Cysteine-Cystine Photoregeneration for Oxygenic Photosynthesis of Acetic Acid from CO₂ by a Tandem Inorganic-Biological Hybrid System

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Supporting Information

Fig. S1-2

Materials and Methods
Figure S1. Reflectance Spectra of TMPc and TiO₂-TMPc. Spectra of TiO₂-TMPc show distinct differences from the neat TMPc. Features in the range of 400-500 nm are diminished on TiO₂, while absorption in the range of >600 nm is enhanced.

Figure S2. Direct Comparison of TMPc and H₂Pc Reflectance Spectra.
Materials and Methods

All reagents were used as received without further purification and were purchased from Sigma-Aldrich with the exception of zinc phthalocyanine (Acros Organics), cobalt(II) phthalocyanine (Alfa Aesar), and nickel(II) phthalocyanine (Eastman Kodak). Reagents related to the growth of *M. thermoacetica* (ATCC 39073) and photosynthesis characterization were described previously.9

Preparation of TiO$_2$-TMPc Photocatalyst

Phthalocyanines were loaded onto TiO$_2$ nanoparticles (Sigma-Aldrich 637254, anatase, <25 nm) by suspending both at the desired ratio in 50 mL of ethanol and 50 µL of 1 M HCl in a 100 mL beaker. The suspension was sonicated with a Microtip Probe Sonicator for 15 min intervals at 21% amplitude and allowed to settle. The solvent was removed by rotary evaporator and stored until further use.

Cystine Photoreduction Assay

Oxygen free 1,4-piperazinediethanesulfonic acid (PIPES, 30 mM) buffered media was made by boiling DI water under a N$_2$ stream for 5 min. The pH was adjusted to 7 by addition of 10 M NaOH. To the cooled PIPES buffer was added 10mM cystine. Each TiO$_2$-Pc composite was suspended in the cystine buffer at 0.1 g L$^{-1}$ and sealed in a Balch type anaerobic tube under an N$_2$ atmosphere. The suspensions were irradiated by a filtered 75 W Xenon lamp (Newport Corp., AM1.5G, 5% sun) for 2 hours with time points taken at 0, 0.25, 0.5, 1 and 2 hrs. Cysteine production was determined photometrically by addition of an equal volume of 5 mM Ellman’s reagent in degassed ethanol (freeze-pump-thaw). The mixture was allowed to react and then
centrifuged to remove the photocatalyst and quantified by UV-Vis spectrophotometry (Shimadzu UV3101PC UV-Vis-NIR Spectrophotometer with an integrating sphere). Reflectance spectra were obtained by suspending the photocatalysts or TMPc in ethanol and drop casting onto glass cover slips until an opaque film formed.

**Preparation and Photosynthesis Assay of *M. thermoacetica*-CdS+TiO₂-MnPc**

*M. thermoacetica*-CdS was prepared and assayed as previously described with modifications noted below.⁹ All techniques were conducted anaerobically under an oxygen-free atmosphere or using standard Hungate technique. Briefly, cryopreserved stocks of *M. thermoacetica* (ATCC 39073) was inoculated at 5 vol.% into undefined precipitation media (UDM) with 25 mM glucose, 0.1 wt.% cysteine·HCl, 250 kPa N₂:CO₂ (80:20) and incubated at 52°C for 3 days until OD₆₀₀=0.16. The suspension was reinoculated at 5 vol.% into fresh UDM with 50 mM glucose and 0.1 wt.% cysteine·HCl. After 24 hrs of incubation as before, 1mM Cd(NO₃)₂ was added. The culture was incubated an additional 2 days with frequent agitation. After 2 days, the yellow suspension was centrifuged under an inert atmosphere and resuspended in half the original volume (5 mL) of defined photosynthesis media (DPM) supplemented with 0.1 wt.% cysteine·HCl and 250 kPa H₂:CO₂ (80:20). The suspension was incubated for 24-36 hrs before the headspace was exchanged for N₂:CO₂ (80:20). Deletional controls (*M. thermoacetica*-CdS, *M. thermoacetica*-CdS+TiO₂-MnPc) were prepared in a similar fashion.

The 0.05 wt.% TiO₂-MnPc was suspended in DPM at 1 g L⁻¹ and sonicated for 1 hr. To the above *M. thermoacetica*-CdS suspension was added 5mL of the TiO₂-MnPc suspension. The complete 10 mL of *M. thermoacetica*-CdS+TiO₂-MnPc was illuminated by a filtered 75 W Xenon lamp (AM1.5G, 5% sun) with a 12 hrs light/12 hrs dark cycle with heating and stirring.
(55 °C, 150 rpm). The suspensions were sampled every 12 hrs. Liquid sample time points were centrifuged to remove cells and nanoparticles and assayed for acetic acid concentration by quantitative $^1$H-NMR with trimethylsilylpropionate as an internal standard. Oxygen production as monitored by a Neofox in-situ optical oxygen monitoring probe (Ocean Optics, Inc.).