Supplementary Information

Methane formation driven by light and heat prior to the origin of life and beyond

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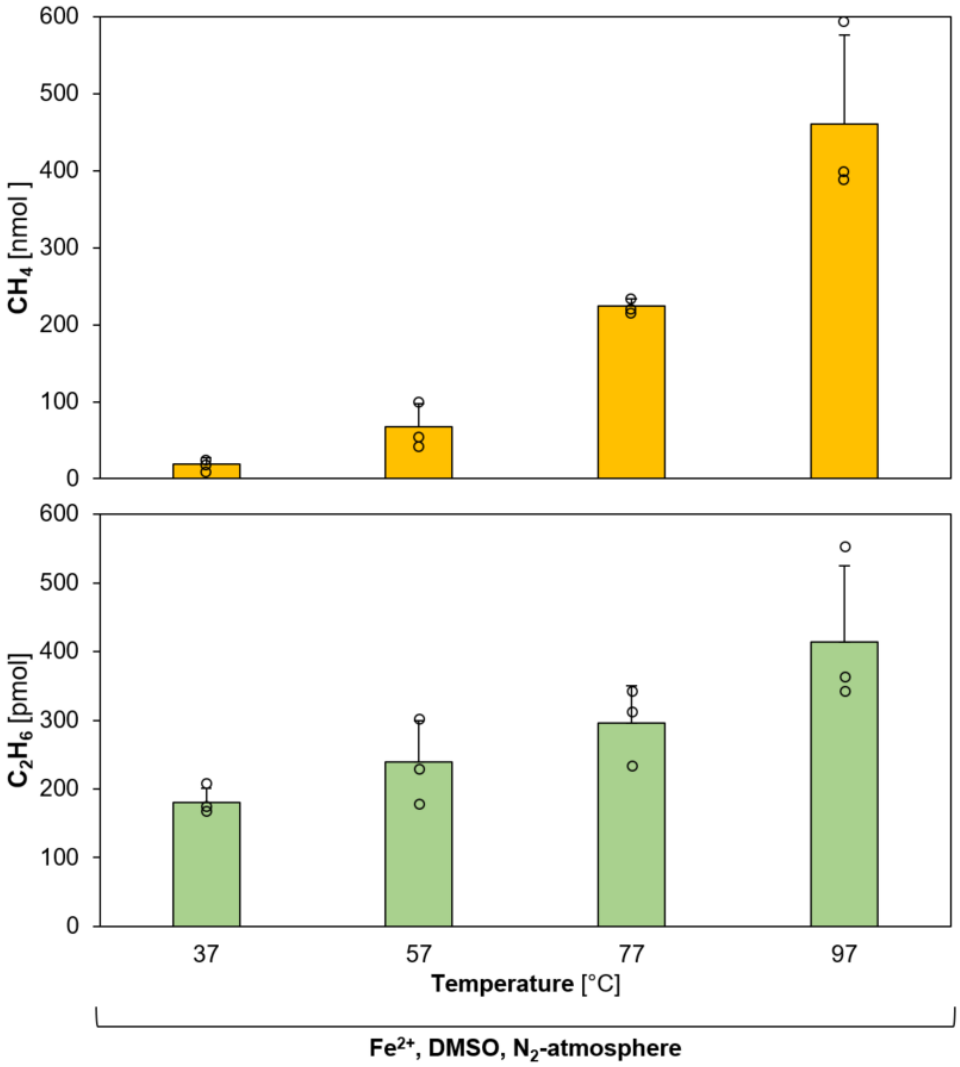
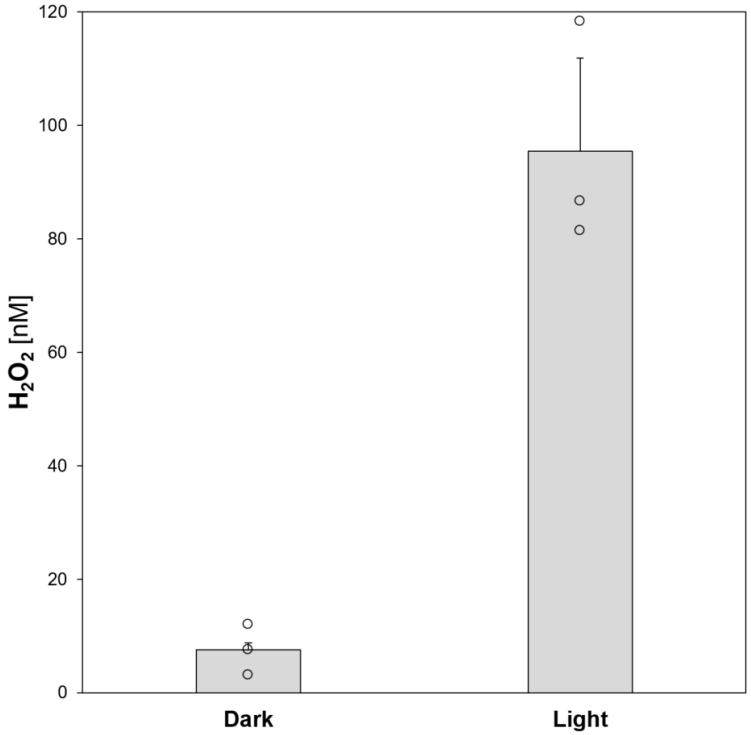
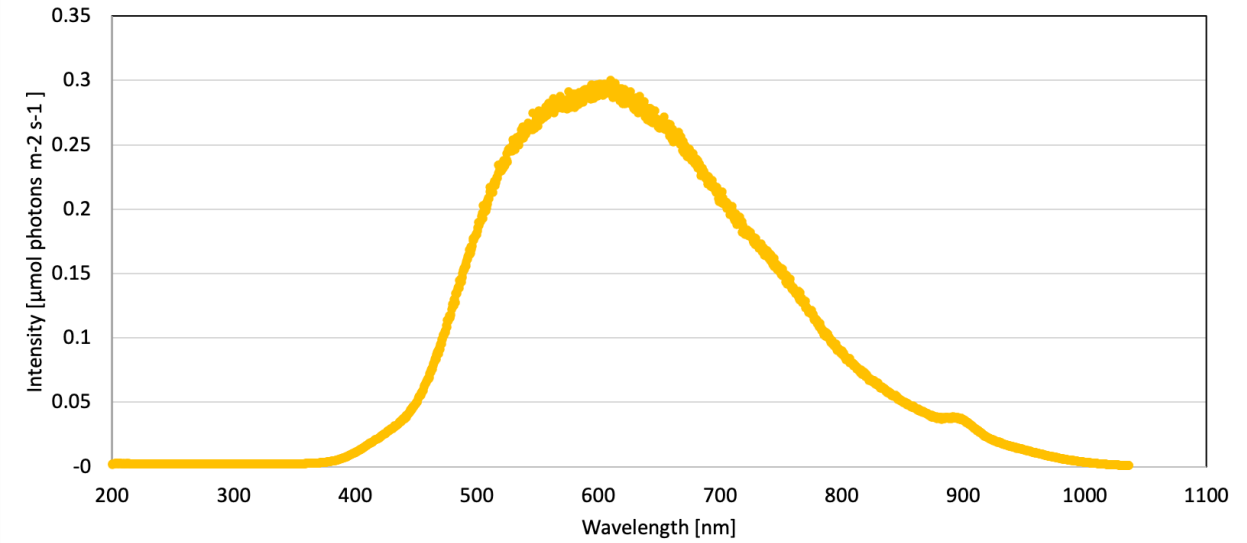


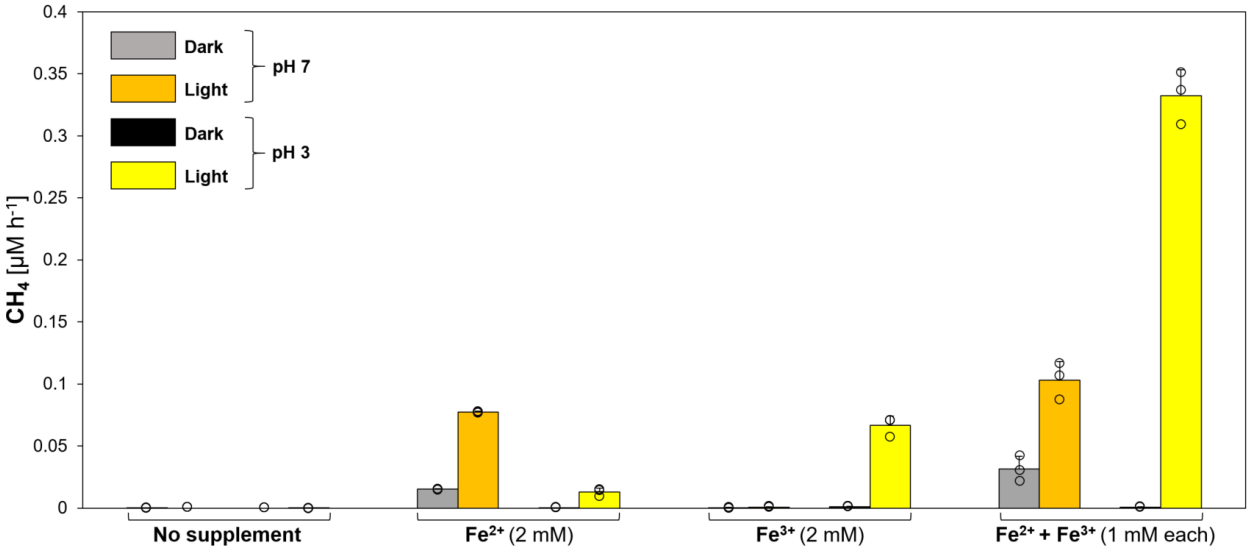
Supplementary Fig. 1. Heat-driven CH_4 and C_2H_6 formation from DMSO. Formed CH_4 amounts increase from ~20 nmol (37 °C) to ~460 nmol (97 °C). Formed C_2H_6 amounts increase