Environ. Chem. **2016**, *13*, 757–766
doi:10.1071/EN15256_AC

Supplementary material

Hydroxyl radical formation from bacteria-assisted Fenton chemistry at neutral pH under environmentally relevant conditions

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Growth medium preparation

Medium components were dissolved in deionised water, placed in an autoclavable container and autoclaved for 15 to 20 min at 121 °C. The medium was then transferred to sterile screw-cap tubes and stored in a refrigerator.

Growth medium composition

In deionised water:

- 1 mM lactic acid
- 2.45 g L^{-1} $NaH_2PO_4 \cdot H_2O$
- 4.58 g L^{-1} Na_2HPO_4
- 0.5 g L^{-1} NH_4Cl
- 0.15 g L^{-1} KCl
- 0.075 g L^{-1} $CaCl_2 \cdot H_2O$
- 1 mL per 100 mL Wolfe's trace minerals solution

In de-ionised water: 0.5 g L^{-1} EDTA, 3.0 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1} $MnSO_4 \cdot H_2O$, 1.0 g L^{-1} NaCl, 0.1 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.1 g L^{-1} $Co(NO_3)_2 \cdot 6H_2O$, 0.1 g L^{-1} $CaCl_2$ (anhydrous), 0.1 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.01 g L^{-1} $CuSO_4 \cdot 5H_2O$, 0.01 g L^{-1} $AlK(SO_4)_2$ (anhydrous), 0.01 g L^{-1} H_3BO_3, 0.01 g L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 0.001 g L^{-1} Na_2SeO_3 (anhydrous), 0.01 g L^{-1} $Na_2WO_4 \cdot 2H_2O$, 0.02 g L^{-1} $NiCl_2 \cdot 6H_2O$.

$OH^{\bullet}$ quantification

Hydroxyl radicals ($OH^{\bullet}$) react with benzoic acid at near-diffusion-limited rates to produce salicylic acid (SA), 3-hydroxybenzoic acid (3-HBA) and 4-hydroxybenzoic acid (4-HBA).[1] We have measured a

product branching ratio of SA : 3-HBA : 4-HBA = 1 : 2 : 1.[2] Salicylic acid can be detected by fluorescence spectroscopy; 3-HBA and 4-HBA do not fluoresce.

Hydroxyl radical production rates were measured by taking fluorescence readings over set time intervals and monitoring SA emission intensity at 407 nm (with 300-nm excitation). To obtain $OH^{\bullet}$ production rates, we multiplied the measured SA formation rate by 4 to account for all three reactions $OH^{\bullet}$ undergoes with BA (because 3-HBA and 4-HBA are not detected by our fluorescence measurements).[2]

We used BA concentrations of 7.5×10^{-4} M in all Fenton experiments in the present study. At this concentration, observed $OH^{\bullet}$ production rates are insensitive to BA concentration when $OH^{\bullet}$ is generated from H_2O_2 photolysis, which is much faster than $OH^{\bullet}$ generation via Fenton chemistry under our experimental conditions.[2] As further confirmation that SA production is insensitive to BA concentration under our experimental conditions, we measured $OH^{\bullet}$ production rates from Fenton chemistry in the presence of 100 μM Fe^{III}, 2×10^{-4} M H_2O_2 and 2.5×10^{-3} M BA. At this higher BA concentration, the observed $OH^{\bullet}$ production rate was $(8.6 \pm 0.4) \times 10^{-12}$ M s^{-1}, which is the same within error as the rate measured in experiments using 7.5×10^{-4} M BA ($(8.4 \pm 0.2) \times 10^{-12}$ M s^{-1}).

Under our experimental conditions, lactic acid concentrations are higher than BA concentrations (1.0×10^{-3} and 7.5×10^{-4} M respectively). Benzoic acid is much more reactive towards $OH^{\bullet}$ than is lactic acid (with rate constants of 5.9×10^{9} and 2.0×10^{6} M^{-1} s^{-1} respectively), so we expect $OH^{\bullet}$ to react almost quantitatively with BA even in the presence of lactic acid.[3] To ensure that this was the case in our experiments, we measured $OH^{\bullet}$ production rates from dark Fenton chemistry (with iron in the form of Fe^{III}) in aqueous solution in the presence and absence of 1 mM lactic acid. Measured rates were $(8.7 \pm 1.0) \times 10^{-12}$ and $(8.4 \pm 0.2) \times 10^{-12}$ M s^{-1} respectively, which leads us to conclude that lactic acid is not a significant $OH^{\bullet}$ sink in our experiments.

