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Supplementary Information for

Acetogenic bacteria utilize light-driven electrons as an energy source for autotrophic growth

Sangrak Jin[a,b], Yale Jeon[c], Min Soo Jeon[c], Jongoh Shin[a,b], Yoseb Song[a,b], Seulgi Kang[a,b], Jiyun Bae[a,b], Suhyung Cho[a,b], Jung-Kul Lee[d], Dong Rip Kim[c], and Byung-Kwan Cho[a,b,e*]

*Corresponding author: B.-K.C. (bcho@kaist.ac.kr)

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Other supplementary materials for this manuscript include the following:

Datasets S1, S2, S3, and S4

SI Appendix Materials and Methods

Synthesis and characterization of cadmium sulfide nanoparticles.

The synthesis of cadmium sulfide nanoparticles (CdS-NPs) was based on the trituration method (1) for CdS-NP-1 and the hydrothermal method (2, 3) for CdS-NP-2, respectively. For CdS-NP-1, we mixed 40 mmol of cadmium acetate and 7 ml of PEG 400 for 10 min using the trituration

technique. Then, 40 mmol of thioacetamide was added and mixed for 30 min. The resulting mixture was left overnight to complete the reaction. For CdS-NP-2, we mixed 1 g of PVP and 4 mmol of cadmium chloride, and 4 mmol of thiourea in 80 mL of DI-water and stirred for 20 min. Then, the mixed solution was transferred to a 100 mL Teflon-lined stainless steel autoclave and heated 150 °C for 8 h. The byproducts were removed by centrifugation with distilled water once and with ethanol twice at 9000 x g for 10 min. Finally, the product was dried at 25 °C. The surface morphology of the CdS-NPs was observed using a field emission scanning electron microscope (FE-SEM, Hitachi, S-4800). The particle size of CdS-NP-1 was in the range of 60-200 nm, and CdS-NP-2 was in the range of 300-600 nm. Selected area electron diffraction was performed using a transmission electron microscope (TEM, JEOL, JEM 2100F) operating at 200 kV. X-ray diffraction (XRD) measurements were performed using an X-ray diffractometer (XRD, Rigaku Corporation, Rigaku Model SmartLab) in the two-theta range of 20°-90°. The UV-VIS absorption spectrum was measured using a UV-VIS spectrophotometer (UV/VIS, Perkin Elmer, Lambda 650S) in the range of 300-800 nm. Photo-luminescence (PL) spectroscopy was performed with a PL spectrometer to measure the absorption of UV followed by the emission of visible light. PL spectrometer using a HeCd laser source at an excitation wavelength of 325 nm. Mott-Schottky plots were determined by using potentiostat (SP-200, Bio-Logic) with three-electrode system, CdS Nanoparticle on FTO, saturated calomel electrode (SCE), graphite rod as the working electrode, reference electrode, counter electrode, respectively. The measurement was carried out from -0.8 V to 0.8 V at 1 kHz, in 0.5 M Na_2SO_4 aqueous solution.

Photocatalytic degradation experiment

100 mg of the CdS-NPs were added to 200 mL of 30 mg L^{-1} Congo Red solution in a 200 mL beaker and stirred for 30 min at 300 x g under dark condition to achieve adsorption−desorption equilibrium between CdS and Congo Red solution. The resulting solution was irradiated for an hour with a 20 W halogen lamp (DECOSTAR® 51 S, China) at a distance of 15 cm, providing primarily visible light source at the surface of the reaction vessel. 5 mL of solution was extracted at different time intervals (0, 10, 20, 30, 45, and 60 min), and the solution was centrifuged to separate the CdS-NPs. The Congo Red concentration was measured using UV−VIS absorption spectroscopy. Distilled water was used as a reference solution for measuring the UV−VIS absorption spectrum. The UV-VIS absorption spectrum of Congo Red solutions was recorded in the range of 250 - 900 nm. The absorption peaks corresponding to Congo Red appeared at 497 nm and 347 nm.

C. autoethanogenum were cultured anaerobically under 5 g L^{-1} fructose condition upto O.D ~ 1.0 and cell collected using centrifugation (6,000 x g, 10 min, 4 °C) in anaerobic chamber. Resupend distilled water and transfer to 50 ml serum bottles. Added 1 mg/ml CdS-NPs, 10 μM $FeCl_3$*$7H_2O$, 5 μM FMN, and 20 mg L^{-1} Congo Red solution. Incubate at 37°C under blue light LEDs for 0, 30, or 60 min. After 1 mL sampling, the solution was centrifuged at 13,000 x g for 5 min to remove NPs. Finally, wavelength specturm were measured in 280 – 880 nm, and caculated the degradation rate in 530 nm.

Bacterial growth conditions

C. autoethanogenum DSM 10061 was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cells were cultivated anaerobically at 37 °C in 97 mL of PETC medium (per 1 L, pH 5.5; 1 g of yeast extract, 1 g of NH_4Cl, 1 g of $NaHCO_3$, 0.2 g of $MgSO_4·7H_2O$, 0.3 g of Cys-HCl·H_2O, 0.15 g of $CaCl_2$, 0.8 g of NaCl, 0.1 g of KCl) supplemented with 20 mL of trace element solution (per 1 L, pH 7.0; 1.5 g of nitrilotriacetic acid, 3.0 g of $MgSO_4·7H_2O$, 0.5 g of $MnSO_4·H_2O$, 1.0 g of NaCl, 0.1 g of $FeSO_4·7H_2O$, 0.18 g of $CoSO_4·7H_2O$, 0.08 g of $CaCl_2$, 0.18 g of $ZnSO_4·7H_2O$, 10 mg of $CuSO_4·5H_2O$, 16 mg of $KAl(SO_4)_2·12H_2O$, 10 mg of H_3BO_3, 10 mg of $Na_2MoO_4·2H_2O$, 30 mg of $NiCl_2·6H_2O$, 0.3 mg of $Na_2SeO_3·5H_2O$, and 0.4 mg of $Na_2WO_4·2H_2O$), 1 mL of phosphate buffer (pH 5.5), 1 mL of Wolfe's vitamin stock solution (per 100 mL, 0.4 mg of biotin, 0.4 mg of folic acid, 2 mg of pyridoxine-HCl, 1 mg of thiamine-HCl, 1 mg of riboflavin, 1 mg of nicotinic acid, 1 mg of pantothenate, 0.02 mg of vitamin B12, 1 mg of p-aminobenzoic acid, and 1 mg of lipoic acid based on ethanol), and 1 mL of L-Cysteine-HCl·H_2O (15 g/L). Cells were grown heterotrophic (5 g

L^{-1} of fructose and headspace pressure of 200 kPa under 100% N_2 gas) or autotrophic (headspace pressure of 200 kPa under 80% H_2 with 20% CO_2 gas) or CdS-treatment (2 mM of CdS NPs and headspace pressure of 200 kPa 100% CO_2 gas under 5W blue light LEDs (460 nm – 470 nm).

