with Teflon-lined screw caps. Concentrated MnO_2 suspension was added to OT solution (in buffer) and mixed continuously using a rotary shaker. Aliquots (1 mL) were removed periodically, and reactions were quenched by dissolving suspended MnO_2 with 8.0 ± 0.5 mg oxalic acid.

Text S5. Evaluation of Quenching Methods

With the exception of experiments examining Mn^{II} evolution and OT sorption, reactions were quenched by dissolving the $\text{MnO}_2(\text{s})$ with oxalic acid. The subset of experiments investigating Mn^{II} evolution and OT sorption employed filtration to halt reactions, followed by acidification of the filtrate. Dissolution of the oxide allowed measurement of total antibiotic in the reactor system. Filtration permitted measurement of dissolved antibiotic and Mn^{II} . The reaction time corresponded to the time at which quenching was complete (see below). In absence of MnO_2 , OT concentrations by both methods were statistically indistinguishable ($p = 0.37$).

Oxalic acid quenching. For oxalic acid quenching, we defined the quench time (t_q) as the time needed to dissolve 90% of the oxide. The quench time depended on the amount of $\text{MnO}_2(\text{s})$ used (e.g., 5 s for $0.05 \text{ mg}\cdot\text{L}^{-1}$, 7 s for $0.2 \text{ mg}\cdot\text{L}^{-1}$). That is, the end of a time interval is the sampling time plus t_q . The practical limit of our sampling technique is ~ 10 s, so the earliest time point measurable would be 15 s for a reaction using $0.05 \text{ mg}\cdot\text{L}^{-1}$ $\text{MnO}_2(\text{s})$ and 17 s for a reaction using $0.2 \text{ mg}\cdot\text{L}^{-1}$ $\text{MnO}_2(\text{s})$ (the lowest and highest oxide concentrations used). Whether oxalic acid could increase reaction of the antibiotic by exposing fresh surface sites during oxide dissolution was not investigated.

Quenching by filtration. Filtration of 5 or 10 mL of reaction suspensions through a $0.2\text{-}\mu\text{m}$ FEP filter (followed by rapid acidification of the filtrate with $0.8 \text{ mg}\cdot\text{mL}^{-1}$ oxalic acid) removed MnO_2 particles and sorbed organic compounds. This method allowed measurement of dissolved OT and Mn^{II} .

Dissolved Mn^{II} and OT were thus operationally defined as that passing the 0.2- μm filter (8, 9). No significant difference was observed between OT solutions filtered using the glass syringe and untreated controls of MnO_2 ($p = 0.35$). Oxalic acid was added following filtration to dissolve any MnO_2 particles $< 0.2 \mu\text{m}$ and reduce propensity for Mn^{II} sorption to surfaces. The filtration method had a quench time of 5 s or 10 s depending on sample volume.

Samples having a final pH ~ 2 provided reproducible OT measurements by HPLC-UV for up to 4 h when using TC as an internal standard. Oxalic acid showed no significant impact on detector response ($p > 0.05$) relative to untreated OT solution.

Text S6. Evaluation of Buffers

We selected sodium acetate and MOPS reaction buffers based on reports by previous investigators that these buffers did not adversely affect reactions of organic compounds with MnO_2 (8-11) and our own assessment of the behavior of these buffers toward reactions of OT with MnO_2 . In two separate experiments we compared r_{init} values for reactions of OT with 490 μM MnO_2 in sodium acetate (10 mM) and MOPS (10 mM) buffers at pH 6.0, a pH intermediate between the pK_a values of acetic acid and MOPS. In the first experiment, 100 μM OT (surface saturating conditions) was reacted in MOPS and acetate buffers in quintuplicate, and initial OT disappearance rates (based on three time points) agreed within 3%. In the second experiment, 25 μM OT were reacted with 490 μM MnO_2 in MOPS (5 time points) and acetate buffers (3 time points) in six replicates, and OT disappearance was monitored; OT disappearance rate was lower in MOPS ($4.1 \pm 0.5 \mu\text{M}\cdot\text{min}^{-1}$) relative to acetate ($7.1 \pm 0.7 \mu\text{M}\cdot\text{min}^{-1}$). Reaction orders with respect to H^+ determined independently for acetate ($\text{pH} \leq 6$) and MOPS ($\text{pH} > 6$) did not differ from that estimated when the data were combined ($p > 0.05$).