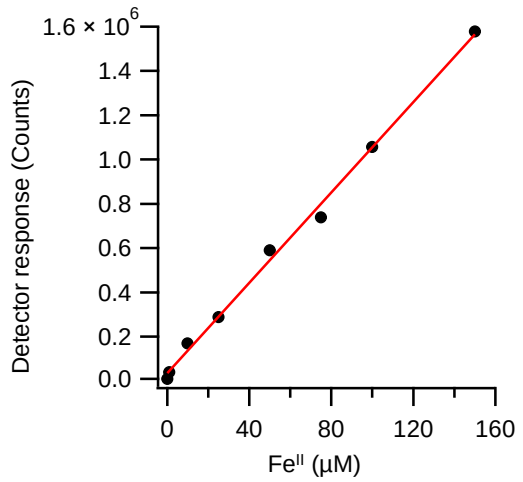

Fig. S1. Effect of Fe^{II} concentration on the detector response to luminol phosphorescence in sterile medium. The slope is 10204 ± 251 counts μM^{-1} with an R^2 value of 0.99.

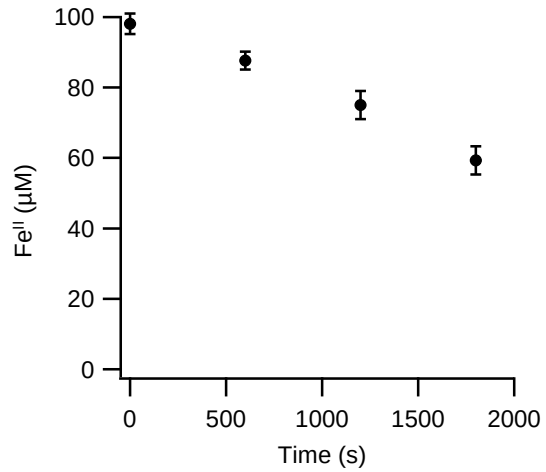

Fig. S2. Fe^{II} oxidation over time in sterile medium at a pH 7 in the absence of hydrogen peroxide and SO.

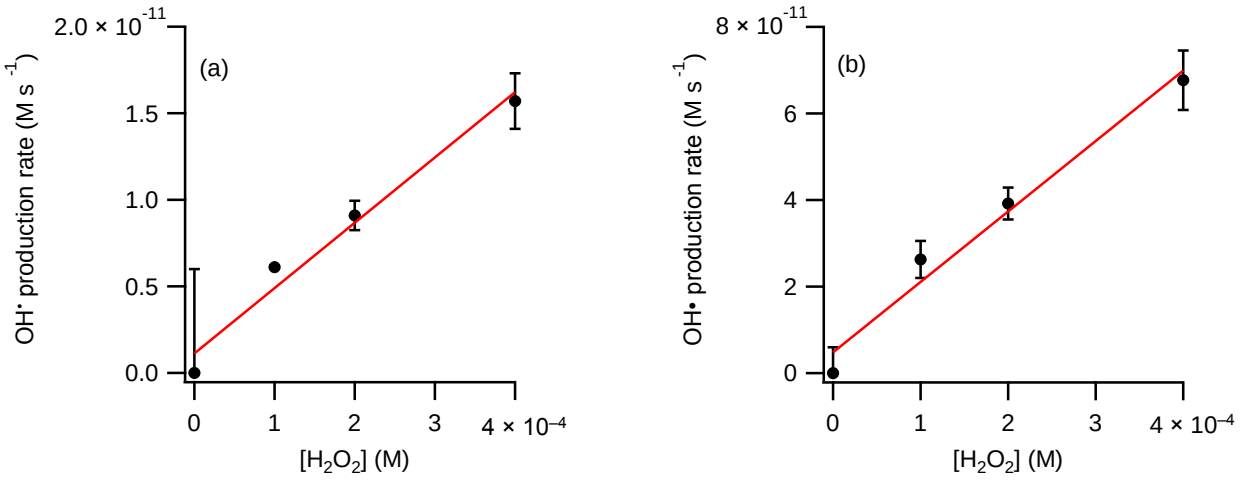

Fig. S3. Effect of H_2O_2 concentration on $OH^{\bullet}$ production rates from Fenton chemistry with iron in the form of Fe^{III} under benchmark conditions in (a) water, and (b) sterile medium. Error bars represent the standard deviation about the mean of three trials. Error bars for 0 M H_2O_2 indicate our limit of detection for $OH^{\bullet}$ production.

References

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