Biosynthesis of CdS-NPs

C. autoethanogenum was cultured strictly anaerobically under 100 mL of PETC medium with 5 g L^{-1} fructose at 37 °C until an optical density (600 nm) of about 0.6 – 0.8. Add 1 mM of $CdCl_2$ and attached CdS-NPs at 37 °C for 24 hours by microbes themselves. After synthesizing the CdS by microbes, the CdS-CA hybrid system was harvested by strictly anaerobic centrifugation at 6000 x g at 4 °C for 10 min and resuspended using fresh PETC medium for three times to eliminate supplemented fructose in the cultured samples. The washed pellets were collected to 5 ml of fresh PETC medium, transferred 1 ml of the collected samples to 100 ml of fresh PETC medium supplemented with 1 ml of phosphate buffer (pH 5.5), 1 ml of Wolfe's vitamin stock solution, 1 ml of L-Cysteine-HCl·H_2O (15 g L^{-1}) and then pressurized with 200 kPa of 100 % CO_2 gas and cultured at 37 °C.

Measure colony forming units in CdS-CA

C. autoethanogenum was anaerobically grown in the PETC medium with 28.8 mg of CdS-NP-1 at 37 °C for 44 h (mid-exponential). We transferred the cultured samples to an anaerobic chamber (H_2; 4%, N_2:96%), and diluted 1 x 10^{-3} by fresh PETC medium. Next, we spread diluted samples onto YT agar plate (per 1 L, pH 5.5; 5 g of yeast extract, 10 g of tryptone, 2 g of NaCl, 15 g of agar), and incubated at 37 °C for 5 days in an anaerobic jar (Oxoid™, pressurized with 50 kPa of H_2; 80% with CO_2; 20%).

Resting assay

C. autoethanogenum was cultured under heterotrophic condition (5 g L^{-1} fructose) until $O.D_{600}$ of about 0.6~1.0, and cell down by centrifugation (6,000 x g, 10 min). In anaerobic chamber (H2; 4%, N2:96%), harvested cell washed 2 times using imidazole buffer (50 mM of imidazole-HCl, 20 mM of MgSO4, 20 mM of KCl, 4 mM of DTE, and 4.4 uM of resazurin, pH 5.5). The washed cell was transferred to 50 ml serum bottles by 1.0 mg/ml of protein concentration and adjusted the final volume to 20 ml by the imidazole buffer. For CdS condition, 5 mg of CdS NP-1 treated to prepared samples. Finally, a headspace gas was exchanged to each N_2, H_2/CO_2 (80/20, v/v), or CO_2 at 200 kPa, and the samples were incubated at 37 °C under light exposed conditions.

Determination of NADH/NAD or NADPH/NADP ratio

C. autoethanogenum was cultivated under H_2-driven (H_2/CO_2:80:20, 200 kPa) or CdS-driven (CO_2 100%, 200 kPa with 28.8 mg of CdS-NP-1) or combinational (H_2/CO_2:80:20, 200 kPa with 28.8 mg of CdS-NP-1) autotrophic condition for 44 h (mid-exponential), and cell down by centrifugation (6,000 x g, 10 min, 4 °C). The cells were washed by the 500 µL of polysome buffer (20 mM Tris-HCl pH 7.4, 140 mM NaCl, 5 mM $MgCl_2$, and 25 µg/mL of lysozyme) and divided washed samples to 1:4 ratio for NADH or NADPH extraction, respectively. Each sample was centrifuged at 6,000 x g, 10 min, 4 °C, and resuspended by 500 µL of Extraction buffer from the NAD/NADH quantification kit (MAK037, SIGMA-ALDRICH) or NADP/NADPH quantification kit (MAK038, SIGMA-ALDRICH). Resuspended samples were rapidly frozen in liquid nitrogen, followed by grinding the frozen cells using a mortar and pestle, and thawed the samples slowly by centrifuge at 3,000 x g, 20 min, 4 °C. To prepare NAD_{total} or $NADP_{total}$ samples, we transferred 150 µL of extracted samples to new microcentrifuge tubes and adjusted up to the final 300 µL volume with fresh Extraction buffer, and transferred 50 µL of NAD_{total} or $NADP_{total}$ samples into a 96 well plate. Also, to prepare $NADH_{only}$ or $NADPH_{only}$, we transferred 150 µL of extracted samples to new microcentrifuge tubes, and heat to 60 °C for 30 minutes in a heating block, cool samples on ice, quickly spined samples to remove any precipitates and adjusted up to 300 µL volume with fresh Extraction buffer, and transferred 50 µL of $NADH_{only}$ or $NADPH_{only}$ samples into a 96 well plate. To convert NAD(P) to NAD(P)H, added 100 µL of the Master reaction mix (98 µL of cycling buffer with 2 µL of cycling enzyme mix) to each well, and incubate for 5 minutes at room temperature. To detect NADH or NADPH, added 10 µL of NADH or NADPH Developer into

each well, measured the absorbance at 450 nm ($A450_{nm}$) by a microplate reader (SynergyTM H1, BioTeck) for 2 hours at 10 minute intervals. Finally, we calculated the NADH/NAD or NADPH/NADP ratio using the highest $A450_{nm}$ in each sample by the following equation:

$$\text{Ratio} = \frac{\text{NAD(P)H}}{\text{NAP(P)total-NAD(P)Honly}}$$

Measurement of ORP value in cultured medium

C. autoethanogenum was cultured in the PETC medium with or without the 10 mM of ethanol under CdS-driven autotrophic condition (28.8 mg of CdS-NP-1, CO_2 100%, 200 kPa) for 5 days, and measured the ORP value of cultured samples using by a pH&ORP probe (Mettler Toledo, InPro3253i/SG/120).