from ~190 pmol (37 °C) to ~415 pmol (97 °C), thereby resulting in CH_4:C_2H_6 ratios from ~110 (37 °C) to ~1100 (97 °C). In a total volume of 4 mL, samples containing a 20 mM potassium phosphate buffer (pH 7), 1 M DMSO, 10 mM $FeSO_4$ were incubated at different temperatures in sealed 20 mL glass vials under N_2 for 4 days. The bars are the mean + standard deviation of triplicates, shown as circles.



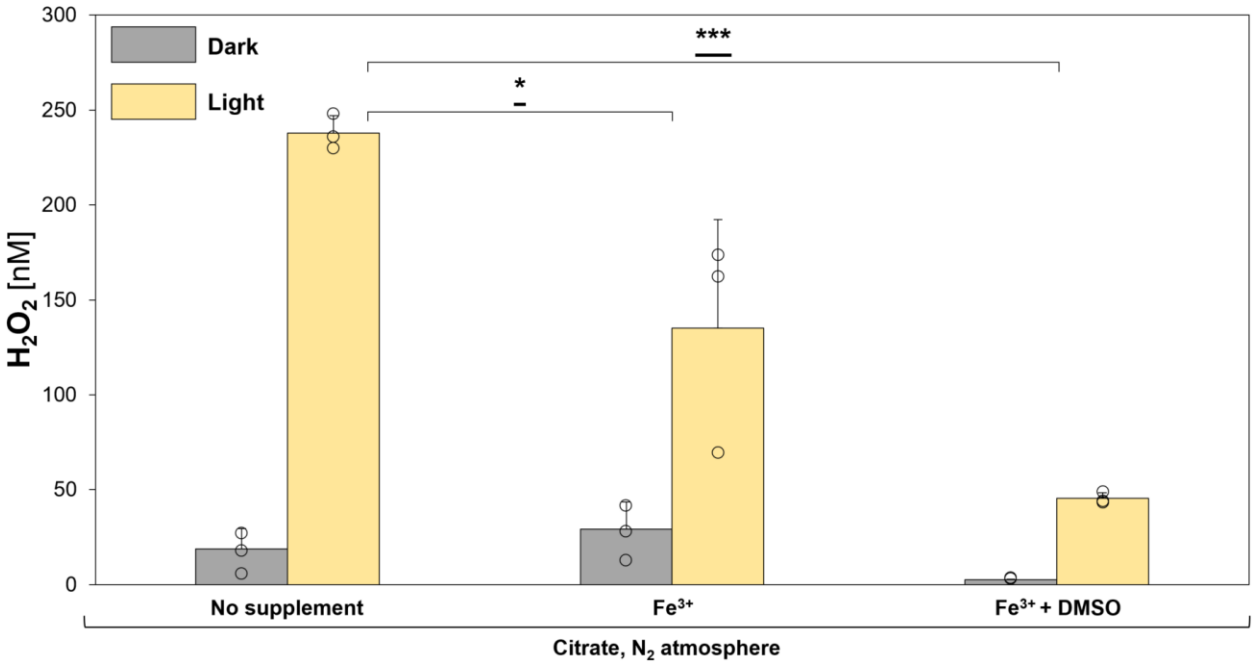
Supplementary Fig. 2. Light-driven H_2O_2 formation in pure buffer. Formed H_2O_2 concentrations increase from ~7 nM in the dark to ~95 nM under light. In a total volume of 4 mL, samples contained 20 mM potassium phosphate buffer (pH 7) that was bubbled with N_2 for 1 h and kept in an anoxic tent for 5 days. Samples were incubated in the dark or under illumination (Supplementary Fig. 3) for 20 h and analysed via fluorescence-based H_2O_2 endpoint measurements (see Methods). The bars are the mean + standard deviation of triplicates, shown as circles.



