Experimental

Construction of *R. rubrum*-CdS biohybrid system

Biomineralization strategy was applied to obtain the biohybrid system of *R. rubrum*-CdS. The stock suspension of *R. rubrum* (ATCC11170) was centrifuged (6000 g, 5 min), and the bacterial cells were inoculated in MMN medium (Table S1) for activation, and the initial cell density (OD_{600}) was adjusted to 1. The cells were cultured to the mid-log phase, centrifuged (6000 g, 5 min) and washed three times with phosphate buffered saline (PBS, pH = 7), resuspended in MMN medium to OD_{600} = 1, and aliquoted into 250 mL anaerobic bottles. The aliquots were supplemented with *L*-cysteine hydrochloride (1 mM) and different concentrations of $Cd(NO_3)_2$ (0.05 mM，0.1 mM，0.2 mM，0.3 mM，respectively), mixed well and cultured in a water bath under the illumination of LED (temperature at 30 °C, light intensity at 30 mW m^{-2}, illumination area 0.002 m^2, magnetic stirring at 200 rpm). After 24 h of incubation, the samples for scanning electron microscopy (SEM) analysis were prepared as the following method: bacterial suspension (1 mL) was centrifuged (13000 g, 5 min) and washed three times with PBS, and fixed with glutaraldehyde (4 %, 1 mL) at 4 °C for 2 h, and then washed twice with PBS. The fixed samples were then dehydrated sequentially with ethanol/water mixtures of gradient concentrations (50 %, 75 %, 85 %, 95 %, and 100 %), dripped onto the polished surface of silicon wafer and air-dried. Samples were analyzed with a desktop field-emission SEM (Phenom Pharos-SED-EDS G2, Thermo Fisher) at an acceleration voltage of 15 kV, the elemental distribution maps of the samples were analyzed with energy-dispersive X-ray spectroscopy (EDS) using an Amptek Fast SDD X123 Ultra High-Performance Silicon Drift Detector at an excitation voltage of 15 kV.

Photosynthetic activities of biohybrid system

The *R. rubrum* suspension was cultured for 24 h, centrifuge (6000 g, 5 min) and washed three times with PBS, and diluted to OD_{600} = 1 with MMG medium (Table S2). Aliquots of the suspensions (100 mL) were filled into the 250 ml anaerobic bottles, the headspace was purged with nitrogen, and the bottle was sealed tight. The suspension was incubated, and samples were taken every 24 h and analyzed.

Hydrogen evolution

A microinjection needle was used to take gas samples (100 μL, each) from the headspace and manually injected them into a gas chromatograph (GC9790II., Fuli, China) to analyze the levels of hydrogen in

samples. Quantification of hydrogen was based on the external standard method, the carrier gas was argon (purity > 99.99 %), the flow rate was 30 mL min^{-1}, the detector was a thermal conductivity detector (TCD), the column chamber temperature was 80 °C, and the retention time was 5 min.

Cell density and dry mass

The OD_{600} values of *R. rubrum* suspension were determined every 24 h using a cell density meter (WPA CO8000, Biochrom, UK). For the measurement of dry mass, the bacterial suspensions (50 mL each) at the beginning of the experiment and at the 7^{th} day of culture were collected and centrifuged (13000 g, 10 min). The supernatant was discarded, the cell pellets were snap-frozen in liquid nitrogen and lyophilized for 48 h (Scientz-10N/A, SCIENTZ, China), and their mass was measured.

Nitrogenase activity

The bacterial suspensions in reaction bottles were purged with Ar or N_2 to remove the residual H_2. A 10 % volume of the headspace gas was replaced with equal volume of acetylene (C_2H_2). The reaction bottles were sealed and placed into a light incubator for 1 h before the analysis of ethylene yield. A microinjection needle was used to take gas samples (100 μL, each) from the headspace and manually injected them into a gas chromatograph (GC9790II, Fuli, China) to analyze the levels of ethylene in samples. Quantification of ethylene was based on the external standard method, the carrier gas was argon (purity > 99.99 %), the flow rate was 30 mL min^{-1}, the detector was a flame ionization detector (FID), the column chamber temperature was 80 °C, and the retention time was 20 min.