Bacterial activity in MnO_2 suspensions. Bacteria were not present at levels expected to cause interference with our experiments. Bacterostatic agents (e.g., NaN_3 , HgCl_2) were not included in our suspensions due to concern over interference with reactions. We did not sterilize the MnO_2 to avoid altering the mineral. We tested for the presence of bacteria in MnO_2 suspensions equilibrated for >13 h

in acetate (10 mM, pH 4.4 and pH 5.6) and MOPS (10 mM, pH 7.5) at 25°C, and for > 13 h in acetate (10 mM, pH 5.6) at 41°C. Aliquots of these suspensions were plated onto yeast-peptone and rich nutrient agars. No colony forming units (CFUs) were observed after 18-h incubation for either acetate or MOPS; one CFU was observed from the MOPS buffer after 36 h incubation. Rapid growth of microorganisms in these buffers was unlikely because the acetate buffer was deficient in both N and P, and the MOPS buffer lacked P. Furthermore, OT is active against a wide range of Gram-negative and Gram-positive bacteria and was employed at concentrations expected to inhibit the growth of bacteria lacking tetracycline resistance genes (12).

Text S7. Instrumental Analysis.

Quantitation of tetracycline antibiotics. Disappearance of tetracycline antibiotics over time was quantified using reverse-phase HPLC with UV absorbance detection at 360 nm using TC or CT as internal standards. Two different HPLC systems and columns were employed for tetracycline antibiotic quantitation: (1) a Gilson model 231 HPLC equipped with a UV-2000 UV absorbance detector (Thermo Separation Products, San Jose, CA); and (2) a Hewlett-Packard 1050 Series HPLC system equipped with an Agilent Technologies 1100 Series diode array detector (DAD). Separation was effected on an Alltech column (4.6 × 250 mm, 5µm Waters Spherisorb® ODS-2 packing) with an isocratic mobile phase composed of 20% methanol, 35% acetonitrile and 45% of aqueous oxalic acid (0.01 M) with 0.001 M Na₂EDTA at a flow rate of 1.4 mL·min⁻¹, or using a Waters ODS-2 column (4.0 × 250 mm, Spherisorb® S5 ODS-2 packing) with an isocratic mobile phase composed of 13% methanol, 22% acetonitrile and 65% aqueous oxalic acid (0.01 M) with 0.001 M Na₂EDTA at a flow rate of 0.8 mL·min⁻¹. Tetracycline antibiotics were quantified using four-point standard curves having R^2 values ≥ 0.99 over a 100-fold concentration range.

Quantitation of dissolved manganese. Samples for Mn analysis were diluted 10-fold with 1% HNO₃. Dissolved Mn^{II} was measured by ICP-OES using a Perkin-Elmer Optima 4300 DV (Wellesley, MA) instrument with quantitation on the 257.6 nm and 259.4 nm emission bands. A three-point standard

curve was prepared over a range of 0.18-18 μM ($R^2 > 0.9999$) by dissolving $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in 10 mM sodium acetate (pH 5.6) containing 1 $\text{mg} \cdot \text{mL}^{-1}$ oxalic acid and diluting 10-fold with 1% HNO_3 . An initial baseline (blank) was acquired, and the injection apparatus was rinsed between samples with Mn-free solution containing 1 mM sodium acetate, 1.1 mM oxalic acid and 1% v/v HNO_3 .