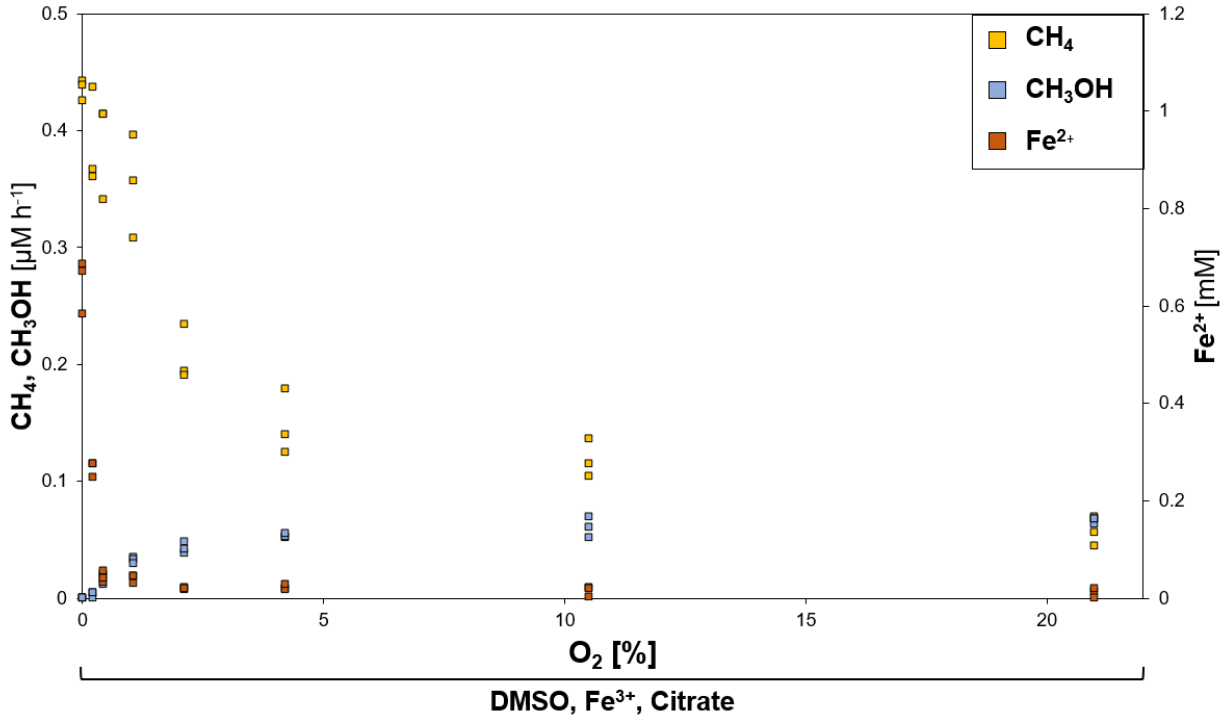
Supplementary Fig. 3. Light spectrum of the used light bulbs. For broad-spectrum sample illumination, Osram (Superlux, Super E SIL 60) light bulbs were used, with an intensity of 82 ± 4 µmol photons m^{-2} s^{-1}, and an energy flux of 52 ± 2 kJ m^{-2} h^{-1}. The light spectrum was determined with a spectrometer (see Methods).