PHB quantification

The lyophilized *R. rubrum* pellets were digested with concentrated sulfuric acid (98 %, 1 mL) in glass tubes and heated in water bath (90 °C, 30 min). The digested mixture was diluted with ultrapure water (4 mL) and filtered through 0.22 μm membrane. The PHB concentration in these samples were measured using high-performance liquid chromatography (1260 Infinity II, Agilent, USA) under the following parameters: the mobile phase was 5 mM sulfuric acid, the column was HPX-87H (Aminex, Bio-rad, USA), the flow rate was 0.6 mL min^{-1}, the injection volume was 10 μL, the column temperature was 60 °C, the diode array detector (DAD) was set to the wavelength at 210 nm.

Malate quantification

The reaction samples (1 mL) were taken every 24 h. They were centrifuged (13000 g, 5 min), the supernatant was collected and filtered through 0.22 μm membrane. The malate concentration in these samples was

measured using the same high-performance liquid chromatography and column as those for the PHB quantification. The refractive index detector (RID) was set to 55 °C, other parameters were identical to those for PHB quantification, and the retention time was 9 min.

NADPH quantification

Quantification of NADPH in reaction samples was performed using the $NADP^+$/NADPH Assay Kit (WST-8 method, Cat. No. S1079, Biyuntian, China). The sample suspensions (200 μL) from day 1 to day 5 were collected and centrifuged (12000 g, 5 min). The supernatant was discarded, and the cell pellets were lysed in an extraction buffer (200 μL). The samples were centrifuged (12000 g, 4 ºC, 5 min), the supernatant was collected (100 μL) and heated at 60ºC for 30 min, and centrifuged again (12000 g, 4 ºC for 5 min), and the supernatant (50 μL) was added into the wells of a 96-well plate. The G6PDH working solution was added (100 μL per well), and the plate was incubated in dark at 37 ºC for 10 min. The color development solution was added (10 μL per well), and the plate was incubated at 37 ºC for 10-20 min to facilitate the formation of an orange-yellow formazan solution, and the absorbance at 450 nm was measured using a microplate reader (SpectraMax iD3, Molecular Devices, Austria). The NADPH concentrations in samples were calculated according to the standard curve.

Photosynthetic pigments quantification

The photosynthetic pigments were extracted using an extraction mixture of methanol: acetone = 2:7 (v/v). The reaction suspension (1 mL) was collected and centrifuged (6000 g, 5 min). The supernatant was discarded, and the cell pellets were washed twice with ultrapure water, and then extracted repeatedly with an extraction mixture (1 mL) until they turned white. The samples were centrifuged (13000 g, 5 min), the supernatant was added to the wells of a 96-well plate, and the absorbance at 496 nm, 515 nm, and 763 nm was measured with a microplate reader (SpectraMax iD3, Molecular Devices, Austria). The pigment concentrations were calculated according to the following formula[1](Bchl a, Bacteriochlorophyll a):

$$C_{Bchl\ a} = \frac{OD_{763}}{\varepsilon_{Bchl\ a} \times l}$$

$$C_{carotenoids} = \frac{\frac{OD_{496} + OD_{515}}{2}}{\varepsilon_{carotenoids} \times l}$$

Where $\varepsilon_{Bchl\ a}$=82.2 g L^{-1} cm^{-1}, $\varepsilon_{carotenoids}$ = 250 g L^{-1} cm^{-1}.

