Electronic Supplementary Material

Biosynthetic CdS–*Thiobacillus thioparus* hybrid for solar-driven carbon dioxide fixation

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Experimental section:

Characterization of CdS–*T. thioparus* biohybrid

After obtaining the optimal Cd^{2+} concentration, 0.1 mM Cd^{2+} and 1 mM cysteine were added into the system to constructing the CdS–*T. thioparus* biohybrid. After 72 h cultivation, a series of characterization methods including high-angle circular dark-field scanning transmission electron microscopy (HAADF-STEM), high-resolution transmission electron microscopy (HRTEM), energy-dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), high-resolution X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) analysis have been used to examine the CdS–*T. thioparus* biohybrid. To prepare HAADF-STEM samples, 1 mL aliquots of CdS–*T. thioparus* biohybrids were centrifuged at 14,000 × g for 10 min. The samples were washed by PBS for three times, with each washing time taking 20 min. 5% glutaraldehyde was added to the sample and placed in a flat-plate shaker (MS-3 digital, IKA, Germany) with 200 rpm. Then the fixed samples were centrifuged at 14,000 × g for 10 min and washed with PBS for three times. The fixed samples were sequentially dehydrated by 25%, 50%, 75%, 90% and 100% ethanol and deposited on 200-mesh copper grids. The sample was observed through the HAADF-STEM system (Talos F200X, FEI, USA), and the composition was analyzed by EDS mapping and energy spectrum. At the same time, the fixed samples were dispersed on silicon wafers and platinum was sprayed on the surface to enhance conductivity. The surface loading of CdS–*T. thioparus* and *T. thioparus* were detected under a scanning electron microscope (Gemini SEM 500, Germany). To prepare the sample for XPS analysis, 100 mL CdS–T. *thioparus* biohybrid was centrifuged and washed three times with PBS. Dried samples and chemically synthesized CdS were dispersed on silicon wafers and detected under a high-resolution X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250Xi, Thermo Fisher Scientific, USA). To prepare samples for HRTEM observation, 1 mL CdS–*T. thioparus* biohybrid was collected and centrifuged at 14,000 × g for 10 min. The sample was washed with PBS for three times and fixed with 5% glutaraldehyde at 4°C for 2 h. The fixed sample was washed twice with PBS and sequentially dehydrated by 30%, 50%, 70%, 80%, 95% and 100% ethanol, and finally dispersed on the ultra-thin copper grid. The samples were examined with a transmission electron microscopy (JEOL 2100 TEM, 200 kV, Japan). To prepare the sample for XRD analysis, 200 mL CdS–*T. thioparus* biohybrid was centrifuged and washed three times with ice-cold PBS. The samples were freeze-dried into powder and measured with an X-ray diffractometer (Smart Labs, JAPAN). The results were compared with standard card PDF-41-1049.

NADPH measurement

The NADPH was measured by the NADP/NADPH assay kit (ab65349, Abcam, UK). 400 μL diluted *T. thioparus* or CdS–*T. thioparus* biohybrid was centrifuged at 13,000 × g for 10 minutes and washed with PBS for three times, followed by adding 800 μL of NADP/NADPH extraction buffer and disposing by freeze/thawed cycles (20 min in liquid nitrogen,10 min at room temperature) for three times. Then the samples were vortexed (VORTEX 2, IKA, Germany) for 10 s and centrifuged at 13,000 × g for 5 min. After transferring 200 μL supernatant of samples into micro-centrifuge tubes and heated to 60°C in a water bath for 30 min, the samples were chilled on ice. Under these conditions, all $NADP^+$ will be decomposed, and NADPH will remain. Then the 50 μL sample, 98 μL NADP cycling buffer and 2 μL NADP cycling enzyme were added in a 96-well flat-bottom plate (Costar, USA). The reaction system was placed on a shaker for mix 5 min. Then, 10 μL NADPH developer was added into the 96-well flat-bottom plate, and the reaction started at room temperature for 1–4 h. A spectrophotometric multi-well plate reader (Bio-Rad iMark, Bio-Rad, USA) was used to monitor the variation at OD_{450} until the value became stable. The NADPH concentration was standardized by the standard curve plotted by the NADPH standard (0–100 pmol). The determination was performed in triplicate, and the standard deviation was calculated accordingly.

