Supplementary Information

Biohybrid Photoheterotrophic Metabolism for Significant Enhancement of Biological Nitrogen Fixation in Pure Microbial Cultures

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Experimental Section

- ***Preparation of the bacterial cell for biohybrid cell construction***

As shown in Fig. S1, The *R. palustris* (ACCC 10649) cells were maintained in 60 mL malate-ammonium (MN) medium[1] (Table S1) in the sealed clear collection vials (60 mL, Thermo Scientific, USA). The bacterial cells were incubated on an orbital incubator (MX-RD-Pro, Scilogex, USA) at 10 revolutions per minute (RPM). All the vials were illuminated by fluorescent tubes with an 80 W/m^2 visible light intensity. The incubating temperature was controlled at 30 ± 1 °C.[2] The bacterial cells were weekly transferred to the fresh prepared MN for maintenance. The maintained *R. palustris* cells were transferred to 60 mL freshly prepared supplemented MN (SMN) medium (Table S1) in the clear collection vials (60 mL, Thermo Scientific, USA) on a rotating incubator (MX-RD-Pro, Scilogex, USA). Light illumination was provided by 80 W/m^2 FTs and the temperature was controlled at 30 ± 1 °C (Fig. S1). The cell density was monitored by measuring the optical density at (OD_{680}) and calculated by standard curves. The pellets of the rapidly accumulated *R. palustris* cells at mid-exponential growth phase (OD_{680} at 1.5) were collected and diluted by the modified MN (MMN) medium (Table S1) to an initial OD_{680} at 0.6.

- ***Surface CdS coating***

The as-prepared MMN-*R. palustris* medium (60 mL) was supplemented with 0 - 0.25 mM $Cd(NO_3)_2$ and 1 mM cysteine as sulfur source. The CdS coating efficiency of *R. palustris* was evaluated by removing the cultured bacterial cells by 0.22 um membrane filtration and quantifying the left Cd^{2+} concentration in the supernatants via an atomic absorption

spectroscopy (AAS, Hitachi Z2300 flame, Japan). The CdS coating efficiency (CE) was calculated by the equation:

$$CE = \left(1 - \frac{Cd^{2+}\ remained\ in\ the\ MMN\ medium}{Cd^{2+}\ added\ into\ the\ MMN\ medium}\right) \times\ 100\%$$

The optimum Cd^{2+} concentration was further confirmed according to the acceptable CE and remained Cd^{2+} in the culture medium (Table S2). Then the optimum Cd^{2+} concentration is used for surface CdS coating. The as-prepared *R. palustris*-MMN medium was filled in the clear collection vials (60 mL) and incubated on the rotating incubator by the same conditions.

- ***Characterization of the R. palustris cells and surface CdS***

The prepared CdS-*R. palustris* culture was collected and fixed by 4 % glutaraldehyde, then sequentially dehydrated by ethanol and deposited on 200-mesh copper grids (Electron Microscopy Sciences, USA). To prepare thin-sectioned samples, a parallel of fixed sample was stained by 1 % OsO_4, then sequentially dehydrated by acetone, followed by the sequential infiltration of resin (Spurr's, Electron Microscopy Sciences, USA). After baking at 80 °C overnight, the embedded samples were sectioned to 70 nm by diamond knife (Diatome Ultra 45°, MF277, Switzerland) and deposited on 100-mesh copper grids (Electron Microscopy Sciences, USA). The HRTEM (Tecnai F20, FEI, USA) at an acceleration voltage of 10 kV was performed to observe the morphology of both the original sample and thin-sectioned sample of the biohybrid cells. The HAADF mode of the HRTEM was applied to obtain HAADF-STEM images of the bacterial membrane with coated NPs. The EDX mode of the HRTEM was performed for EDS mapping and analyzing the elemental composition of the surface particles. To isolate the CdS NPs from the cell surface, the cell pellet of the CdS-*R. palustris* culture (10

L) was collect and lysed by QIAGEN Buffer P2[3] and then ultrasonicated by a ultrasonicator (Branson 2510E, Gemini, USA) overnight. The lysed sample was centrifuged and the pellet in yellowish layer was collected and washed by ethanol and ultra-pure water for multiple times. Finally, the sample was freeze-dried and grinded into power. The UV-vis spectroscopic measurement of the isolated CdS NPs powder was performed on a UV-vis spectrophotometer (BlueStar A Split Beam, LabTech, USA). The valence band (VB) of the isolated CdS NPs powder was measured by the X-ray photoelectron spectroscopy (XPS, ESCALAB MK, Thermo Fisher, USA). To test the photocurrent density under fixed potentials, the isolated CdS NPs (5 mg) was fixed onto fluorine-doped tin oxide (FTO) glass as photocathode and measured by an electrochemical workstation (CHI650E, CH Instruments, USA). A xenon lamp (PLS-SXE300C, PerfectLight, China) with 420 nm filter was used to provide visible light irradiation (1000 W/m^2).