ATP quantification

Quantification of ATP in reaction samples was performed using the ATP Assay Kit (Cat No. S0026, Biyuntian, China). The sample suspensions (200 μL) of day 3 and day 5 were collected and centrifuged (12000 g, 5 min). The supernatant was discarded, and the cell pellets were lysed in extraction buffer (200 μL). The supernatant was collected (100 μL) and mixed with detection solution (100 μL), and the luminescence was measured using a microplate reader (SpectraMax iD3, Molecular Devices, Austria). The ATP concentrations in samples were calculated according to the standard curve.

NH_4^+ quantification

The sample suspensions (200 μL) of day 3 and day 5 were collected and centrifuged (12000 g, 5 min). The supernatant was discarded, and the cell pellets were washed in PBS twice. The supernatant was discarded and the cell pellets were lysed in lysis buffer (10 % sucrose, 300 mM NaCl, 90 mM EDTA, 3 mg mL^{-1} lysozyme, 50 mM Tris-HCL, pH 7.5).[2] Next, the suspension was mixed and incubated on ice for 2 h. The cell suspension was then frozen and thawed five times by cycling between 37°C water bath and liquid nitrogen. Finally, the suspension was sonicated (30 % power) for 3 min using an Ultrasonic broken instrument. The sample suspensions were centrifuged (12000 g, 5 min), and the supernatant (20 μL) was mixed with solutionIand solutionII, left to stand at 25 °C for 1 hour, and mixed with solution III. The absorbance at 625 nm were measured with a microplate reader (SpectraMax iD3, Molecular Devices, Austria).

Electrochemical studies

The *R. rubrum* suspension (40 μL of OD_{600} = 5) was uniformly applied on one side of the carbon paper (1 cm × 1 cm size). The suspension was dried, and 20 μL of Nafion (0.05% in absolute ethanol) was added onto the carbon paper, which was then clamped with electrode clips. The working electrode (carbon paper with *R. rubrum* cells), reference electrode (Ag/AgCl reference electrode), and counter electrode (Pt sheets) was installed into a quartz electrolyte cell (50 mL volume). The cell was filled with electrolyte solution (PBS, 40 mL), purged with nitrogen gas (10 min) and sealed. The light reactions were irradiated under the LED source of white light, or monochromatic light at 450 nm or 620 nm with bandpass filter, respectively (perfect light, China), the light intensity was 20k lux. The following photoelectrochemical analysis were performed on an electrochemical workstation (CHI760E, Shanghai Chenhua, China): (1) For linear-sweep voltammetry (LSV), the voltage range was 0 to - 1 V and 0 to 1 V, the sweep speed was 0.01 V s^{-1}; (2) For

amperometric-time (I-t) response, the current values at the -0.6 V and 0.6V potential (versus Ag/AgCl) were recorded; (3) For alternate-current AC impedance test, the initial voltage was the open circuit potential, the high-frequency parameter was set to 10^6 Hz, the low-frequency parameter was set to 10^{-2} Hz, the AC impedance spectrum under this parameter was recorded, and the equivalent circuit diagram was fits using ZSimp Win software.

Supplementary References

1. S. Condori, S. Atkinson, N. Leys, R. Wattiez and F. Mastroleo, *Res. Microbiol.*, 2016, **167**, 380-392.
2. C. Wang, A. Lum, S. Ozuna, D. Clark and J. Keasling, *Appl. Microbiol. Biotechnol.*, 2001, **56**, 425-430.