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Glyceraldehyde 3-phosphate measurement

GAPDH (glyceraldehyde 3-phosphate dehydrogenase) can catalyze the conversion of glyceraldehyde 3-phosphate (GAP) to 1,3-bisphosphoglycerate (BPG). This reaction produces a colorimetric ratio (450 nm) that is proportional to the enzyme activity. Therefore, the GAP concentration in the system can be quantified by the absorbance at 450 nm. A 200 μL aliquot of the sample was centrifuged at 13,000 × g for 5 min and washed three times with PBS. The same centrifugation was used to collect the sediment. Then 200 μL of assay buffer (MAK-277A, Sigma-Aldrich, USA) was added to the system for homogenizing 10 min, and centrifuged at 11,000 × g for 5 min. After that, 96 μL of the supernatant was mixed with 2 μL GADPH Developer (MAK-277C, Sigma-Aldrich, USA) and 2 μL GADPH (MAK-277E, Sigma-Aldrich, USA) in a 96-well flat-bottom plate. The reaction system was incubated at 37°C in the dark for 60 min, and then a spectrophotometric multi-well plate reader was used to measure the absorbance at OD_{450}. Meanwhile, the GAP concentration was standardized by the standard curve plotted by the GAP standard solution (G5251, Sigma-Aldrich, USA). The determination was performed in triplicate, and the standard deviation was calculated accordingly.

Glutamate Synthase (GOGAT) measurement

GOGAT mainly exists in prokaryotes, yeasts, and non-green tissue precursors of higher plants. GOGAT forms the GS/GOGAT cycle with glutamine synthetase (GS) and participates in the regulation of ammonia assimilation. GOGAT uses NADH as an electron donor to catalyze the amino group of glutamine to α-ketoglutarate to form two glutamic acid molecules. The decrease rate of NADH absorbance at 340 nm can reflect the activity of GOGAT. GOGAT assay kit (Sangon Biotech, D799301, China) was used to detect the changes in solar-driven CO_2 fixation. *T. thioparus* and CdS–*T. thioparus* biohybrid were collected into the centrifuge tube and centrifuged at 13,000 × g for 5 min, followed by discard the supernatant. The obtained samples were washed three times with PBS and homogenized with 500 μL extract buffer for 10 min. Then the samples were broken by an ultrasonic cell breaker (JY92-IIN, Xinzhi, Ningbo) in ice bath, followed by centrifuging at 10,000 × g for 10 min and taking the supernatant on ice. Finally, 100 μL sample and 900 μL working solution were added to the reaction tube, and the absorbance changes before and after the reaction were recorded at OD_{340}. GOGAT activity was measured every 12 h in a transfer cycle and took the average value of triplicate measurements.

Bacterial dry weight measurement

1 mL aliquots of the samples from experimental and control groups were collected and centrifuged at 13,000 × g for 5 min, followed by carefully removing the supernatant. The obtained samples were placed in an electro-heating standing-temperature cultivator (DHP-9082, Yiheng, Shanghai) at 80°C. The dry mass of each dilution was measured by subtracting the mass of the pre-weighed centrifuge tube from the total mass. For the CdS–*T. thioparus* experimental group, the dry mass was calculated by subtracting added Cd^{2+} and cysteine. The data were collected by taking the average value for triplicate determinations.

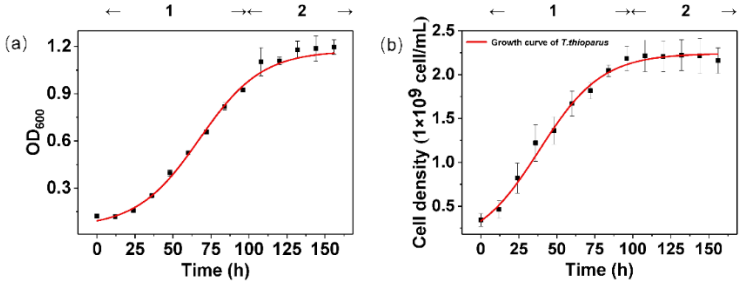

Figure S1 (a) Standard curve of cell density (cell/mL) against optical density at 600 nm (OD_{600}) (b). Growth curve of *T. thioparus* in Medium 1#. (1): the mid-exponential growth phase, (2): early stationary growth phase. Boltzmann model was used to perform a non-linear fit.

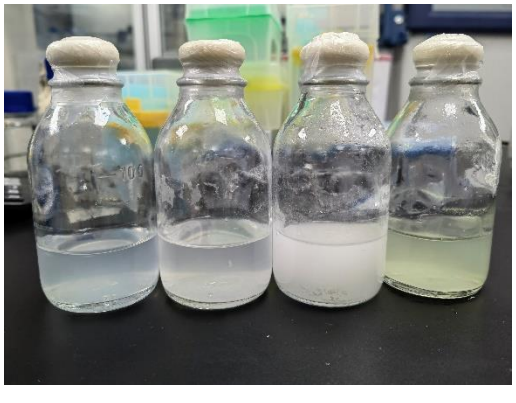

Figure S2 Photograph of the *T. thioparus* with only Cd^{2+}, *T. thioparus* with only cysteine, *T. thioparus*, and CdS–*T. thioparus* biohybrid with Cd^{2+} and cysteine, as depicted from left to right.