- ***N_2 fixation study***

The cell pellets of the CdS-coated *R. palustris* and natural cells were collected from the early stationary phase and then diluted by the fresh prepared modified malate-glutamate (MMG) medium (Table S1), supplemented with 1 mM cysteine as hole sacrificial agent. The initial OD_{680} of the CdS-*R. palustris*-MMG medium was adjusted to 1.0 (cell density = 6.5×10^8 cell mL^{-1}, dry weight of biomass = 0.25 g L^{-1}). Parallel groups of 30 mL prepared mediums were aliquoted into the 60 mL clear collection vials. The vials were purged with pure N_2 gas (purity > 99.9 %) at atmospheric pressure and incubated on the orbital incubator at 10 RPM under FT (80 W/m^2) (Fig. S1). For comparison, parallel groups of the biohybrid and natural cells purged

with pure Argon gas and under the same light irradiation or in dark condition were added.

- ***Measurement of Nitrogenase activity and generated H_2***

The acetylene-ethylene assay[4] is employed to measure the nitrogenase activity of the CdS-coated *R. palustris* and the natural cells during the 120 h N_2 fixation study. The gaseous phase in the head space of the parallel groups of the samples collected at difference time points are exchanged by pure acetylene (C_2H_2) at atmospheric pressure. The generated ethylene (C_2H_4) in 2 hours was measured by a gas chromatography (GC-7806, Shiweipx, China) using FID mode in triplicate. The produced ethylene is standardized to the unit of nmol h^{-1} mg^{-1} cell. The H_2 evolution in the 30 mL gaseous phase of the parallel vials was detected by the GC (GC-7806, Shiweipx, China) using TCD mode, then quantified by standard curves.

- ***Assay of the NADPH level, Ammonium concentration and total L-amino acids.***

At fixed time intervals, the cellular NADPH level, ammonia concentration and total L-amino acid concentration were measured in triplicate by collecting 1 mL sample by a sterile syringe from the reactions. The NADP/NADPH Assay Kit (ab65349, abcam, UK), Ammonia Assay Kit (ab83360, abcam, UK) and L-Amino Acid Quantification Kit (K639-100, BioVision, USA) were used for the corresponding assays following the provided protocols.

- ***15-N_2 isotopic labelling study***

The prepared biohybrid and natural cells in the MMN mediums were transferred to the MMG mediums (30 mL) in the 60 mL sealed collection vials as previously described. The gaseous

phase of the biohybrid and natural cells were purged with 15-N labelled N_2 gas (purity > 99.9 %) and the N_2 fixation study under the same conditions was conducted. At the early stationary phase, the samples in the 30 mL MMN medium were filtered by 0.22 um membranes. The 15N (m/z=29)/14N (m/z=28) ratio was measured by an Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS, Thermo Scientific DELTA V, USA).

- ***Measurement of the produced solid biomass***

The pellets of CdS-*R. palustris* and natural cells after 120 h N_2 fixation were collected and heated at 80 °C for 72 h to measure the dry weight of obtained solid biomass. The mass of the pre-weighted centrifuge tube and the solid biomass in the beginning of the experiment are subtracted. For reference, the natural *R. palustris* directly supplied with different levels of ammonium (2.5 to 10 mM) under Ar are also investigated.

- ***Calculation of the photosynthetic efficiency (PE)***

The PE was defined as the ratio of the light energy transferred to the biomass to the total received energy from light and organic source. The photosynthetic efficiency (PE) was calculated by the equation:[5]

$$PE = \frac{P \times (-\Delta H)}{(-\Delta H^{'}) \times MCE + TLEI}$$

Where P is the biomass productivity;

Biomass productivity of *R. palustris*

= (1.3536 g L^{-1} – 0.2 g L^{-1}) × 60 × 10^{-3} L ÷ 72 h = 0.9613 × 10^{-3} g h^{-1};

Biomass productivity of the CdS-coated *R. palustris*

= (3.4357 g L^{-1} – 0.2 g L^{-1}) × 60 × 10^{-3} L ÷ 72 h = 2.6964 × 10^{-3} g h^{-1};

$-\Delta H$ is the heat of combustion of the ash-free biomass, which is calculated according to the following equation:[6]

$$-\Delta H = \frac{(\%C \times 2.664 + \%H \times 7.936 - \%O) \times 100}{398.9} \times 13.2586\ (Kcal\ g^{-1})$$

The biomass composition (% of C, H, O, N) of *R. palustris* and CdS-coated *R. palustris* was analyzed by an elemental analyzer (vario EL Ⅲ, German) and listed in Table S3.

