Supporting Information

Iron complex facilitated copper redox cycling for nitric oxide generation as non-toxic nitrifying biofilm inhibitor

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DTTCT NMR Spectrum

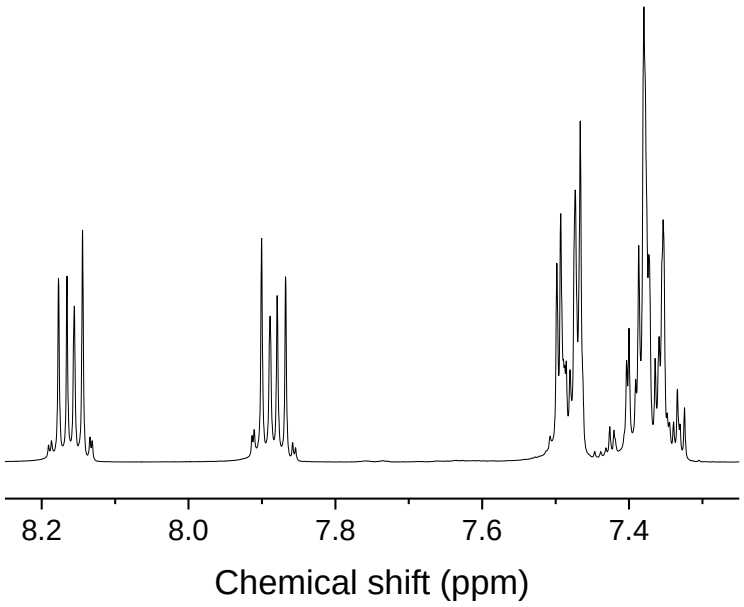

Figure S1. ^{1}H-NMR spectrum of DTTCT in d-DMSO.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 – 8.10 (m, 1H), 7.89 (dt, J = 6.4, 3.2 Hz, 1H), 7.55 – 7.43 (m, 2H), 7.43 – 7.29 (m, 3H).

XPS spectra of as-synthesized FeDTTCT

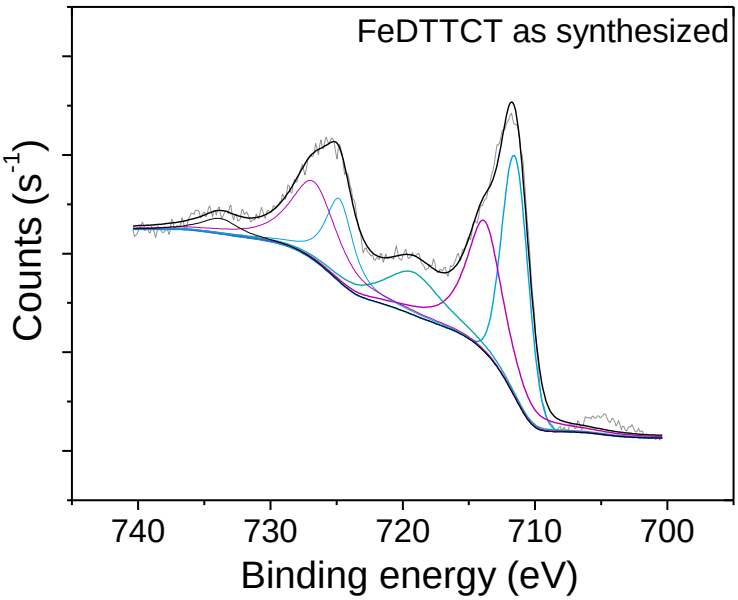

Figure S2. XPS spectra of Fe 2p region of as-synthesized FeDTTCT.

Nitric oxide generation measurement from metal complex in the presence of nitrite and ascorbic acid

Nitric oxide measurement was performed following the method described in the manuscript. In brief, various coupons (bare, 1 mg CuDTTCT, or 1 mg of FeDTTCT) was placed in a 20 mL glass vials equipped with stir bars. A 10 mL aliquots of 10 mM phosphate buffer solution pH 6 was filled into the vials. When stable baseline was observed, 1 mM of sodium nitrite was added into the glass vials, which is immediately followed by the addition of 1 mM of ascorbic acid (black arrow; Sigma-Aldrich).