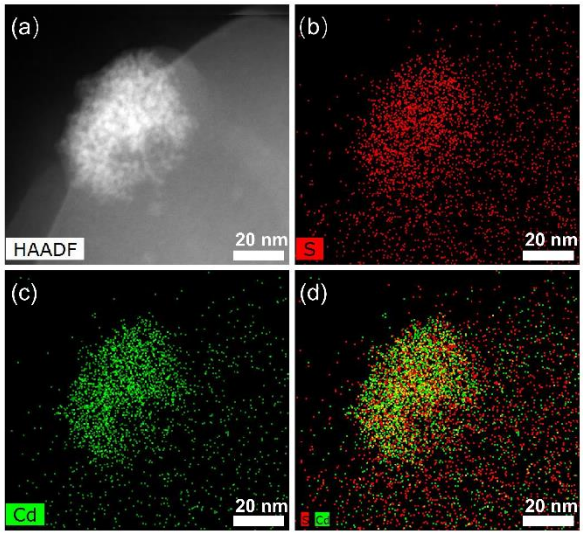

Figure S3 (a) HAADF-STEM image of the CdS–*T. thioparus* biohybrid. EDS mapping of the CdS–*T. thioparus* biohybrid showing the (b) S, (c) Cd and (f) S and Cd elements.

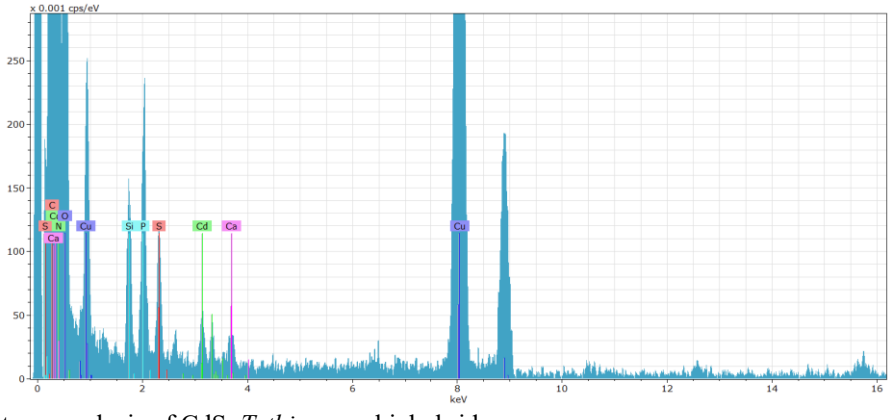

Figure S4 EDS spectrum analysis of CdS–*T. thioparus* biohybrid.

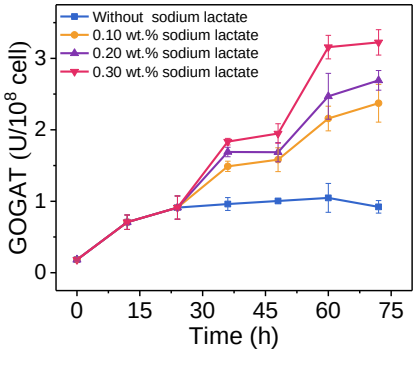

Figure S5 Sodium lactate-dependent GOGAT yield in solar-driven CO_2 fixation by CdS–*T. thioparus* biohybrid, during which the sodium lactate is added after 24 h of irradiation.

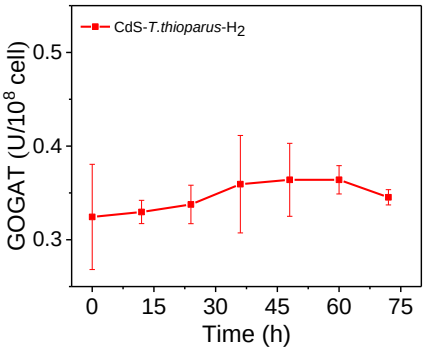

Figure S6 H_2-dependent GOGAT yield in solar-driven CO_2 fixation by CdS–*T. thioparus* biohybrid.

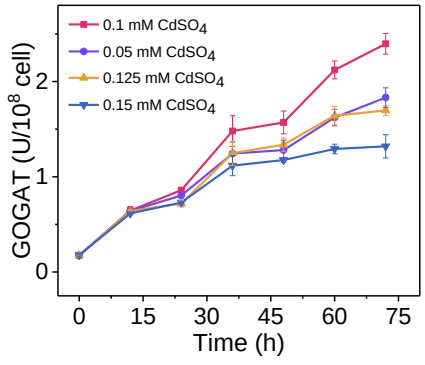

Figure S7 The effect of $CdSO_4$ concentration on the solar-driven CO_2 fixation performance by CdS–*T. thioparus* biohybrid.