Preliminary characterization of reaction products. Antibiotic concentrations of 100-1000 μM were reacted with MnO_2 to facilitate product analysis by HPLC-DAD ($\lambda = 200\text{-}400\text{ nm}$) or quadrupole mass spectrometry (MS) (Applied Biosystems API 4000, Perkin-Elmer) with positive electrospray ionization (5.5 kV, 35 psig nebulizer gas, 30 psig drying gas). Products were separated for UV spectroscopy on an Alltech column ($4.6 \times 250\text{ mm}$, $5\mu\text{m}$ Waters Spherisorb[®] ODS-2 packing) with a gradient from 80% aqueous (0.01 M oxalic acid/0.001 M Na_2EDTA)/7% methanol/13% acetonitrile to 20% aqueous/29% methanol/51% acetonitrile over 20 min and a flow gradient from 1.2 to 1.5 $\text{mL} \cdot \text{min}^{-1}$ to maintain approximately isobaric conditions. Product separation for MS was conducted at 30°C on a Luna C_{18} column with a mobile phase gradient of 0.1% formic acid and 5% to 100% methanol over 25 min at 0.3 $\text{mL} \cdot \text{min}^{-1}$.

Text S8. Interpretation of Kinetic Data.

Initial reaction rates. A least squares linear regression model forced through the initial antibiotic concentration (y-intercept) on a concentration-versus-time curve was used to determine the initial reaction rate (r_{init}) over the time increments for which pseudo-first-order kinetics appeared to be reasonably approximated. Standard errors of the slopes of these regressions were calculated using Sigma Plot 9.0 (SYSTAT, Inc.). The number of time increments used for each initial rate determination is given in Tables S1-S4.

Initial rate constants. Initial rate constants (k_{init}) were calculated using a rate expression corresponding to the determined reaction orders (see eq 2 in the main text). Expected random error (ε_k) of initial rate constants (k_{init}) was calculated using the equation for n^{th} order reactions given by Hill (13):

$$\left(\frac{\varepsilon_k}{k_{init}}\right)^2 = \left(\frac{\Delta t_1}{t_2 - t_1}\right)^2 + \left(\frac{\Delta t_2}{t_2 - t_1}\right)^2 + \left[\frac{(n-1)C_2^{n-1}}{C_1^{n-1} - C_2^{n-1}}\right]^2 \times \left(\frac{\Delta C_1}{C_1}\right)^2 + \left[\frac{(1-n)C_1^{n-1}}{C_1^{n-1} - C_2^{n-1}}\right]^2 \times \left(\frac{\Delta C_2}{C_2}\right)^2 \quad (S1)$$

and, assuming absolute initial measurements, simplified to express absolute uncertainty with respect to precision of time and concentration measurements as:

$$\varepsilon_k = k_{init} \times \sqrt{\left(\frac{\Delta t_2}{t_2 - t_1}\right)^2 + \left[\frac{(1-n)C_1^{n-1}}{C_1^{n-1} - C_2^{n-1}}\right]^2 \times \left(\frac{\Delta C_2}{C_2}\right)^2} \quad (S2)$$

Subscripts 1 and 2 denote conditions at initial and final measurements, and uncertainty associated with each time measurement (t_n , min) is given by Δt_n . The instrumental precision (ΔC_2) of concentration measurement (C_2 , μM) associated with the latest time point used in determination of the rate constant was calculated as described by Harris (14).

Temperature dependence of reaction rate. Initial rate constants were calculated for six temperatures between 7 and 44°C. The highest (44°C) and lowest (7°C) temperatures investigated were omitted from thermodynamic calculations because they deviated substantially from the linear relationship apparent from 12 to 36°C. Temperature dependence of rate constants was fitted to the Arrhenius equation:

$$\ln(k_{init}) = \frac{E_a}{RT} + \ln(A) \quad (S3)$$

where R is the universal gas constant, E_a ($\text{J}\cdot\text{mol}^{-1}$) is the activation energy, T (K) is temperature, and A is the pre-exponential factor. The relative error associated with the activation energy was calculated according to Hill (13):