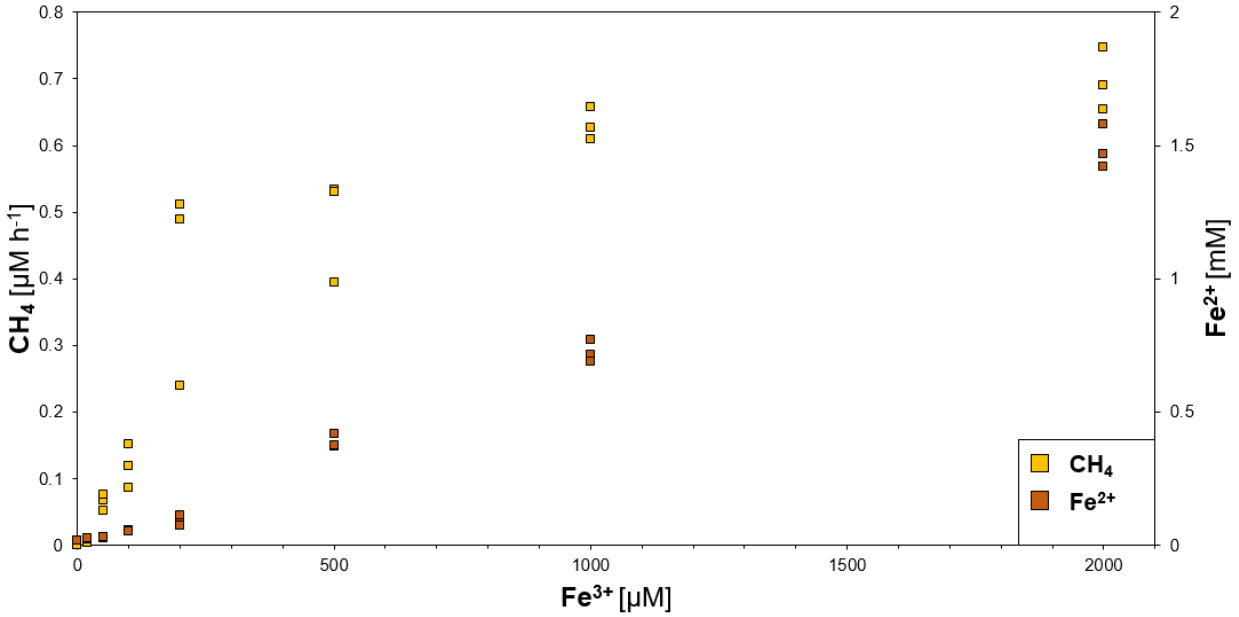
Supplementary Fig. 4. Light-driven CH_4 formation is enhanced upon $[Fe(H_2O)_6]^{3+}$ photolysis under acidic conditions. Only trace CH_4 levels (<0.0002 µM h^{-1}) were measured without iron addition. Upon Fe^{2+}-supplementation, CH_4 formation rates increased at pH 7 from ~0.015 µM h^{-1} in the dark to ~0.077 µM h^{-1} under light and at pH 3 from <0.0002 µM h^{-1} in the dark to ~0.013 µM h^{-1} under light, suggesting a light-driven ·OH formation from OH^-. Conversely, ~0.67 µM CH_4 h^{-1} are formed upon Fe^{3+}-supplementation in the light under acidic conditions, while only trace amounts of CH_4 were detected under pH-neutral conditions or in the dark. At pH 7, a stoichiometric 1:1 ratio of Fe^{3+} and Fe^{2+} increased CH_4 formation rates by ~1.3-fold to ~0.1 µM h^{-1} in comparison to Fe^{2+}-supplemented samples, while the corresponding CH_4 formation rates at pH 3 increased ~25-fold to ~0.316 µM h^{-1}. Samples consisting of buffered solutions (pH 3 or 7) were incubated in the dark or under broad spectrum light (Supplementary Fig. 3) for 24 h under N_2. The bars are the mean + standard deviation of triplicates, shown as circles.



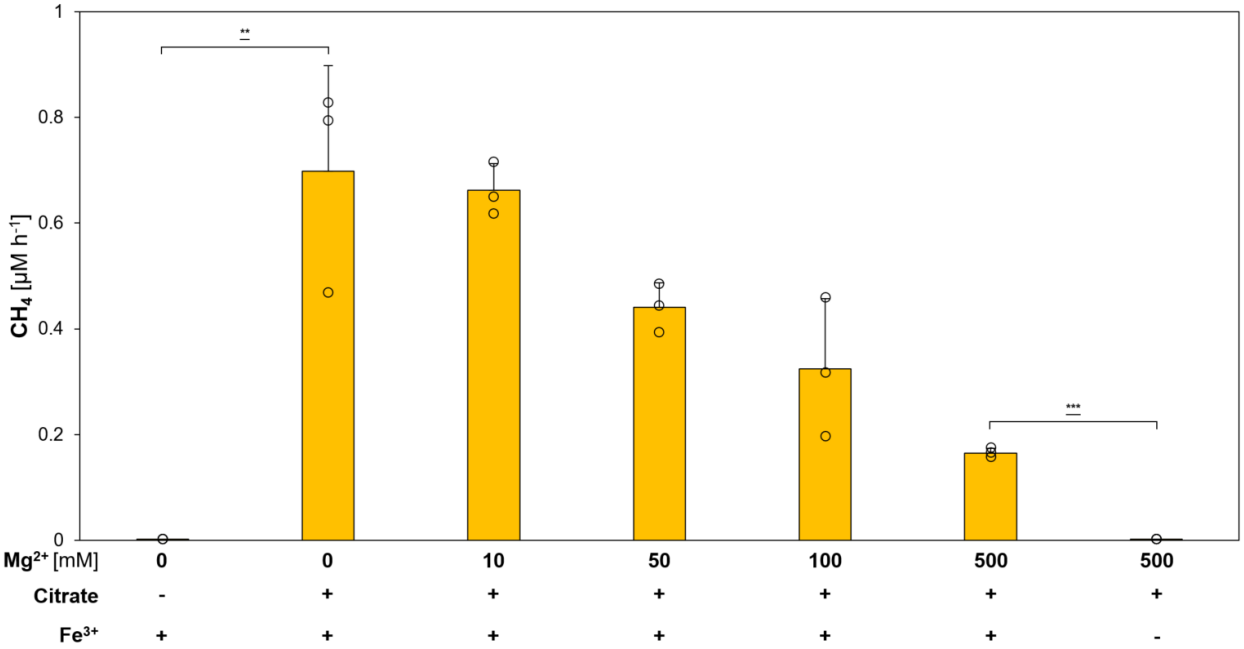
Supplementary Fig. 5. Iron and DMSO reduce H_2O_2 concentrations generated by light. Under light, H_2O_2 concentrations decrease from ~238 nM (buffer + citrate) to ~135 nM in the presence of iron (buffer + citrate + Fe^{3+}). This decrease in H_2O_2 concentration indicates the reaction of H_2O_2 with Fe^{2+} formed by LMCT. Upon addition of DMSO, H_2O_2 levels further decrease to ~45 nM under light, underlining the ROS-scavenging effect of DMSO. In a total volume of 4 mL, samples contained 20 mM potassium phosphate