$-\Delta H'$ is the heat combustion of malate: 2.387 (Kcal g^{-1});

MCE is the malate consuming efficiency (g h^{-1}), which is calculated by the data in Table S4;

TLEI is the total light energy input, which is estimated by the total solar radiation impinging on horizontal surface per m^2 using the following formula:

$$TLEI = TSR \times 2.75 \times 10^{-3} \times 0.64 \times 0.89$$
$$= 80 \times 0.85986 \times 2.75 \times 10^{-3} \times 0.64 \times 0.89$$
$$= 0.10775\ (Kcal\ h^{-1})$$

Where TSR is the total solar radiation impinging on horizontal surface per m^2 (Kcal h^{-1} m^{-2}); 80 is the light intensity of the fluorescent tubes (W m^{-2}); 0.85986 is the conversion factor from W m^{-2} to Kcal h^{-1} m^{-2}; 2.75 × 10^{-3} is the horizontal surface area of the 60 mL clear collection vials; 0.64 is the coefficient factor of the photosynthetic available radiation of *R. palustris* from 400 nm to 900 nm; 0.89 is the glass transmittivity;

The calculated parameter and PE value are concluded in Table S5.

The individual contribution from organic carbon to the PE is calculated as follows:

$$PE(contributed\ by\ organic\ carbon) = PE_{R.\ palustris} \times \frac{Energy\ from\ organic\ carbon}{TLEI + Energy\ from\ organic\ carbon}$$

For the natural cells,

$$PE(contributed\ by\ organic\ carbon\ alone) = 2.35\% \times \frac{0.0565 \times 2.387}{0.10775 + 0.0565 \times 2.387} = 1.31\ \%$$

$$PE(contributed\ by\ light\ alone) = 2.35\% - 1.31\% = 1.04\ \%$$

The consuming of organic carbon in the biohybrid and natural cells is similar (Table S4), therefore, the contribution of the organic carbon to the $PE_{CdS\text{-}R.\ palustris}$ is roughly evaluated to be equal to that of the natural cells. Therefore, the contribution of the light originated energy including the light alone and light-induced electrons to the $PE_{CdS\text{-}R.\ palustris}$ is calculated:
$PE(contributed\ by\ light\ originated\ energy) = 6.73\% - 1.31\% = 5.42\ \%$.
In the CdS-coated *R. palustris* cells, the contribution (%) from light originated energy to the biomass $= 5.42\% \div (5.42\% + 1.31\%) \times 100\% = 80.5\ \%$. The contribution (%) from the organic carbon to the biomass $= 100\% - 80.5\% = 19.5\%$.

- ***Investigation of the effect of the amount of loaded CdS NPs***

To investigate the solid biomass productivity of the *R. palustris* coated with different amount of CdS NPs on the cell surface, the *R. palustris* cells supplemented with different initial Cd^{2+} concentration (0 - 0.25 mM) was prepared. Using the same culture conditions as the N_2 fixation study, the biomass productivity both under VL irradiation and in the dark were measured. The pellets from different groups after 120 h N_2 fixation were collected and heated at 80 °C for 72 h to measure the dry weight of obtained solid biomass. The variation of intracellular NADPH level in the 0.5 mM, 0.15 mM and 0.25 mM groups was monitored by the NADP/NADPH Assay Kit (ab65349, abcam, UK).

- ***Investigation of the cytotoxicity of CdS NPs***

To investigate the cytotoxicity of the CdS NPs, the chemoheterotrophic growth curve of *R. palustris* coated with different amount of CdS NPs was studied in the 60 mL clear collection vials filled with nutrient broth medium (Lab M, Lancashire, UK) in darkness. At fixed time intervals, 1 mL cell sample was collected by the sterile syringe to measure the cell density. The

cell density was monitored by measuring the optical density at (OD_{680}) and calculated by standard curves.