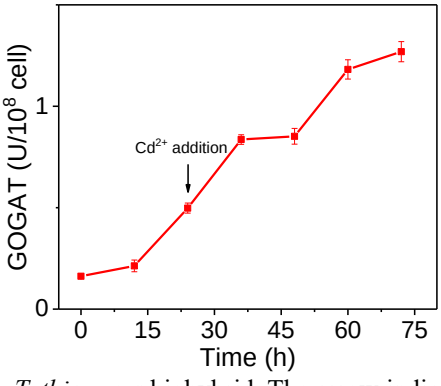

Figure S8 Cellular GOGAT level of CdS–*T. thioparus* biohybrid. The arrow indicates the time when CdS is generated. The experiment is conducted by the light/dark cycle (12h/12h). The GOGAT activity is measured every 12h in a transfer cycle and takes the average value in triplicate determination.

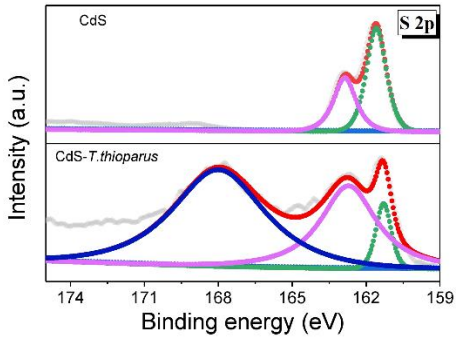

Figure S9 High-resolution XPS spectra of S 2p for the CdS–*T. thioparus* biohybrid system after 12 h solar-driven CO_2 fixation.

The emerging peak at about 168 eV is attributed to the formation of SO_4^{2-}.

Table S1 Mediums for cell culture, rapid biomass accumulation, CdS coating, CO_2 fixation and mechanism studies.

Culture Medium 1#	1000 mL	Reagent brand
ddH_2O	1000 mL	Milli-Q
$(NH_4)_2SO_4$	0.1 g	Aladdin
K_2HPO_4	4.0 g	Macklin
KH_2PO_4	4.0 g	Macklin
$MgSO_4 \times 7H_2O$	0.1 g	Macklin
$CaCl_2$	0.1 g	Macklin
$FeCl_3 \times 6H_2O$	0.02 g	Macklin
$MnSO_4 \times H_2O$	0.02 g	Macklin
$Na_2S_2O_3 \times 5H_2O$	10 g	Macklin
KOH	Adjust pH to 6.6	Aladdin

Culture Medium 2#	1000 mL	Reagent brand
ddH_2O	1000 mL	Milli-Q
K_2HPO_4	4.0 g	Macklin
KH_2PO_4	4.0 g	Macklin
$MgSO_4 \times 7H_2O$	0.1 g	Macklin
$CaCl_2$	0.1 g	Macklin
$FeCl_3 \times 6H_2O$	0.02 g	Macklin
$MnSO_4 \times H_2O$	0.02 g	Macklin
KOH	Adjust pH to 6.6	Aladdin

Culture Medium 3#	1000 mL	Reagent brand
ddH_2O	1000 mL	Milli-Q
K_2HPO_4	4.0 g	Macklin
KH_2PO_4	4.0 g	Macklin
$MgSO_4 \times 7H_2O$	0.1 g	Macklin
$CaCl_2$	0.1 g	Macklin
$FeCl_3 \times 6H_2O$	0.02 g	Macklin
$MnSO_4 \times H_2O$	0.02 g	Macklin
Agar	12 g	Macklin
KOH	Adjust pH to 6.6	Aladdin

Table S2 The contents of sulfur and cadmium in CdS–*T. thioparus* biohybrid.

Elements	Series	Net	[wt.%]	[norm. wt.%]	[norm. at.%]	Error in wt.% (3 Sigma)
Sulfur	K-series	1802	24.567	24.567	53.309	2.920
Cadmium	L-series	2906	75.433	75.433	46.691	23.338

Table S3 CdS coating efficiency of the *T. thioparus* cells collected at the mid-exponential growth phase.

Cd^{2+} concentration	Remained Cd^{2+} in medium	Coating efficiency (CE, 80 mL system)
0.1 mM	0.165 μg/mL	98.532 %
0.2 mM	6.220 μg/mL	72.330 %
0.3 mM	2.029 μg/mL	79.155 %
0.4 mM	9.558 μg/mL	78.740 %
0.5 mM	12.143 μg/mL	78.393 %