Metabolite analysis

After removing cultured microbes and treated CdS-NPs by filtering with a 0.2-μm Minisart® RC15 Syringe Filters (Sartorius), acetate and other organic acids in supernatants were analyzed by high-performance liquid chromatography 1525 system (Waters) using a MetaCarb 87H organic acid column (Agilent Technologies) at 37 °C and detected using a refractive index detector (RID, Waters).

Measurement of Hydrogen production in CdS-CA systems

To analyze H_2 gas in the headspace of the CdS-CA systems, 100 ul of headspace gas is sampled using a syringe, and then it is analyzed on Gas chromatograph GC-2014 (Shimadzu) using packed column (Restek, C57271-03). While increasing the temperature from 30°C to 100°C, the headspace gas was analyzed using 30 ml/min, 540 kPa of N_2 carrier gas.

Total RNA extraction and RNA-Seq library preparation

The cultured microbes were sampled at 44 h (mid-exponential) by centrifugation immediately at 6000 *g* at 4 °C for 10 min. The pellets were resuspended by the 500 mL of lysis buffer (20 mM Tris-HCl pH 7.4, 140 mM NaCl, 5 mM $MgCl_2$, and 20% of Triton X-100) and were rapidly frozen in liquid nitrogen, followed by grinding the frozen cells using a mortar and pestle. Total RNA was then isolated using TRIzol reagent (Thermo Scientific). To remove fragmented DNA in the isolated RNA samples, rDNase I (Ambion) was treated to RNA samples for 30 min at 37 °C. Ribosomal RNAs (rRNAs) were eliminated through the Ribo-Zero™ rRNA Removal Kit for Meta-bacteria (Epicentre). The RNA-Seq libraries were constructed from rRNA depleted RNA samples using the TruSeq Stranded mRNA Library Prep Kit (Illumina) and then sequenced by a 100-bp single-end sequencing recipe in an Illumina Hi-Seq 2500 instrument (Rapid-Run mode).

Data processing

RNA-Seq reads were analyzed using the CLC Genomics Workbench 6.5.1 (Qiagen). After trimming low quality reads, remaining reads were aligned to the genome of *C. autoethanogenum* DSM 1006 obtained from NCBI (NC_022592.1) with the following parameters: mismatch cost = 2, insertion cost = 3, deletion cost = 3, length fraction = 0.9, and similarity fraction = 0.9. For analyzing of transcriptome profiles, the raw read counts per gene were calculated by CLC RNA-Seq analysis module. The normalization of read counts per gene was performed using the DEseq2 method in R(4).

SI Appendix Figures

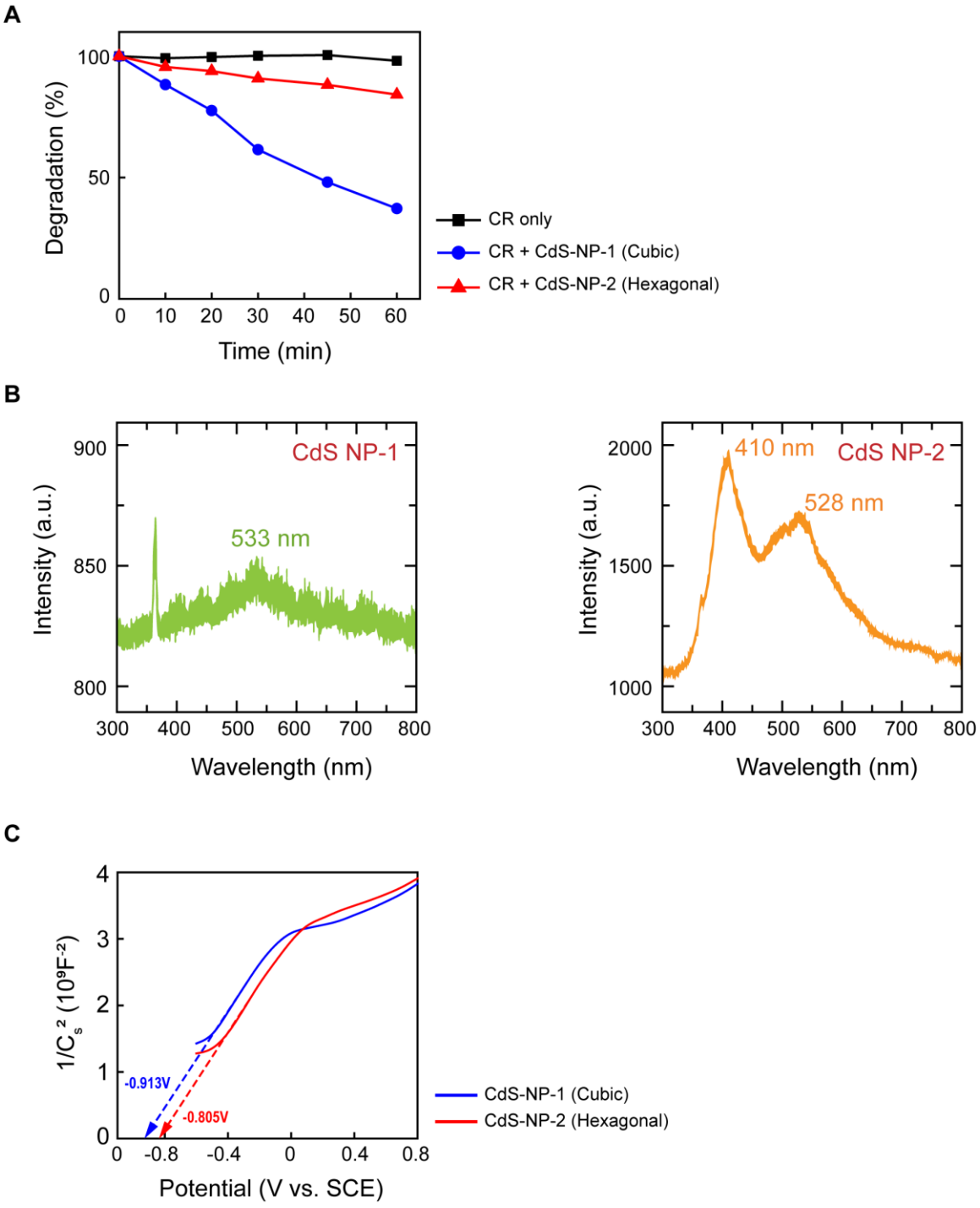


Fig. S1. Physical characterization of CdS NP-1 or NP-2. (A) Photocatalytic degradation efficiency of the two types of CdS-NPs. **(B)** Photoluminescence spectra of CdS-NP-1 and CdS-NP-2. **(C)** Measurements potential energy of conduction band edge via Mott-Schottky curves of CdS NP-1 and NP-2.