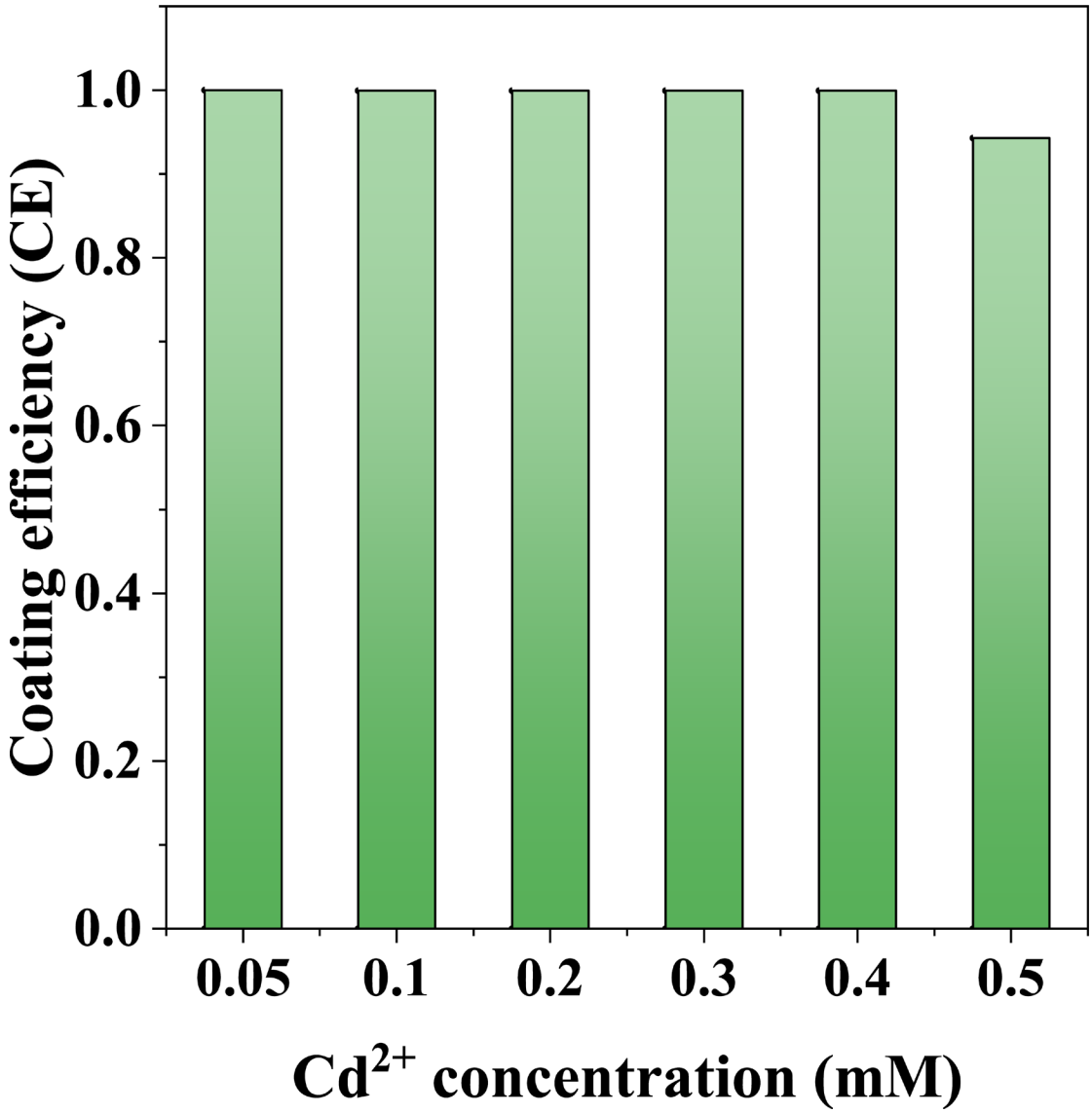

Fig. S1 CdS coating efficiencies for biomineralization on *R. rubrum* cells using different concentrations of $Cd(NO_3)_2$ for 24 h