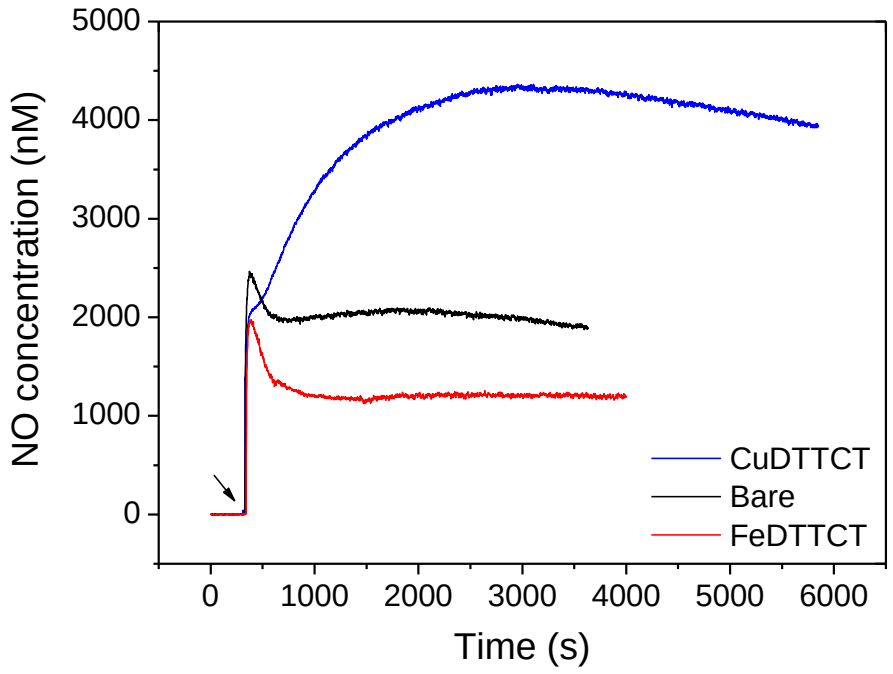

Figure S3. Amperometric measurements of nitric oxide generation from CuDTTCT coupon (1 mg loading) or FeDTTCT coupon (1 mg loading) in the presence of 1 mM nitrite and 1 mM ascorbic acid. Bare coupon (PVC coupon without the addition of metal complex) was used as the control. Black arrow denotes the addition of ascorbic acid.

Effect of FeDTTCT coupon on nitrifying bacteria growth

The effect of FeDTTCT coupon on nitrifying bacteria growth was investigated following the method detailed in the manuscript. Coupons containing various loading of FeDTTCT were incubated with the nitrifying bacteria culture in 12-well plates for 3 days in the dark (30 °C, 100 rpm) and the biomass was determined using BCA assay as described in the manuscript.

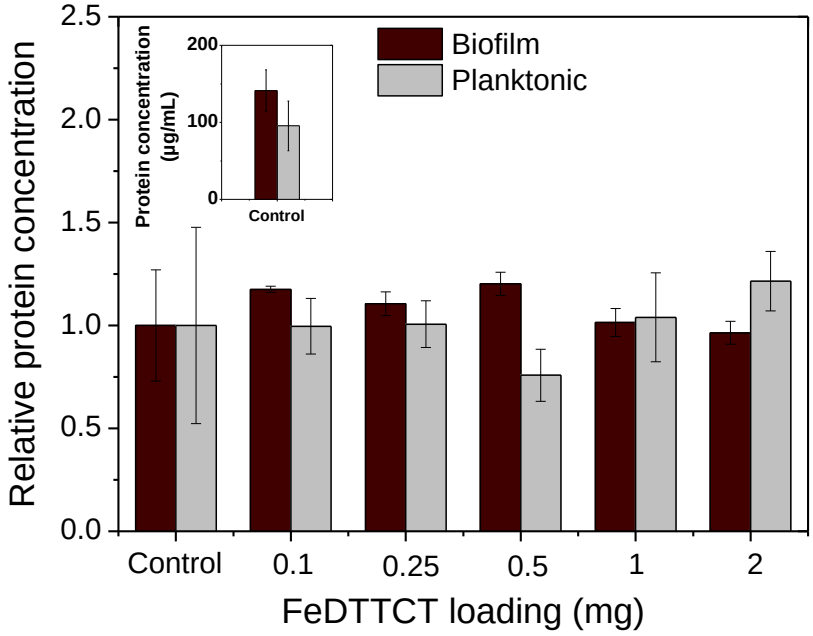

Figure S4. Protein measurements of nitrifying bacteria biomass grown in the presence of coupons containing various loading of FeDTTCT. All values shown are normalized to the bacteria biomass grown in the presence of bare coupon (control, inset). Error bars indicate standard error between replicates (n = 4); *$p \leq 0.05$ against the control.

Table S1. ICP-OES measurements of leached iron ions concentration after 3 days incubation with nitrifying bacteria from coupons containing various loading of FeDTTCT

FeDTTCT loading (mg)	Leached iron ions concentration (mg/L)
0.1	0.58
0.25	0.59
0.5	0.59
1	0.52
2	0.55

Effect of CuDTTCT coupon on nitrifying bacteria growth

The effect of CuDTTCT coupon on nitrifying bacteria growth was investigated following the method detailed in the manuscript. Coupons containing various loading of CuDTTCT were incubated with the nitrifying bacteria culture in 12-well plates for 3 days in the dark (30 °C, 100 rpm) and the biomass was determined using BCA assay as described in the manuscript.