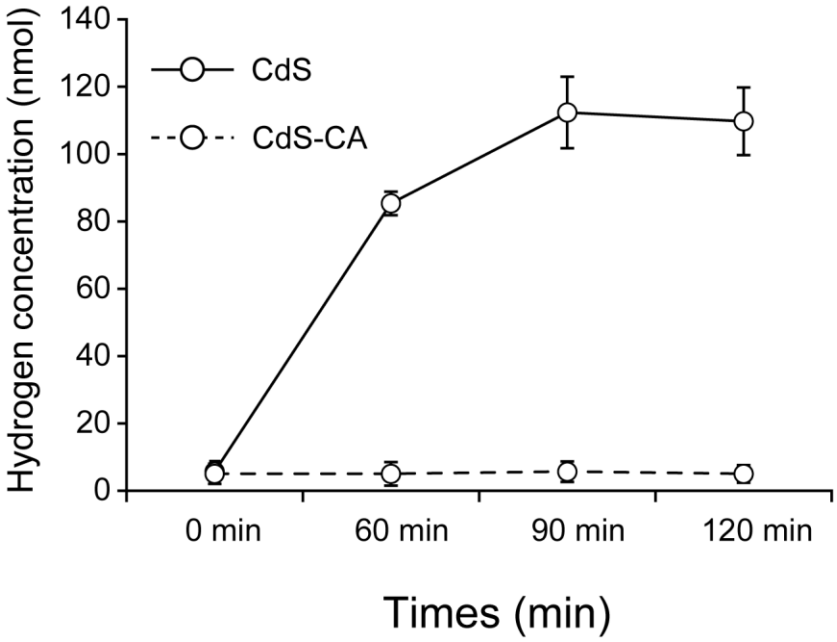
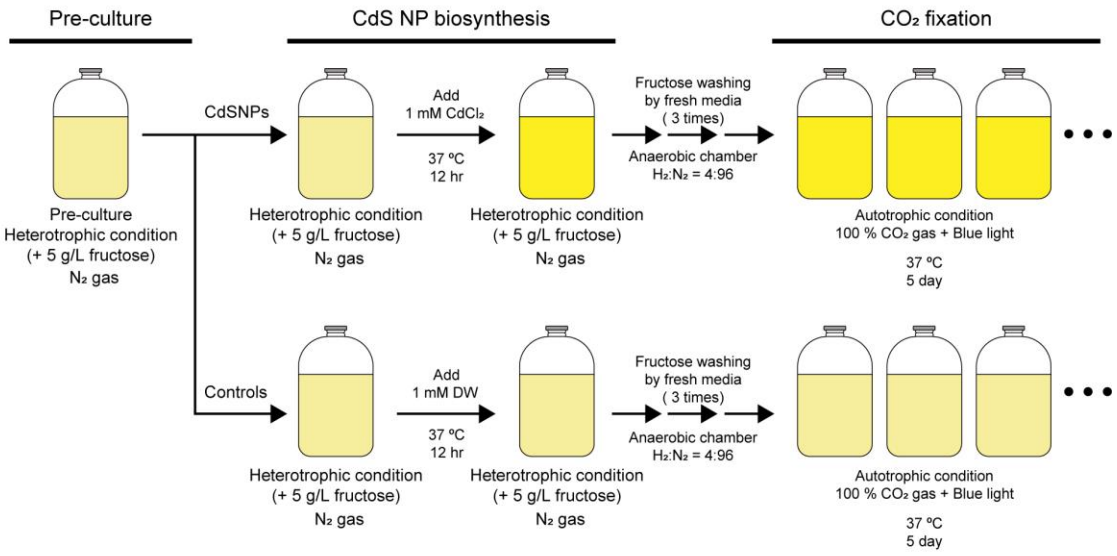


Fig. S2. Measurement photo-generated hydrogen concentration in CdS-CA.

A



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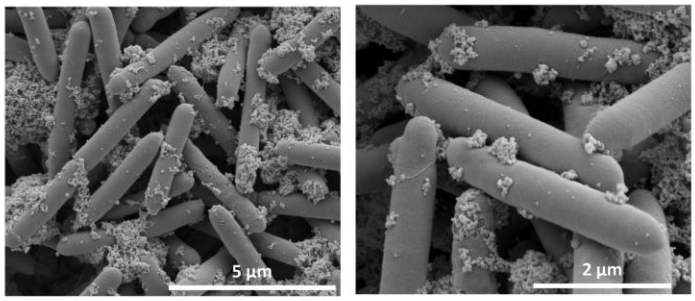

Fig. S3. Development of CdS-CA hybrid system using in vivo CdS biosynthesis. (A) The method to construct the CdS-CA hybrid system using in vivo CdS biosynthesis. **(B)** Scanning Electron Microscope (SEM) analysis of the CdS-CA hybrid system.