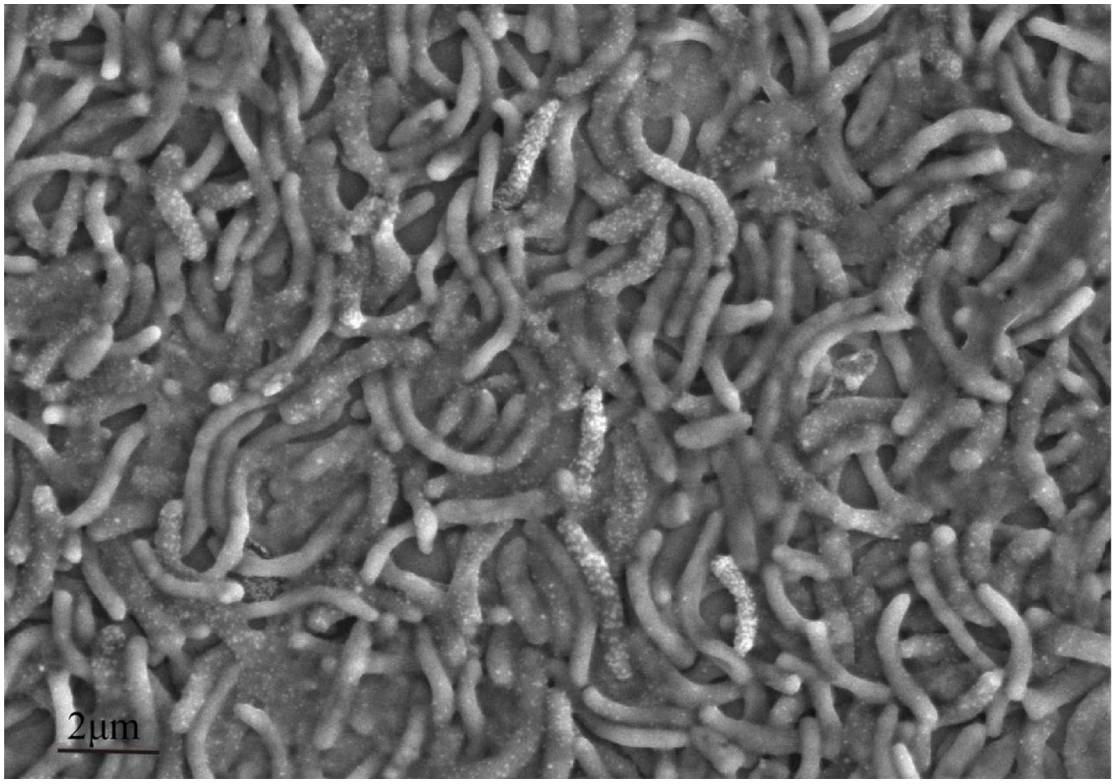

Fig. S2 SEM image of *R. rubrum*-CdS biohybrid system prepared under a Cd^{2+} concentration of 0.2 mM.