- ***Investigation of the role of cysteine***

To investigate the hole sacrificing function and potential nutritional function of the cysteine in the medium, different initial concentration (0 - 1.0 mM) of the cysteine were added in the MMG medium. Using the same culture conditions as the N_2 fixation study, the biomass productivity of the biohybrid cells and natural cells under VL irradiation were measured. The pellets from different groups after 120 h N_2 fixation were collected and heated at 80 °C for 72 h to measure the dry weight of obtained solid biomass.

- ***Investigation of the role of the cross-membrane interface***

To investigate the role of the cross-membrane interface between the CdS NPs and the bacterial membrane, 0.25 mM of the isolated CdS NPs and the chemically synthetic CdS (prepared by mixing 16.2 mmol $Cd(NO_3)_2 \cdot 4H_2O$ with 48.6 mmol thiourea in 40 mL ethylenediamine, then heating at 160 °C in furnace for 12 h) were simply mixed with the natural cells in the MMG medium. The pellets from different groups after 120 h N_2 fixation were collected and heated at 80 °C for 72 h to measure the dry weight of obtained solid biomass.

- ***Investigation of the electron transduction***

To investigate the transduction of photo-induced electron, different biosafe concentration of (0.1 and 0.2 mM) Cr(VI) were added into the CdS-coated *R. palustris* in MMG medium. Using

the same culture conditions as the N_2 fixation study, the final productivity of the biohybrid cells under VL irradiation was measured. All the experiments were conducted in triplicates.

- ***Investigation of the N_2 fixation in sufficient-ammonium circumstance***

To study the N_2 fixation in ammonium-sufficient environment, the prepared CdS-*R. palustris* and natural cells were collected and diluted by fresh MMN medium (Table S1) with 1 mM cysteine to an initial OD_{680} at 1.0. The culturing condition is the same as the study in MMG medium. Parallel groups filled with Ar at atmospheric pressure were conducted. The variation of intracellular ammonia and obtained dry weight of solid biomass were measured. All the experiments were conducted in triplicates.

- ***Investigation of the contribution of Calvin cycle to the solid biomass synthesis***

To study the contribution of Calvin cycle, the prepared CdS-*R. palustris* and natural cells were collected and diluted by modified propionate-glutamate medium (MPG, Table S1) with 1 mM cysteine, then adjusted to OD_{680} at 1.0. Parallel groups of 30 mL of the prepared solution were aliquoted into the 60 mL clear collection vials filled with pure N_2 or Ar gas at atmospheric pressure and incubated by the same conditions. The dry weight of the obtained solid biomass of all the experimental groups were measured. All the experiments were conducted in triplicates.

- ***Investigation of the photoautotrophic growth of the natural and biohybrid cells***

The prepared CdS-coated *R. palustris* cells and the natural cells were collected and diluted by

the photoautotrophic medium (Table S1) with 1mM cysteine, then adjusted to OD_{680} at 1.0. Parallel groups of 30 mL of the prepared solution were aliquoted into the 60 mL clear collection vials filled with N_2/Ar and 5 mL pure CO_2 in the head space. All the vials were incubated by the same conditions as N_2 fixation study. The dry weight of the obtained solid biomass of all the experimental groups were measured. All the experiments were conducted in triplicates.

- ***Feasibility study of the CdS-coated R. palustris for practical application***

To investigate the feasibility of the biohybrid cells for practical application, equal molar of carbon of the glycerol and crude glycerol from industry are used to substitute the malate (45 mM) in the MMG medium ((crude) glycerol medium, Table S1). The simulated solar provided by a 300W xenon lamp with solar simulator accessories (Air Mass 1.5 Filters, 100 W m^{-2}) is used to substitute the VL irradiation. The combination of VL or solar with the different carbon sources were set. The vials were incubated by the same conditions as N_2 fixation study. The dry weight of the obtained solid biomass of all the experimental groups were measured. In addition, a 12h:12h solar/dark cycle was applied to both the CdS-coated *R. palustris* cells and natural cells using crude glycerol as carbon source. The experiment last for 9 days. The variation of cell density in the experimental groups were monitored, and the final dry weight of soli biomass are measured.