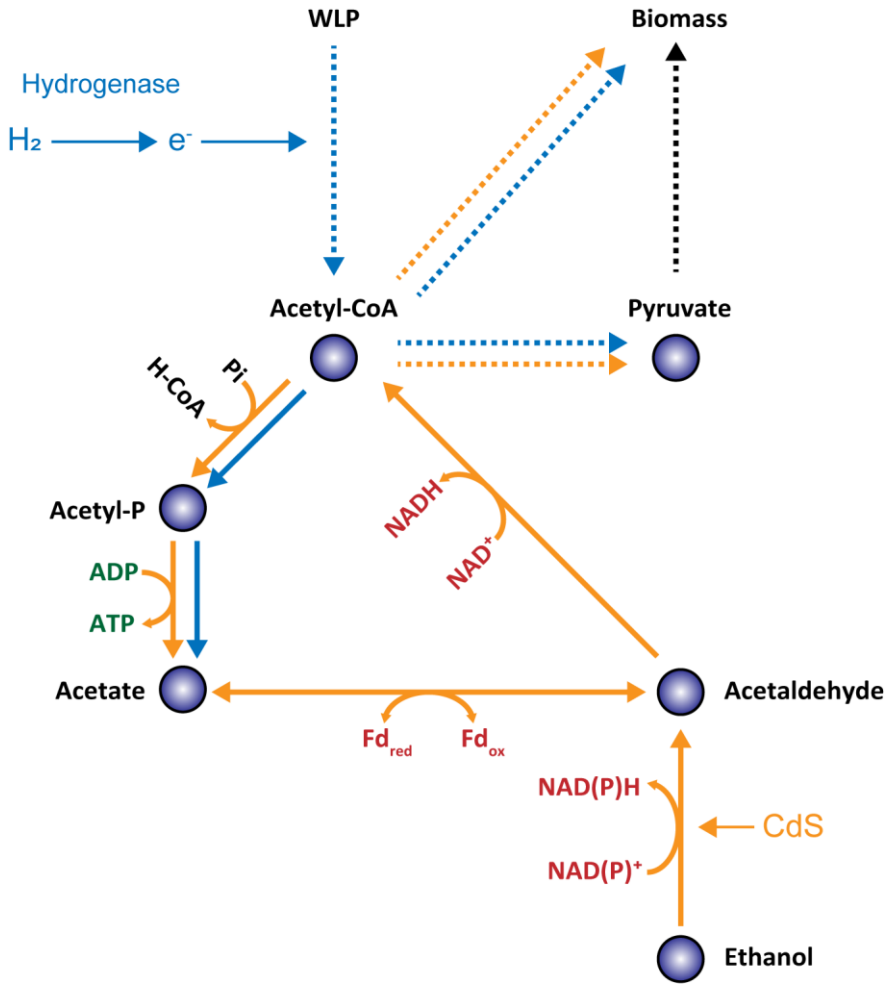

Fig. S4. Acetate-Ethanol metabolism in the CdS-CA system.

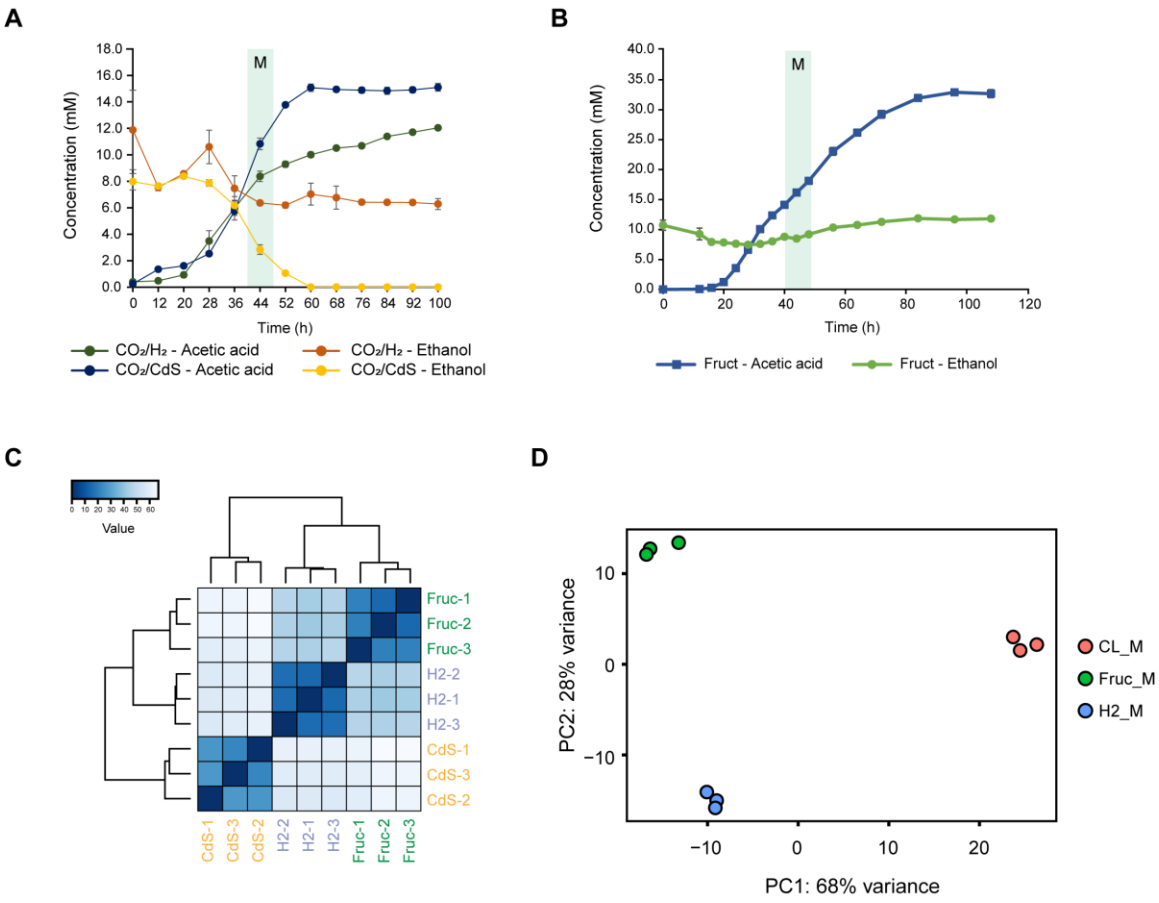

Fig. S5. Analysis of significant or reproducible difference of transcriptome data. (A) Acetate or ethanol production curve under H_2- or CdS-driven autotrophic conditions. The green bar indicates RNA sampling points (mid-exponential phase) for transcriptome analysis. **(B)** Acetate or ethanol production curve under fructose growth condition. The green bar indicates RNA sampling points (mid-exponential phase) for transcriptome analysis. **(C)** Hierarchical clustering analysis for calculating the distance between transcriptome data. **(D)** Principal component analysis (PCA) of transcriptome data.