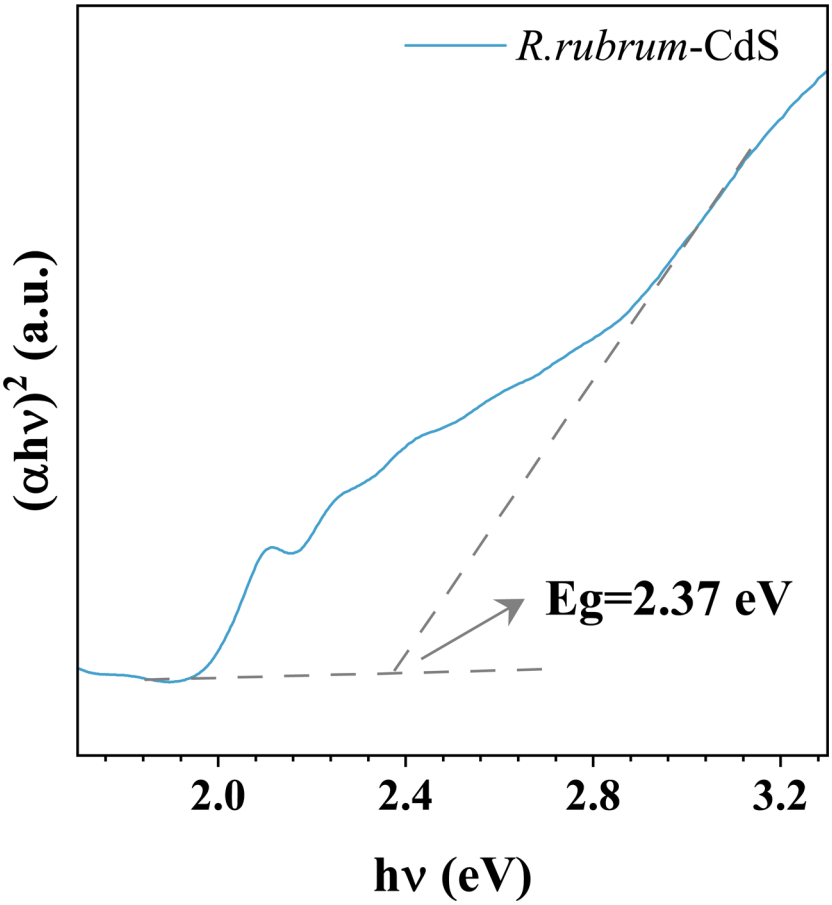

Fig. S3 Tauc plots for band gap (*Eg*) determination of *R. rubrum*-CdS biohybrid system.

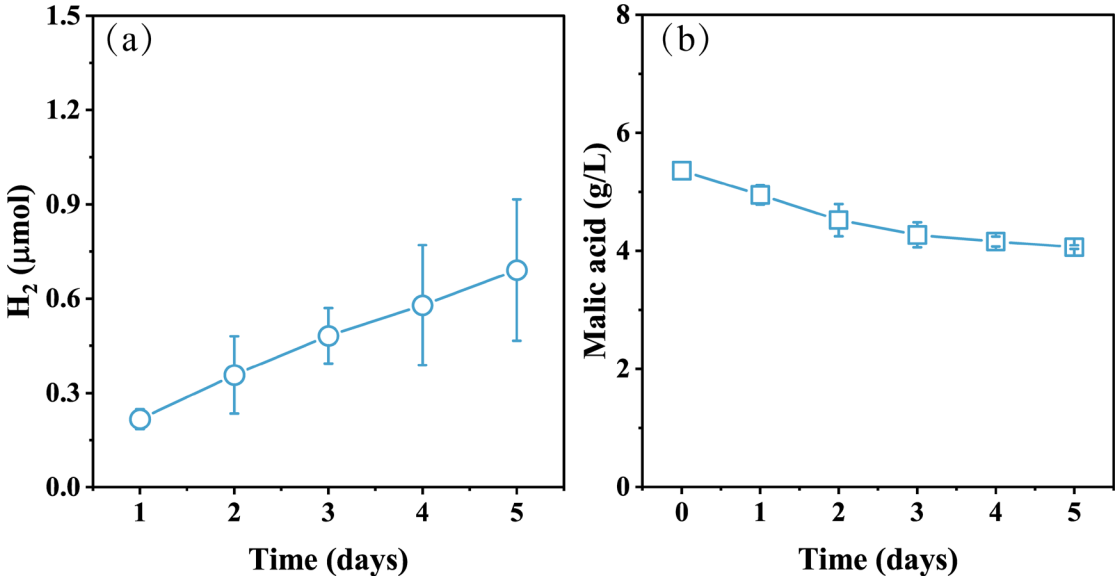

Fig. S4 a Photocatalytic H_2 evolution of chemically synthesized CdS. **b** Changes of malic acid concentration in cell-free MMG medium at the presence of chemically synthesized CdS.