$$\frac{\varepsilon_E}{E_a} = \left(\frac{T_2}{T_2 - T_1}\right)^2 \left(\frac{\Delta T_1}{T_1}\right)^2 + \left(\frac{T_1}{T_2 - T_1}\right)^2 \left(\frac{\Delta T_2}{T_2}\right)^2 + \left[\ln\left(\frac{k_2}{k_1}\right)\right]^{-2} \times \left[\left(\frac{\varepsilon_{k1}}{k_1}\right)^2 + \left(\frac{\varepsilon_{k2}}{k_2}\right)^2\right] \quad (S4)$$

where subscripts 1 and 2 denote conditions at the initial and final measurements, respectively. Precision of temperature control (ΔT) at a specific temperature was defined as the larger of the absolute

differences between the average reactor temperature and the highest or lowest temperature encountered during that reaction.

On the Arrhenius plot (Figure S5), the deviations from the linear relationship between $\ln k_{\text{init, pH5.6}}$ and T^{-1} at the temperature extremes (7 and 44°C) were not surprising since temperature control was less precise at these boundaries, and small variations in temperature are substantially propagated in the Arrhenius method (13). OT was stable in pH 5.6 buffer for over 3 h at 48°C, indicating thermal degradation was not responsible for the lack of linearity of the Arrhenius plot at 44°C. We cannot exclude the possibility that thermally induced changes in the MnO₂ crystal structure caused a loss in surface reactivity at high temperature.

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I

Products of OT reaction with Mn oxide for ~ 20 s

FIGURE S1. Transformation of (I) OT and (II) CT by MnO_2 produced a variety of reaction products. Chromatograms from 20-s reaction of 1000 μM antibiotic with 490 μM MnO_2 in 10 mM sodium acetate (pH 5.6) (top), MnO_2 -free control (middle) and antibiotic-free control (bottom). Reactions were quenched with 44 mM oxalic acid. Products were separated by HPLC and detected with a diode array detector (see Text S7). (OT) and (CT), UV spectra of unaltered oxytetracycline and chlortetracycline; (OT'), UV spectrum of dominant OT reaction product monitored over time; (A) – (D), UV spectra of selected transformation products. The chromatograms illustrate that products with a wide range of characteristics were formed as a result of OT and CT reaction with $\text{MnO}_2(\text{s})$. Asterisks denote peaks for which UV spectra were acquired. Loss or shifting of the OT peak at ~ 360 nm indicates alteration of the molecule on the phenolic ring (Figure 1, left) and/or along the backbone from carbon 11 to 12a, while alteration of the peak at ~ 260 nm suggests changes to the tricarbonyl ring containing carbons 1 – 4a and 12a (12). The OT peak (10.76 min) height in (I) was truncated to improve appearance of the chromatogram.

FIGURE S2. Time courses for OT (10 and 25 μM) degradation by MnO₂ (95 μM and 380 μM surface sites) at pH 5.6 (10 mM sodium acetate) and 25°C. Solid lines are linear fits to initial data points used to estimate initial reaction rates; error bars represent one standard deviation of triplicate experiments.

FIGURE S3. Sorption of OT to the MnO_2 surface at pH 6.0 (10 mM sodium acetate) and 25°C initially increased, then decreased. Dissolved OT (initial concentration = 25 μM) and suspended MnO_2 (initial concentration 490 μM) were reacted, and the total [OT] and free [OT] measurements were subtracted to estimate the bound fraction of OT. Error bars represent one standard deviation of triplicate measurements of total OT and duplicate measurements of free OT. Experimental details are presented in Text S5.

FIGURE S4. Loss of OT with concurrent dissolved Mn^{II} dynamics. An apparent drop in dissolved Mn (right axis) was observed at pH 5.6 (in 10 mM sodium acetate) following initiation of reaction with OT (89 μM) and followed by an increase in dissolved Mn when OT and MnO₂ (95 μM sites) are present at approximately a 1:1 ratio. See Text S4 for experimental details.