- ***Investigation of the stability of the biohybrid cells***

The cells at the beginning of the experiment and after 9 days were collected for study. The relative respiration activity of the used biohybrid cells was measured by mixing the sample

with 2,3,5-triphenyl tetrazolium chloride (TTC) and measuring the absorbance at 484 nm by a spectrophotometer (BlueStar A Split Beam, LabTech, USA).[7] Partial sample of the biohybrid cells was also prepared for the observation under HRTEM. To measure the bacterial membrane integrity, LIVE/DEAD BacLight Bacterial Viability Kit (L13152, Life technologies, USA) was applied to stain the cells and observed under a fluorescence microscope (Nikon E80i, Japan). The potential leakage of the Cd^{2+} into the solution after the N2 fixation study was measured by the AAS (Hitachi Z2300 flame, Japan).

For all the experimental results, the means and standard deviations from triplicate experiments were calculated by Microsoft Excel 2016 and Origin Lab 8.5. The Student's t-test in SPSS 20.0 was used to detect significant differences in experiments. A p value < 0.05 was defined as statistically significant.

Supplementary Figures

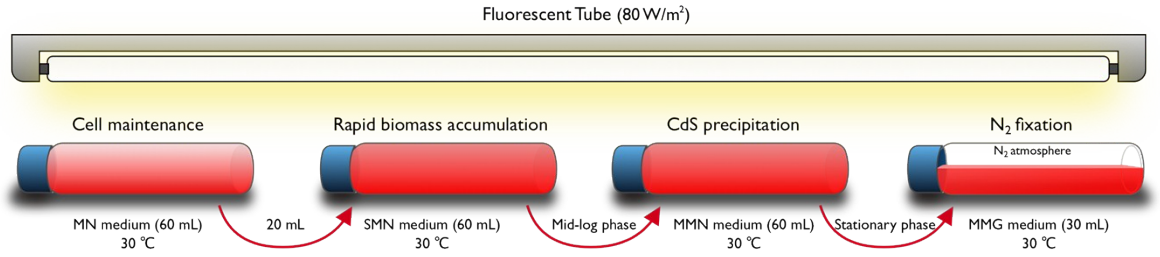

Fig. S1 Graphic illustration of cell maintenance, rapid cell accumulation, biohybrid cell preparation and N_2 fixation study.

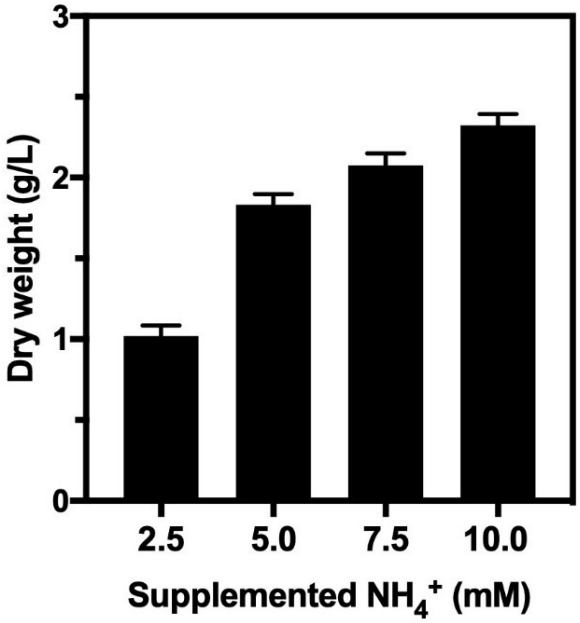

Fig. S2 The solid biomass productivity of the natural cells supplemented with different concentration of ammonium (2.5-10 mM)

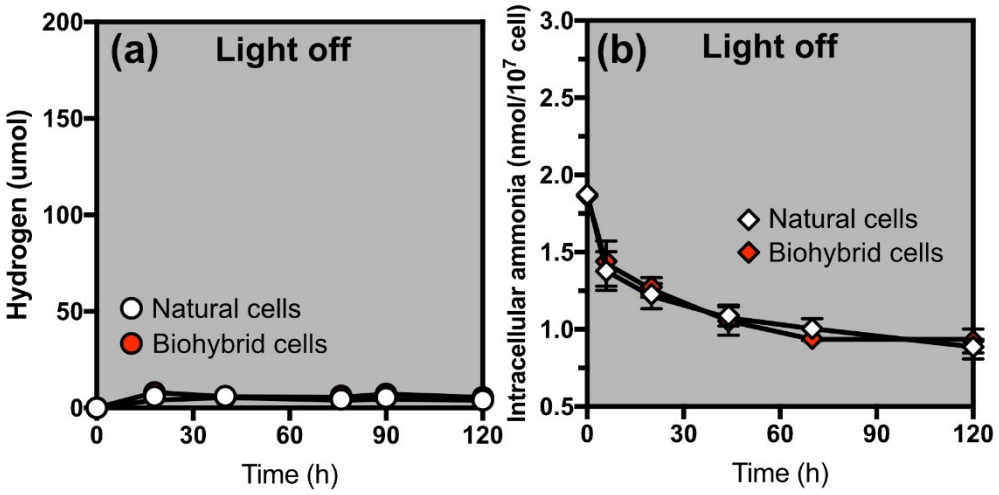

Fig. S3 (a) H_2 production in the head space of the vial and (b) intracellular ammonia concentration of the natural and biohybrid cells in the N_2 fixation study in N-deficient medium without light illumination.