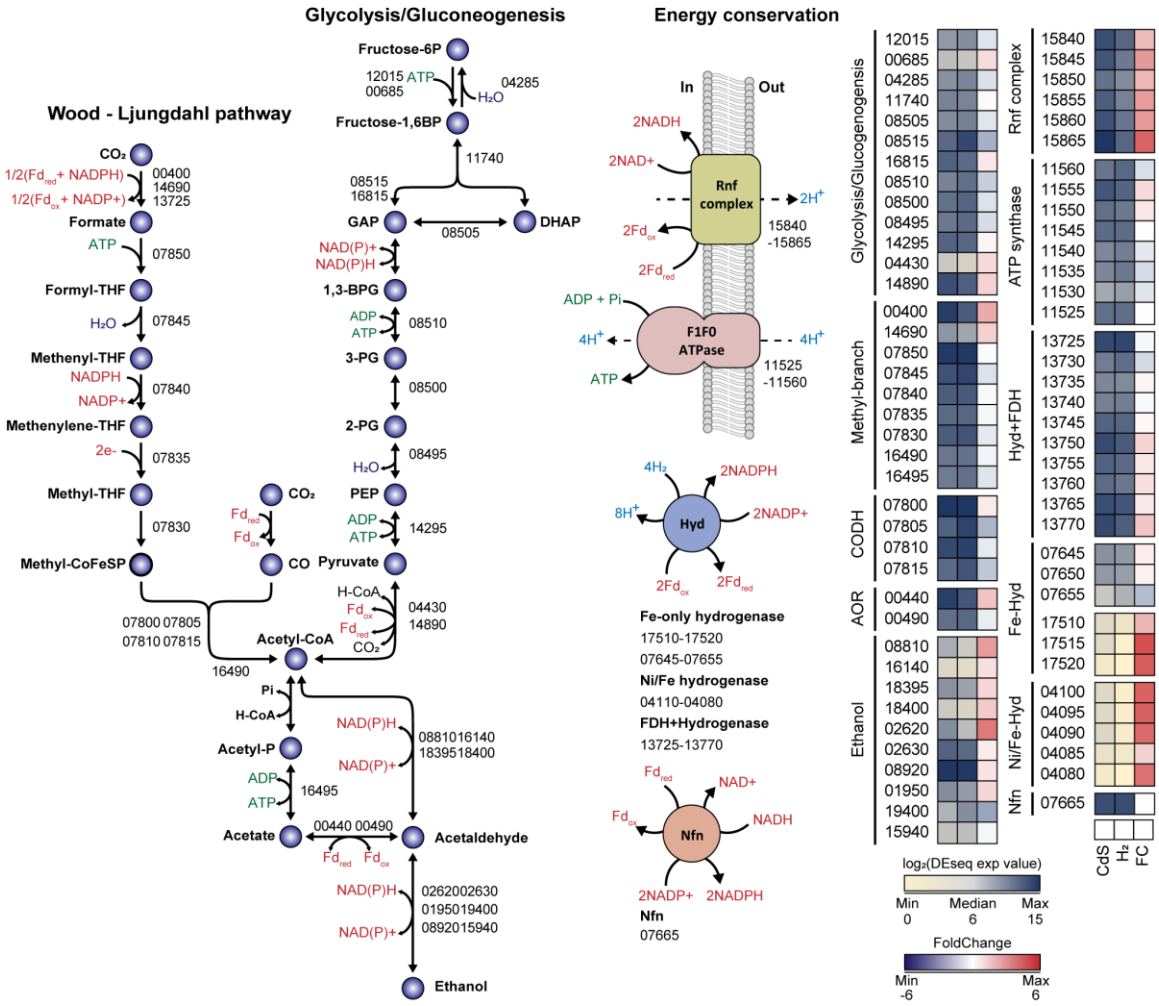

Fig. S6. Transcriptome profile of the *C. autoethanogenum* system under CdS- or H_2-driven autotrophic conditions.

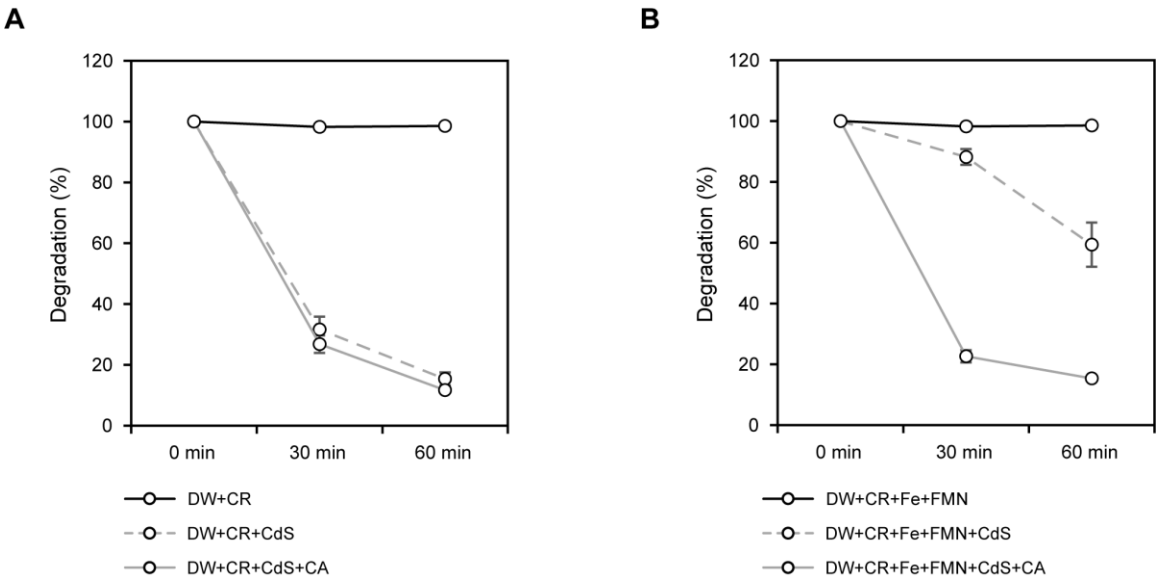

Fig. S7. Photocatalytic degradation of CdS-CA under with or without mediators. (A) Photocatalytic degradation efficiency of CdS-CA system in distilled water. **(B)** Photocatalytic degradation efficiency of CdS-CA system in distilled water plus 10 μM of Fe^{3+} and 5 μM of FMN.