FIGURE S5. Arrhenius plot for the reaction of OT (25 μM) with MnO_2 (490 μM) at pH 5.6 (10 mM sodium acetate). Error bars represent absolute uncertainty (eq S2) computed from initial rate constants determined in triplicate reactions. Rate constants ($\mu\text{M}^{-0.5} \cdot \text{min}^{-1}$) expressed in terms of moles of MnO_2 surface sites; See Table S2 for numerical values of rate constants. Regression was limited to data points represented by open circles (○).

TABLE S1. Initial Rate Parameters for OT and MnO₂(s) Reaction Order Determination^a

[OT] ₀ (μ M)	[MnO ₂] ₀ (μ M)	molar ratio of MnO ₂ surface sites to OT	r_{init} ^b (μ M $\cdot$ min ⁻¹)	time increments ^c	time course used to determine r_{init} (min)
10	490	9.5	4.4 $\pm$ 0.5	3	1.58
10	740	14	8 $\pm$ 1	2	0.77
10	990	19	8.3 $\pm$ 0.7	2	0.76
10	1970	38	13 $\pm$ 2 ^d	1	0.45
25	490	3.8	8 $\pm$ 2	3	1.58
25	740	5.7	15 $\pm$ 3	2	0.76
25	990	7.6	16 $\pm$ 2	2	0.77
25	1970	15	25 $\pm$ 3	2	0.78
50	490	1.9	14 $\pm$ 3	3	1.5
50	740	2.9	20 $\pm$ 4	3	1.09
50	990	3.8	24 $\pm$ 4	2	0.77
50	1970	7.6	42 $\pm$ 5	2	0.78
100	490	0.95	9 $\pm$ 2	6	3.08
100	490	0.95	13 $\pm$ 3	5	1.58
100	490	0.95	7 $\pm$ 3	5	1.58
100	990	1.9	32 $\pm$ 4	2	0.77
100	1970	3.8	57 $\pm$ 10	2	0.78
200	490	0.48	22 $\pm$ 2	5	3.08

^a Abbreviations: [MnO₂]₀, initial MnO₂(s) concentration; [OT]₀, initial OT concentration. Experiments were conducted at pH 5.6 and 25°C in triplicate.

^b Mean $\pm$ standard error.

^c Number of time increments after $t = 0$ used to calculate k_{init} .

^d Represents lower bound estimate of reaction rate.

TABLE S2. Initial Rate Parameters for Temperature Dependence of OT Transformation^a

temperature ^b (°C)	r_{init} ^c ($\mu\text{M}\cdot\text{min}^{-1}$)	$k_{\text{init, pH5.6}}$ ^d ($\mu\text{M}^{-0.5}\cdot\text{min}^{-1}$)	time increments	time course used to determine r_{init} (min)
7 (4 – 8)	7 ± 1	0.025 ± 0.004	3	1.08
12 (10 – 14)	4.0 ± 0.5	0.011 ± 0.001	5	2.08
17 (16 – 18)	5.4 ± 0.8	0.015 ± 0.001	4	1.58
25 (24 – 26)	8 ± 2	0.02 ± 0.01	3	1.58
36 (36 – 36)	26 ± 7 ^e	0.07 ± 0.02	1	0.33
44 (42 – 45)	19 ± 2	0.052 ± 0.004	3	0.58

^a Experiments were conducted at by reacting 25 μM OT with 490 μM MnO_2 at pH 5.6 and 25°C (molar ratio of MnO_2 surface sites to OT was 3.8).

^b Mean with range reported in parentheses.

^c Mean ± standard error.

^d Initial rate constant defined in terms of moles of MnO_2 surface sites (95 μM) and presented as mean ± expected random error (eq S2).

^e Represents lower bound estimate of reaction rate.