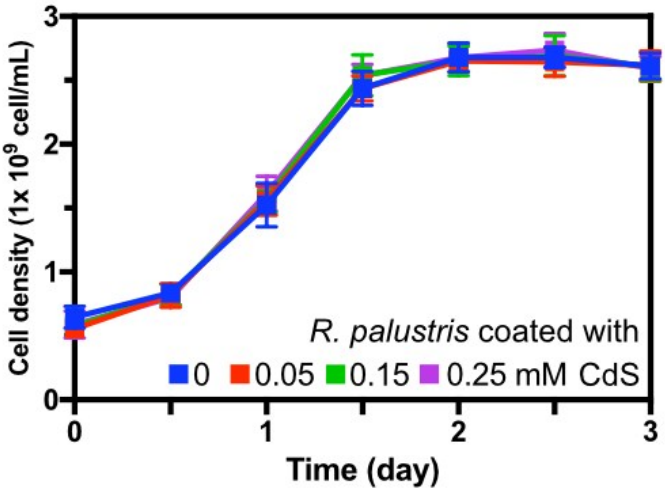

Fig. S4 The chemoheterotrophic growth curves of the biohybrid cells with different amount of CdS NPs in darkness. Nutrient broth is used as the culture medium.

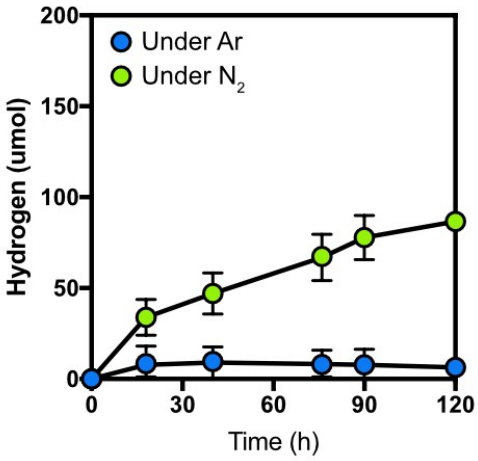

Fig. S5 Hydrogen production (in the head space of the vial) of the biohybrid cells during N_2 fixation study in the N-sufficient medium under Ar and N_2.

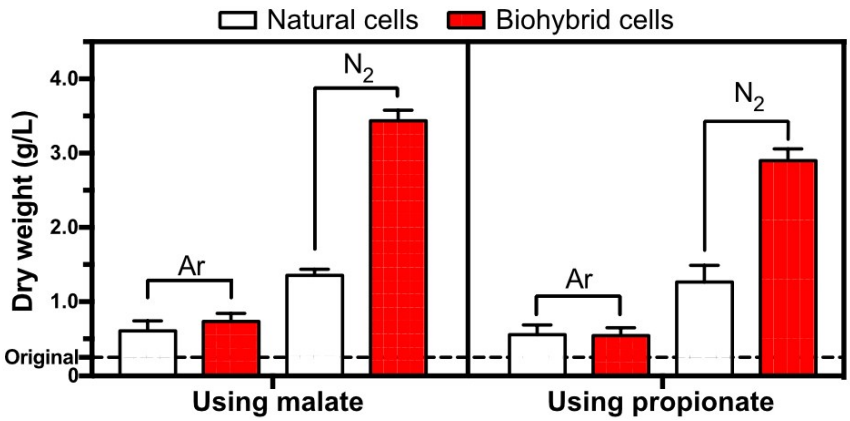

Fig. S6 Biomass production of natural and biohybrid cells using malate/propionate without NH_4^+ in Ar/N_2 atmosphere.