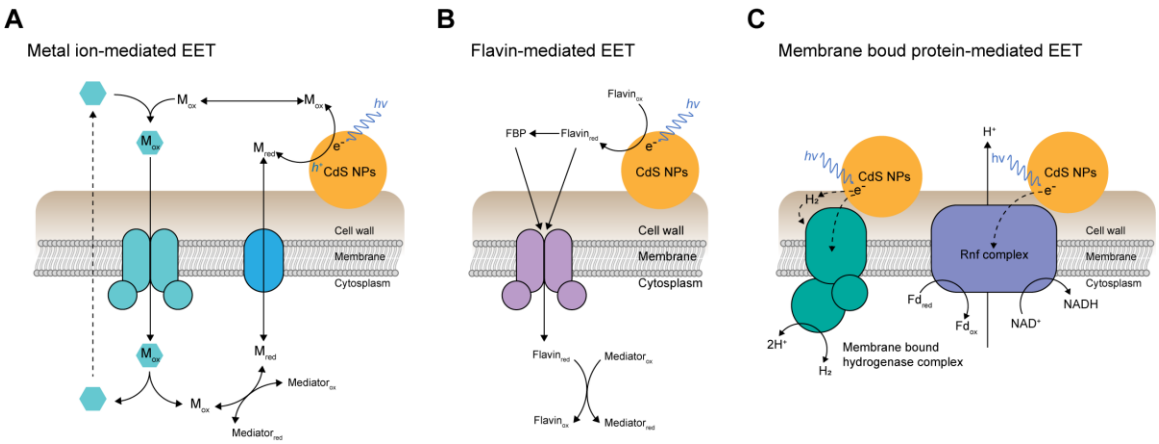

Fig. S8. Hypothesis of extracellular electron transfer (EET) mechanisms in CdS-CA systems. (A) Metal ion-mediated EET, M; metal ion. **(B)** Flavin-mediated EET, FBP; flavin binding protein. **(C)** Membrance bound protein-mediated EET

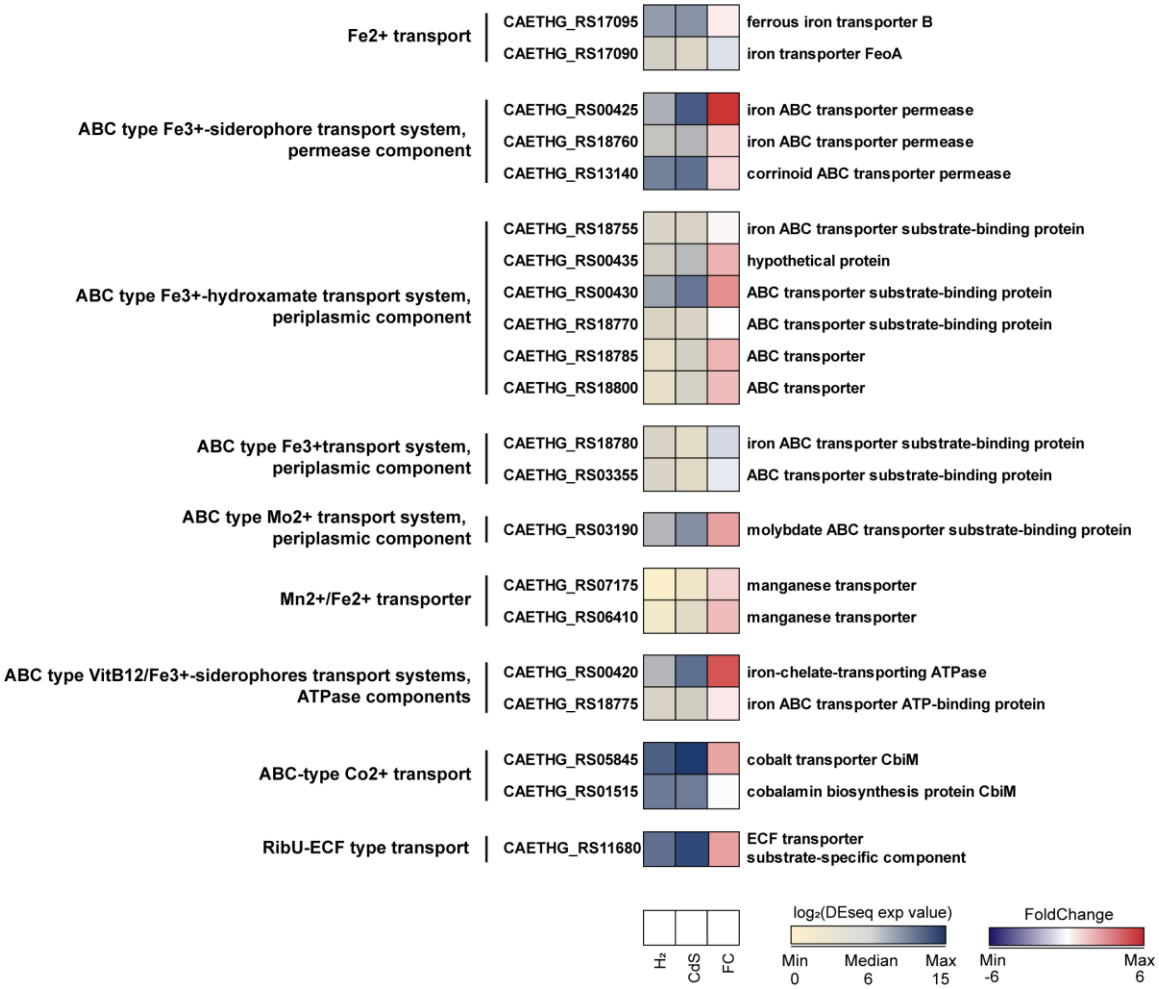

Fig. S9. Transcriptome profile of EET-associated genes in *C. autoethanogenum*. Transcript expression levels of genes involved in metal ion transporter or, RibU riboflavin transporter in *C. autoethanogenum* under CdS-driven autotrophic condition compared with H_2 autotrophic condition.