buffer (pH 7), 10 mM citrate and, optionally, 2 mM $FeCl_3$ and 500 mM DMSO that were previously degassed with N_2 and kept in an anoxic tent overnight. Samples were incubated in the dark or under illumination (Supplementary Fig. 3) for 3 h and directly analysed via fluorescence-based H_2O_2 endpoint measurements (see Methods). Statistical analysis was performed using paired two-tailed *t*-tests, *: $p \leq 0.05$, ***: $p \leq 0.001$. The bars are the mean + standard deviation of triplicates, shown as circles.



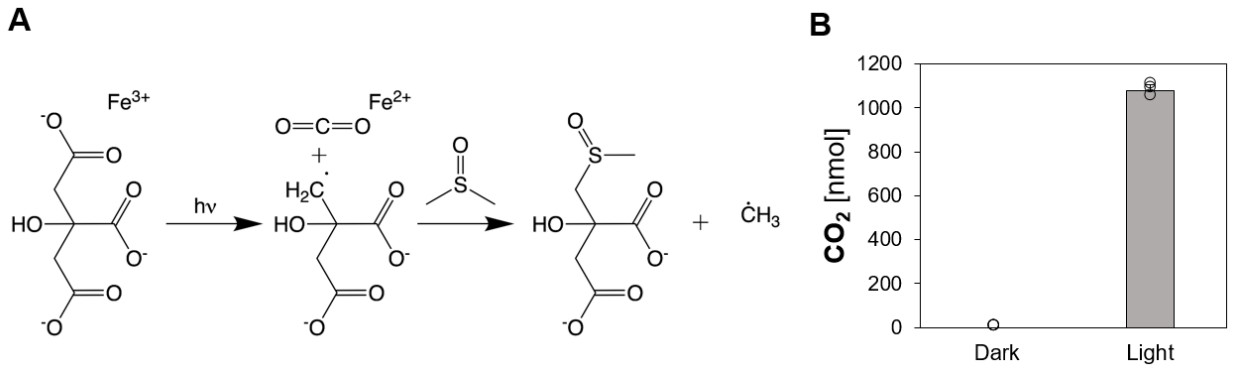
Supplementary Fig. 6. Light-driven formation of CH_4, CH_3OH and Fe^{2+} from DMSO and Fe^{3+} under varying O_2 concentrations. In a total volume of 4 mL, samples containing 20 mM potassium phosphate buffer (pH 7), 500 mM DMSO, 2 mM $FeCl_3$ and 10 mM citrate were incubated for one day in sealed 20 mL glass vials under constant broad-spectrum illumination (Supplementary Fig. 3). Mixtures of air and N_2 were generated and adjusted to 1 bar, ranging from ~21 % O_2 to pure N_2 (0 % O_2). From 0 % O_2 to ~1 % O_2, CH_4 rates decreased from ~0.44 µM h^{-1} to ~0.35 µM h^{-1}, while Fe^{2+} levels decreased from ~1 mM to ~0.07 mM. From ~1 % O_2 to 21 % O_2, CH_4 rates further decreased by an additional ~0.2 µM h^{-1}, while Fe^{2+} levels further decreased from ~0.07 mM to ~0.04 mM, indicating an immediate Fe^{2+} oxidation by O_2 or the Fenton reaction at high O_2 concentrations and a formation of excess Fe^{2+} under suboxic and anoxic conditions. Apart from the anoxic sample (0 % O_2), formation of CH_3OH was detected in the presence of O_2, ranging from ~0.003 µM h^{-1} (~0.2 % O_2) to ~0.07 µM h^{-1} (21 % O_2). Experiments were conducted in triplicates; individual values are shown.