TABLE S3. Initial Rate Parameters for pH Dependence of OT Transformation^a

$[OT]_0$ (μM)	$[MnO_2]_0$ (μM)	molar ratio of MnO_2 surface sites to OT	pH ^b	r_{init}^b ($\mu M \cdot min^{-1}$)	time increments ^d	time course used to determine r_{init} (min)
25	490	3.8	4.4 $\pm$ 0.1	19 $\pm$ 2	2	0.75
25	490	3.8	5.0 $\pm$ 0.1	18 $\pm$ 2	2	0.75
25	490	3.8	5.6 $\pm$ 0.1	8 $\pm$ 2	3	1.58
25	490	3.8	6.0 $\pm$ 0.1	2 $\pm$ 1 ^d	4	0.75
25	490	3.8	6.5 $\pm$ 0.1	1.3 $\pm$ 0.7	5	1.08
25	490	3.8	7.0 $\pm$ 0.1	1.7 $\pm$ 0.5 ^d	4	2.08
25	490	3.8	7.5 $\pm$ 0.1	0.3 $\pm$ 0.3	5	10.08
25	490	3.8	7.9 $\pm$ 0.1	0.14 $\pm$ 0.03	5	60.08
25	490	3.8	8.1 $\pm$ 0.3	0.13 $\pm$ 0.03	8 ^e	60.08
25	490	3.8	9.2 $\pm$ 0.3	0.05 $\pm$ 0.01	5	120.08
25	490	3.8	9.8 $\pm$ 0.3	0.06 $\pm$ 0.02	3	90.08
25	490	3.8	10.9 $\pm$ 0.1	0.06 $\pm$ 0.02	4	120.08
10	490	9.5	5.6 $\pm$ 0.1	4.4 $\pm$ 0.5	3	1.58
10	490	9.5	8.8 $\pm$ 0.2	0.018 $\pm$ 0.003	5	199.08
25	990	7.6	5.6 $\pm$ 0.1	16 $\pm$ 2	2	0.77
25	990	7.6	8.8 $\pm$ 0.2	0.09 $\pm$ 0.02	5 ^e	60.10

^a Abbreviations: $[MnO_2]_0$, initial $MnO_2(s)$ concentration; $[OT]_0$, initial OT concentration.

^b Median pH $\pm$ range.

^c Mean $\pm$ standard error.

^d Number of time increments after $t = 0$ used to calculate k_{init} .

^e A single datum was omitted as an outlier ($>$ two SD from mean).

TABLE S4. Initial Rate Parameters for Transformation of Selected Tetracycline Antibiotics by MnO₂(s)^a

antibiotic	$r_{\text{init}}^{\text{b}}$ ($\mu\text{M}\cdot\text{min}^{-1}$)	time increments ^c	time course used to determine r_{init} (min)
OT	8 ± 2	3	1.58
TC	11 ± 3	3	1.08
MC	13 ± 3	4	1.08
RT	7 ± 2	4 ^d	1.08
CT	$35 \pm 7^{\text{e}}$	1	0.42

^a Selected tetracycline antibiotics (25 μM) were reacted with 490 μM MnO₂ at pH 5.6 and 25°C (molar ratio of MnO₂ surface sites to OT was 3.8).

^b Mean $\pm$ standard error.

^c Number of time increments after $t = 0$ used to calculating k_{init} .

^d A single datum was omitted as an outlier (> two SD from mean).

^e Represents lower bound estimate of reaction rate.

TABLE S5. Retarded Rate Model Parameters^a

temperature (°C)	k_{app}^b (min ⁻¹)	α^b (min ⁻¹)	model fit (R^2)
5	0.017 ± 0.006	0.05 ± 0.03	0.93
10	0.013 ± 0.003	0.05 ± 0.01	0.98
15	0.011 ± 0.002	0.03 ± 0.01	0.97
20	0.015 ± 0.003	0.03 ± 0.01	0.98
25	0.018 ± 0.004	0.03 ± 0.01	0.98
30	0.018 ± 0.003	0.02 ± 0.01	0.99
40	0.08 ± 0.01	0.19 ± 0.04	0.98

^a Based on triplicate reactions of OT (100 μM) with MnO₂ (490 μM, 95 μM reactive surface sites) in 10 mM sodium acetate buffer (pH 5.6) were conducted for up to 3 d. Parameters shown were determined expressing OT in μM and MnO₂ in g·L⁻¹.

^b Mean ± standard error.