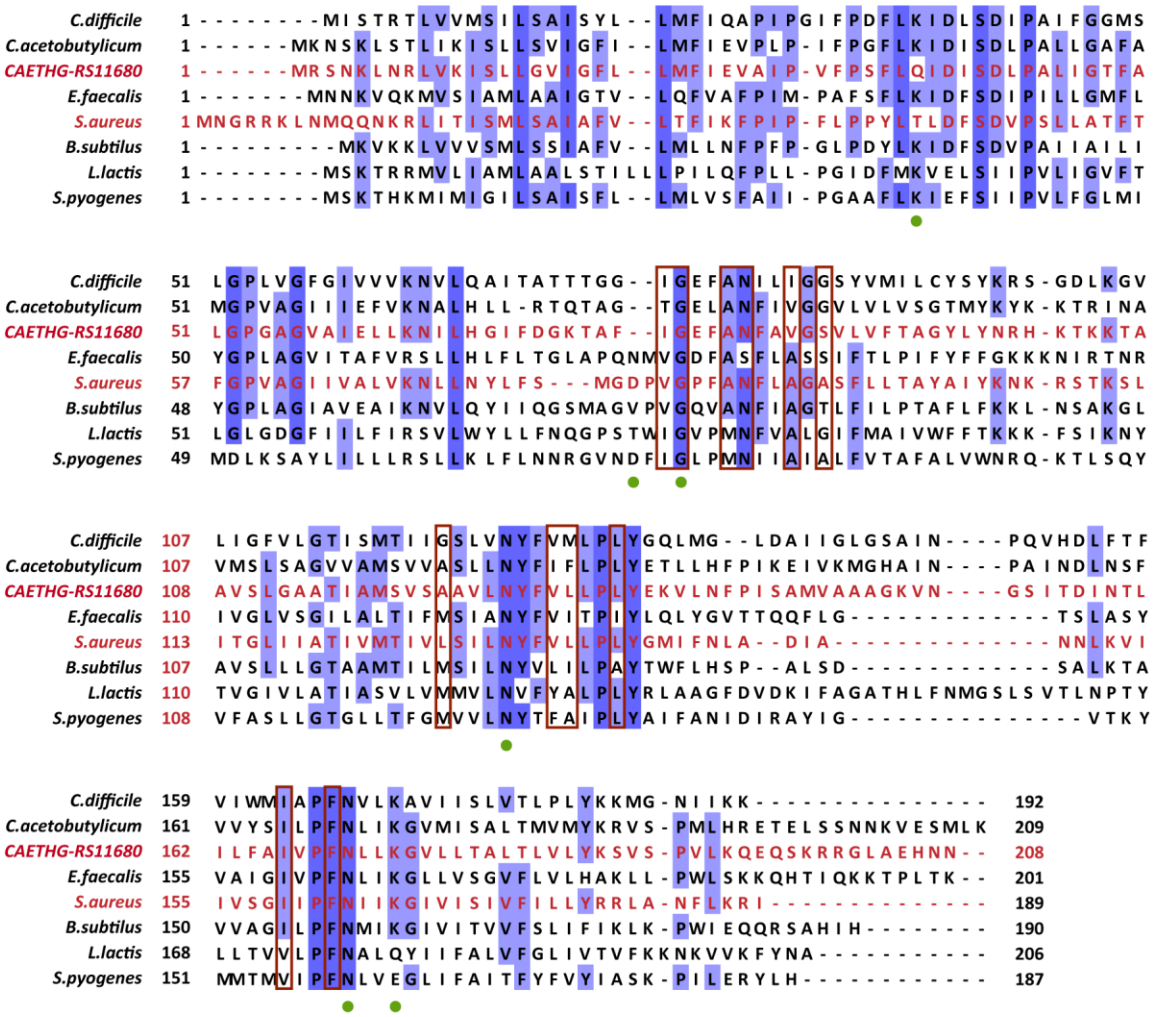

Fig. S10. Amino acid sequence alignment of RibU transporter. Amino acid sequence analysis of RibU transporter candidate in *C. autoethanogenum* compared with the identified RibU transporters in *Clostridium difficile* (*C. difficile*) (5), *Clostridium acetobutylicum* (*C. acetobutylicum*) (6, 7), *Enterococcus faecalis* (*E. faecalis*) (7, 8), *Staphylococcus aureus* (*S. aureus*) (7), *Bacillus subtilis* (*B. subtilis*) (7, 9), *Lactococuus lactis* (*L. lactis*) (10) and *Streptococcus pyogenes* (*S. pyogenes*) (7, 11). The blue boxes indicate highly conserved amino acid sequences in RibU transporters. The red boxes indicate a hydrophobic pocket of the riboflavin binding site in RibU transporter from *Staphylococcus aureus* (7). The Green circles indicate important amino acids to recognize riboflavin by forming multiple hydrogen bonds in RibU of *Staphylococcus aureus* (7).

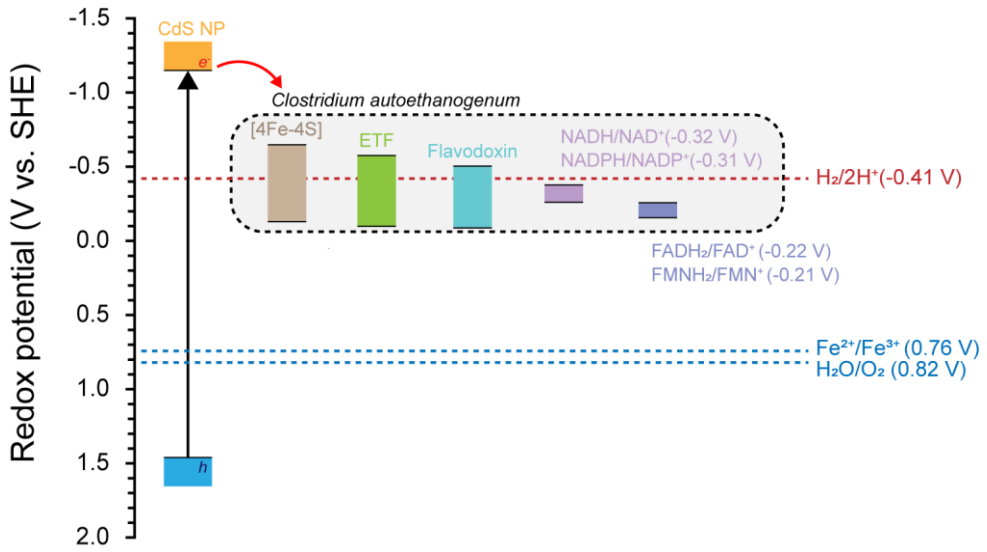

Fig. S11. Redox potential of major electron transfer components in CdS-CA systems.

Dataset S1. Information of the RNA-seq mapped reads.

Dataset S2. Results of DEseq2 normalization.

Dataset S3. Results of DEseq expression values and foldchange in carbon and energy metabolism of *C. autoethanogenum* DSM 10061.

Dataset S4. Information of DEG results under CdS-driven autotrophic condition compared with H_2-driven autotrophic condition.

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