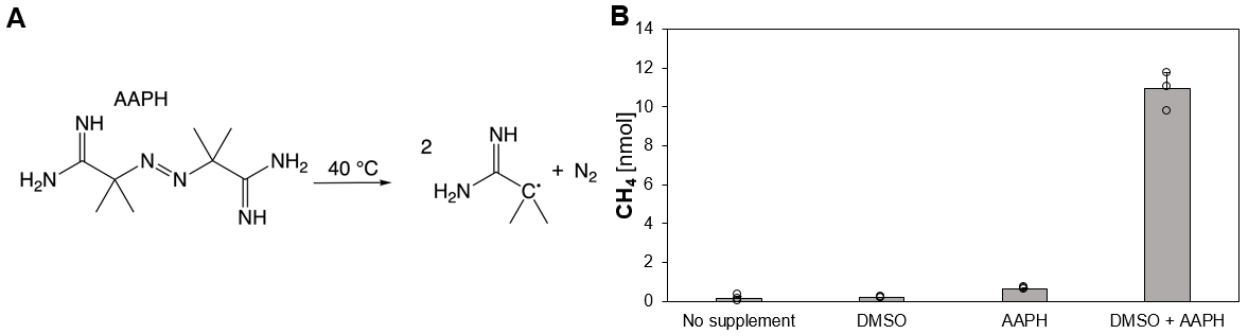
Supplementary Fig. 7. Light-driven CH_4 formation from DMSO correlates with the iron concentration. In a total volume of 4 mL, samples containing 20 mM potassium phosphate buffer (pH 7), 500 mM DMSO and 10 mM citrate were incubated for one day under N_2 in sealed 20 mL glass vials under constant broad-spectrum illumination (Supplementary Fig. 3). Different amounts of Fe^{3+} (as $FeCl_3$) were added, ranging from 0 µM to 2000 µM. Only trace amounts of CH_4 (<0.01 µM h^{-1}) were formed upon supplementation with 0 or 20 µM Fe^{3+}. Methane amounts then increased from ~0.06 µM h^{-1} CH_4 (50 µM Fe^{3+}) to ~0.4 µM h^{-1} (200 µM Fe^{3+}), with Fe^{2+} levels also increasing from ~0.03 mM to ~0.11 mM. From 200 µM Fe^{3+} to 2000 µM Fe^{3+} supplementation, CH_4 levels increased by ~0.3 µM h^{-1}. In the same range, Fe^{2+} levels increased from ~0.11 mM to ~1.5 mM, indicating that LMCT-driven Fe^{2+} formation occurred at higher rates as its consumption to Fe^{3+} via the photochemical Fenton reaction. Experiments were conducted in triplicates; individual values are shown.