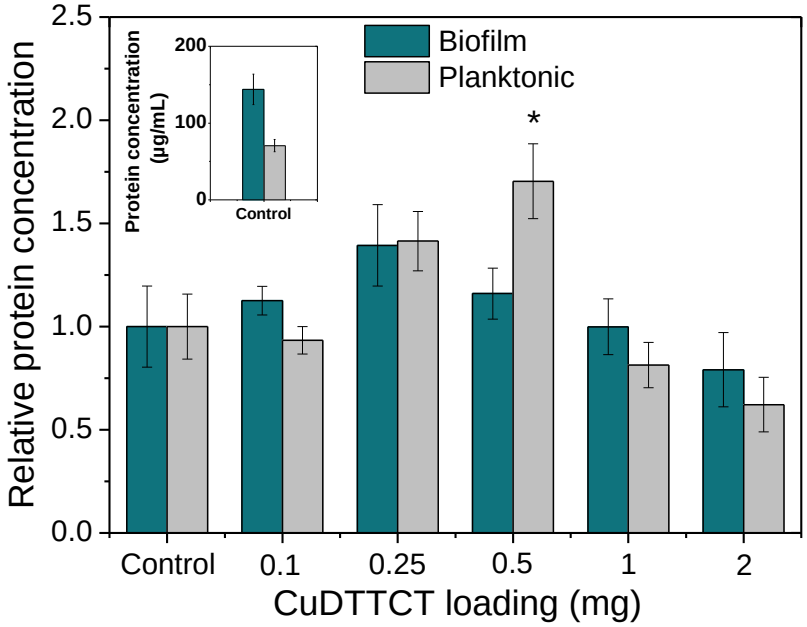

Figure S5. Protein measurements of nitrifying bacteria biomass grown in the presence of coupons containing various loading of CuDTTCT. All values shown are normalized to the bacteria biomass grown in the presence of bare coupon (control, inset). Error bars indicate standard error between replicates (n = 4); *$p \leq 0.05$ against the control.

Table S2. ICP-OES measurements of leached copper ions concentration after 3 days incubation with nitrifying bacteria from coupons containing various loading of CuDTTCT

CuDTTCT loading (mg)	Leached copper ions concentration (mg/L)
0.1	0.08
0.25	0.45
0.5	0.71
1	0.87
2	1.18

Effect of PTIO on nitrifying bacteria growth

Various concentration of PTIO were added into nitrifying bacteria culture in a 12-well plates and incubated for 3 days in the dark (30 °C, 100 rpm). Bacteria biomass was determined using BCA assay as described in the manuscript.

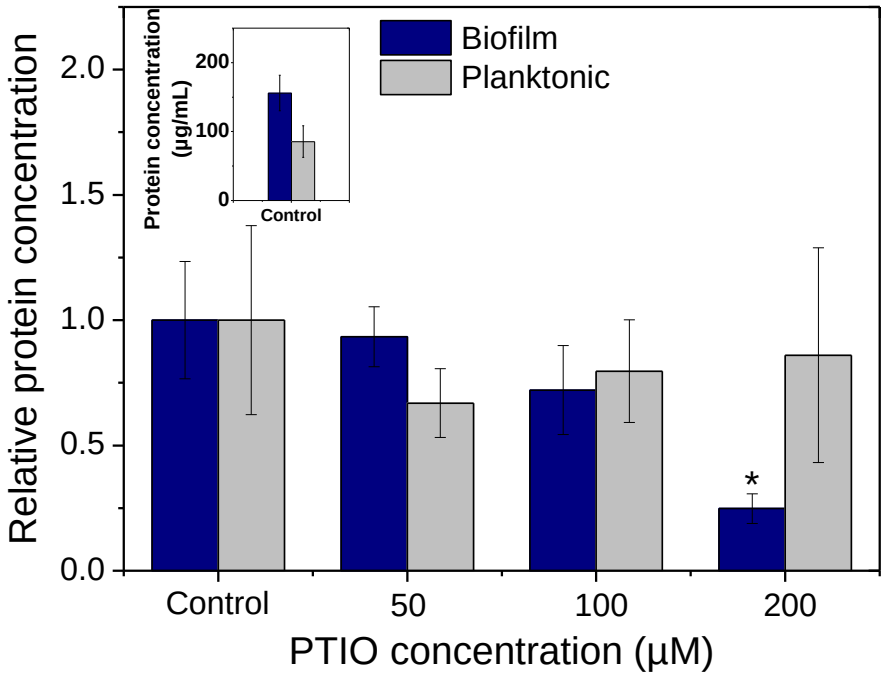

Figure S6. Protein measurements of nitrifying bacteria biomass grown in the presence of PTIO. All values shown are normalized to the bacteria biomass grown in the presence of bare coupon (control, inset). Error bars indicate standard error between replicates (n = 4); *$p \leq$ 0.05 against the control.