Table S1 Components of MMN medium

MMN	**1000 mL**
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (PAPA, 30 mM)	0.1 mL
Malic acid	5.36 g
$(NH_4)_2SO_4$	1.25 g
MOPS	8.37 g
Tricine	0.72 g
β-glycerophosphate·2Na	0.344 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	75 mg
$FeSO_4 \cdot 7H_2O$	11.8 mg
K_2SO_4	20 mg
H_3BO_3	2.8 mg
$MnSO_4 \cdot 4H_2O$	2.1 mg
$Na_2MoO_4 \cdot 2H_2O$	0.75 mg
$ZnSO_4 \cdot 7H_2O$	0.24 mg
$Cu(NO_3)_2 \cdot 3H_2O$	0.04 mg
KOH	Adjust pH to 6.8

Table S2 Components of MMG medium

MMG	**1000 mL**
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (PAPA, 30 mM)	0.1 mL
Malic acid	5.36 g
Glutamic acid	2 g
MOPS	8.37 g
Tricine	0.72 g
β-glycerophosphate·2Na	0.344 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	75 mg
$FeSO_4 \cdot 7H_2O$	11.8 mg
K_2SO_4	20 mg
H_3BO_3	2.8 mg
$MnSO_4 \cdot 4H_2O$	2.1 mg
$Na_2MoO_4 \cdot 2H_2O$	0.75 mg
$ZnSO_4 \cdot 7H_2O$	0.24 mg
$Cu(NO_3)_2 \cdot 3H_2O$	0.04 mg
KOH	Adjust pH to 6.8

Formulas for calculating solar-to-hydrogen energy conversion efficiencies:

$$\text{Photochemical Efficiency(PE\%)} = \frac{\text{H}_2 \text{ production rate} \times \text{H}_2 \text{ energy content}}{\text{absorbed light energy}} \times 100\%$$

$$\text{PE\%(Ar} - R.rubrum - \text{CdS)} = \frac{0.29\left(\frac{MJ}{mol}\right) \times 171.06\left(\frac{\mu mol}{L}\right) \times 0.18(L)}{30\left(\frac{W}{m^2}\right) \times 0.002(m^2) \times 86400(s)} \times 100\%$$

$$\text{PE\%(Ar} - R.rubrum) = \frac{0.29\left(\frac{MJ}{mol}\right) \times 102.25\left(\frac{\mu mol}{L}\right) \times 0.18(L)}{30\left(\frac{W}{m^2}\right) \times 0.002(m^2) \times 86400(s)} \times 100\%$$

$$\text{PE\%(N2} - R.rubrum - \text{CdS)} = \frac{0.29\left(\frac{MJ}{mol}\right) \times 92.07\left(\frac{\mu mol}{L}\right) \times 0.18(L)}{30\left(\frac{W}{m^2}\right) \times 0.002(m^2) \times 86400(s)} \times 100\%$$

$$\text{PE\%(N2} - R.rubrum) = \frac{0.29\left(\frac{MJ}{mol}\right) \times 68.58\left(\frac{\mu mol}{L}\right) \times 0.18(L)}{30\left(\frac{W}{m^2}\right) \times 0.002(m^2) \times 86400(s)} \times 100\%$$

The standard molar enthalpy of combustion for H_2 is 0.29 MJ mol^{-1}. The native *R. rubrum* cells produced H_2 under Ar atmosphere for 7 days, the daily H_2 yield was 102.25 μmol L^{-1} OD_{600}^{-1}. The CdS-*R. rubrum* biohybrid cells produced H_2 under Ar atmosphere for 7 days, the daily H_2 yield was 171.06 μmol L^{-1} OD_{600}^{-1}. The native *R. rubrum* cells produced H_2 under N_2 atmosphere for 7 days, the daily H_2 yield was 68.58 μmol L^{-1} OD_{600}^{-1}. The CdS-*R. rubrum* biohybrid cells produced H_2 under N_2 atmosphere for 7 days, the daily H_2 yield was 92.07 μmol L^{-1} OD_{600}^{-1}. The headspace volume was 0.18 L, light illuminance was 30 Wm^{-2}, illumination area was 0.002 m^2, total illumination time was 86400 s.