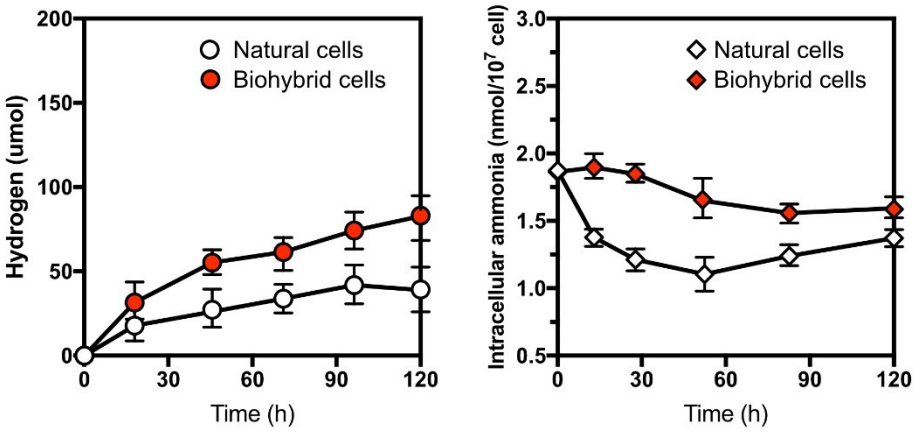

Fig. S7 (a) Hydrogen production and (b) intracellular NH_4^+ variation of the natural and biohybrid cells in the photoautotrophic medium under N_2.

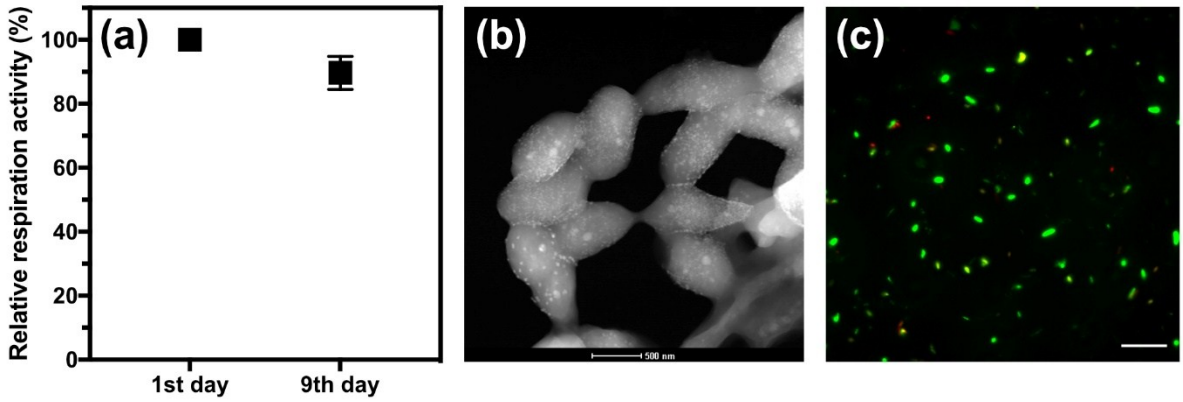

Fig. S8 (a) Relative respiration activity (%) test at the 1st day and 9th day of the practical application study using crude glycerol under solar/dark cycle, (b) HRTEM and (c) fluorescent microscope figure of the *R. palustris* coated with CdS NPs at the 9th day of the practical application study. The cells were stained by Syto9/PI dyes. Scale bar in (c): 10 um.

Supplementary Tables

Table S1. Media used in this study.

Basic medium (10 ×)	1000 mL
KH_2PO_4	6 g
K_2HPO_4	9 g
$MgSO_4 \cdot 7H_2O$	2 g
$CaCl_2 \cdot 2H_2O$	750 mg
$FeSO_4 \cdot 7H_2O$	118 mg
Ultrapure H_2O	to 1000 mL

Modified Basic medium (10 ×)	1000 mL
MOPS	83.72 g
Tricine	7.17 g
β-glycerophosphate·2Na	3.44 g
$MgSO_4 \cdot 7H_2O$	2 g
$CaCl_2 \cdot 2H_2O$	750 mg
$FeSO_4 \cdot 7H_2O$	118 mg
K_2SO_4	480 mg
Ultrapure H_2O	to 1000 mL

Trace element solution	100 mL
H_3BO_3	280 mg
$MnSO_4 \cdot 4H_2O$	210 mg
$Na_2MoO_4 \cdot 2H_2O$	75 mg
$ZnSO_4 \cdot 7H_2O$	24 mg
$Cu(NO_3)_2 \cdot 3H_2O$	4 mg