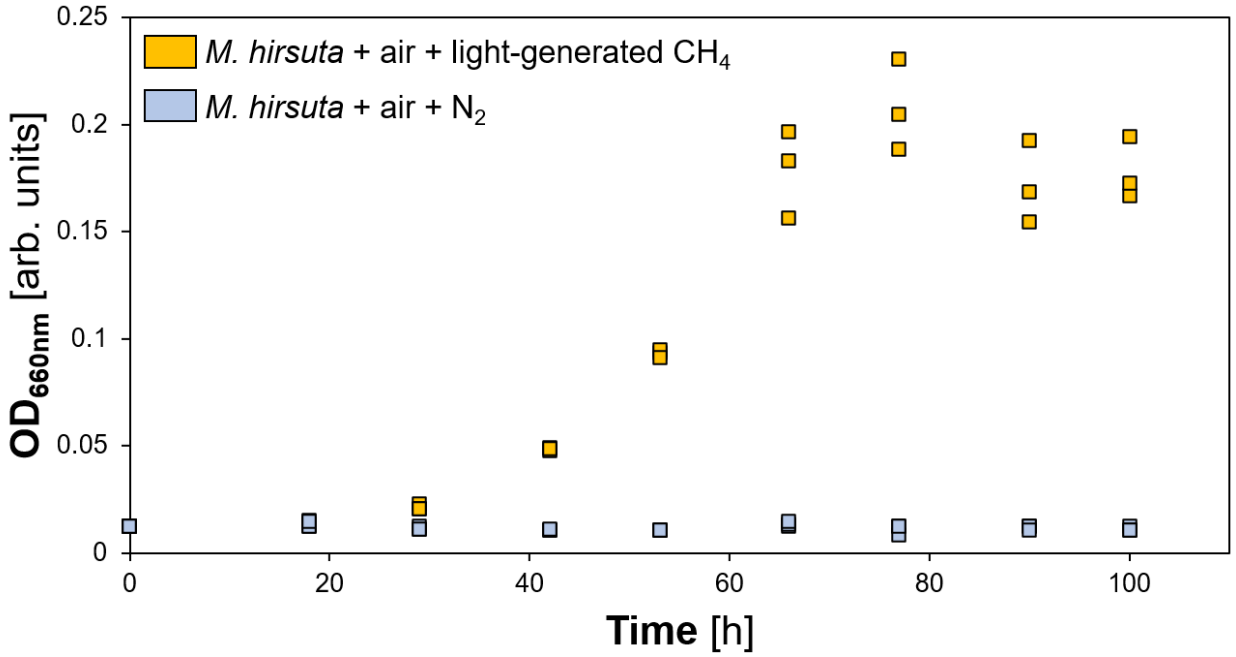
Supplementary Fig. 8. Magnesium decreases light-driven CH_4 formation from DMSO. In a total volume of 4 mL, samples containing 20 mM potassium phosphate buffer (pH 7), 500 mM DMSO and, optionally, 2 mM Fe^{3+} (as $FeCl_3$) and 10 mM citrate were incubated for one day under N_2 in sealed 20 mL glass vials under constant broad-spectrum illumination (Supplementary Fig. 3). Different amounts of Mg^{2+} (as $MgCl_2$) were added, ranging from 0 mM to 500 mM. Only trace amounts of CH_4 (<0.01 µM h^{-1}) were formed in the presence of Mg^{2+} and citrate Upon supplementation with Fe^{3+} without addition of citrate trace amounts of CH_4 (<0.01 µM h^{-1}) were detected. Upon citrate and Fe^{3+} supplementation, CH_4 rates increased to ~0.7 µM h^{-1}, while increasing Mg^{2+} amounts decreased CH_4 rates until ~0.16 µM h^{-1} at 500 mM Mg^{2+}. Experiments were conducted in triplicates; individual values are shown as cirlces.



Supplementary Fig. 9. Decomposition of the LMCT-induced citrate radical drives ·CH_3 formation from DMSO. (A) Illumination of a Fe^{3+}-citrate complex results in Fe^{2+} and a citrate radical, disassembling into CO_2 and a carbon-centered radical that further reacts with DMSO by cleaving off a ·CH_3. **(B)** While no CO_2 is formed in the dark, ~1080 nmol CO_2 is detected from illuminated samples containing 2 mM $FeCl_3$, 10 mM citrate, 500 mM DMSO in 20 mM potassium phosphate buffer (pH 7) under N_2, after 24 h. The bars are the mean + standard deviation of three independent measurements, shown as circles.



Supplementary Fig. 10. Organic radicals drive CH_4 formation from DMSO. (A) At 40 °C, the compound 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) decomposes into 2 carbon-centered organic radicals and N_2. **(B)** CH_4 is formed upon the interaction between DMSO and AAPH. While only ~0.2 nmol or ~0.6 nmol CH_4 are formed from DMSO or AAPH alone, respectively, ~11 nmol CH_4 are formed upon combining both substances, indicating CH_4 formation driven by carbon-centered radicals. 500 mM DMSO was mixed with 20 mM AAPH and incubated for 3 h in N_2-saturated DPBS buffer at 40°C under N_2. The bars are the mean + standard deviation of triplicates, shown as circles.



Supplementary Fig. 11. Light-driven CH_4 formation sustains methanotrophic growth of *Methylocystis hirsuta*. Methanotrophic growth of *M. hirsuta* is sustained by light-generated CH_4. While CH_4-supplemented samples (yellow squares) grew, N_2-supplemented samples (blue squares) did not exhibit bacterial growth (see Methods). Experiments were conducted in triplicates; individual values are shown.