MN (malate-ammonium) medium[1]	1000 mL
Basic medium (10 ×)	100 mL
DL-malic acid	45 mM
$(NH_4)_2SO_4$	10 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	to 1000 mL

SMN (supplemented malate-ammonium) medium	1000 mL
MN medium	1000 mL
Casein hydrolysate	3 g
Yeast extract	3 g

MMN (modified malate-ammonium) medium	1000 mL
Modified Basic medium (10 ×)	100 mL
DL-malic acid	45 mM
$(NH_4)_2SO_4$	2.5, 5.0, 7.5, 10 mM
Cysteine	1 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	To 1000 mL

MMG (modified malate-glutamate) medium	1000 mL
Modified Basic medium (10 ×)	100 mL
DL-malic acid	45 mM
DL-glutamic acid	2 g
Cysteine	1 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	To 1000 mL

MPN (modified propionate-ammonium) medium	1000 mL
Modified Basic medium (10 ×)	100 mL
Propionic acid	45 mM
$(NH_4)_2SO_4$	2.5, 5.0, 7.5, 10 mM
Cysteine	1 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	To 1000 mL

MPG (modified propionate-glutamate) medium	1000 mL
Modified Basic medium (10 ×)	100 mL
Propionic acid	45 mM
DL-glutamic acid	2 g
Cysteine	1 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	To 1000 mL

Photoautotrophic medium	1000 mL
Modified Basic medium (10 ×)	100 mL
DL-glutamic acid	2 g
Cysteine	1 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	To 1000 mL

(Crude) glycerol medium	1000 mL
Modified Basic medium (10 ×)	100 mL
(Crude) glycerol	60 mM
DL-glutamic acid	2 g
Cysteine	1 mM
Trace element solution	1 mL
EDTA (2% w/v)	1 mL
4-Aminobenzoic acid (30 mM)	0.1 mL
NaOH	Adjust pH to 6.8
Ultrapure H_2O	To 1000 mL

Table S2. CdS coating efficiency (CE)

Cd^{2+} concentration	Remained Cd^{2+} in medium	CE
0.05 mM (5.62 mg/L)	0.06 mg/L	98.93 %
0.10 mM (11.24 mg/L)	0.28 mg/L	97.51 %
0.15 mM (16.86 mg/L)	0.93 mg/L	94.48 %
0.20 mM (22.48 mg/L)	1.41 mg/L	93.75 %
0.25 mM (28.10 mg/L)	2.74 mg/L	90.26 %
0.30 mM (33.72 mg/L)	9.02 mg/L	74.23 %
0.35 mM (39.34 mg/L)	13.21 mg/L	66.41 %
0.40 mM (44.96 mg/L)	22.24 mg/L	50.54 %

Table S3. Elemental composition and the calculated heat energy of combustion of the natural *R. palustris* cells and CdS-coated *R. palustris*.

Biomass composition	***R. palustris***	**CdS-*R. palustris***
C (%)	51.17	51.89
H (%)	9.15	10.08
O (%)	30.12	29.06
N (%)	9.56	8.97
Molecular formula	$CH_{2.13}N_{0.16}O_{0.44}$	$CH_{2.33}N_{0.15}O_{0.42}$
Heat energy of combustion (Kcal g^{-1})	5.9433	6.2876

Table S4. Malate consuming efficiency (MCE) of the biohybrid cells and natural cells.

Time (h)	Malate concentration (mM)		Consuming Efficiency (g h^{-1})	
	Natural cells	Biohybrid cells	Natural cells	Biohybrid cells
0	45	45	-	-
12	39.19	40.33	0.0649	0.0522
24	33.16	34.23	0.0674	0.0682
36	27.44	28.69	0.0639	0.0619
48	23.54	23.96	0.0436	0.0529
60	19.18	17.95	0.0487	0.0672
72	14.68	12.59	0.0503	0.0599
Average malate consuming efficiency (MCE)			0.0565	0.0604

Table S5. Photosynthetic efficiency (PE) calculation of the untreated *R. palustris* and CdS-coated *R. palustris* cultured in ammonium-insufficient MMG medium.

	Natural cells	**Biohybrid cells**
Biomass productivity (g h^{-1})	0.9613×10^{-3}	2.6964×10^{-3}
$-\Delta H$ (Kcal g^{-1})	5.9433	6.2876
TLEI (Kcal h^{-1})	0.10775	0.10775
$-\Delta H'$ (Kcal g^{-1})	2.387	2.387
MCE (g h^{-1})	0.0565	0.0604
PE (%)	2.35	6.73

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