Supplementary information

Photobiocatalytic H_2 evolution of GaN:ZnO and [FeFe]-hydrogenase recombinant *Escherichia coli*

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Fig. S1 Spectral distribution of a 300-W xenon lamp (Model: CX-04E with an R300-3J lamp housing, INOTEX Co., LTD., Japan) was utilized as a full arc light source for the results in **Fig. 1**, **Fig. 4**, **Fig. 7** and **Fig. S2**. In addition, the results of **Fig. 8** under visible-light irradiation were obtained by using the xenon lamp equipped with a cut-off filter λ ≥ 422 nm (Model: LUO422, Asahi Spectra, Japan).

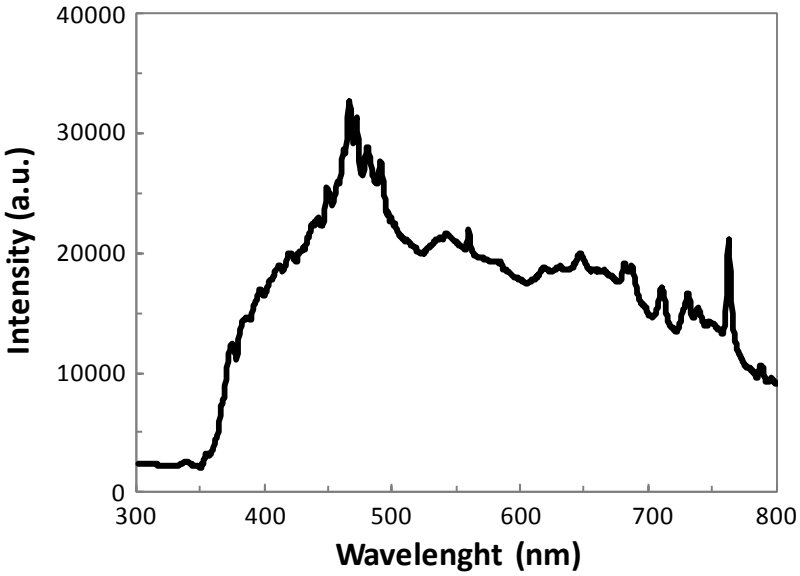


Fig. S2 UV-Vis spectrum of photocatalytic reduced $MV^{\bullet+}$ by different oxynitrides in the presence of 10% TEOA pH 8 and 5 mM methyl viologen (MV^{2+}) irradiated by a full arc light source of 1.7 W/cm^2 using a 300-W xenon lamp (CX-04E with an R300-3J lamp housing, INOTEX Co., LTD., Japan): A) TEOA+MV^{2+} only as a negative control, B) $LaTiO_2N$, C) $BaTaO_2N$, D) Ta_3N_5 and E) GaN:ZnO. Amount of reduced $MV^{\bullet+}$ was calculated from the absorbance at 605 nm using a molar conversion coefficient, ε, of 1.3×10^4 $M^{-1}.cm^{-1}$. The analysed results were presented as shown in **Fig. 1A** (amount of reduced $MV^{\bullet+}$ in a function of time) and **Fig. 1B** (MV^{2+} reduction rate).

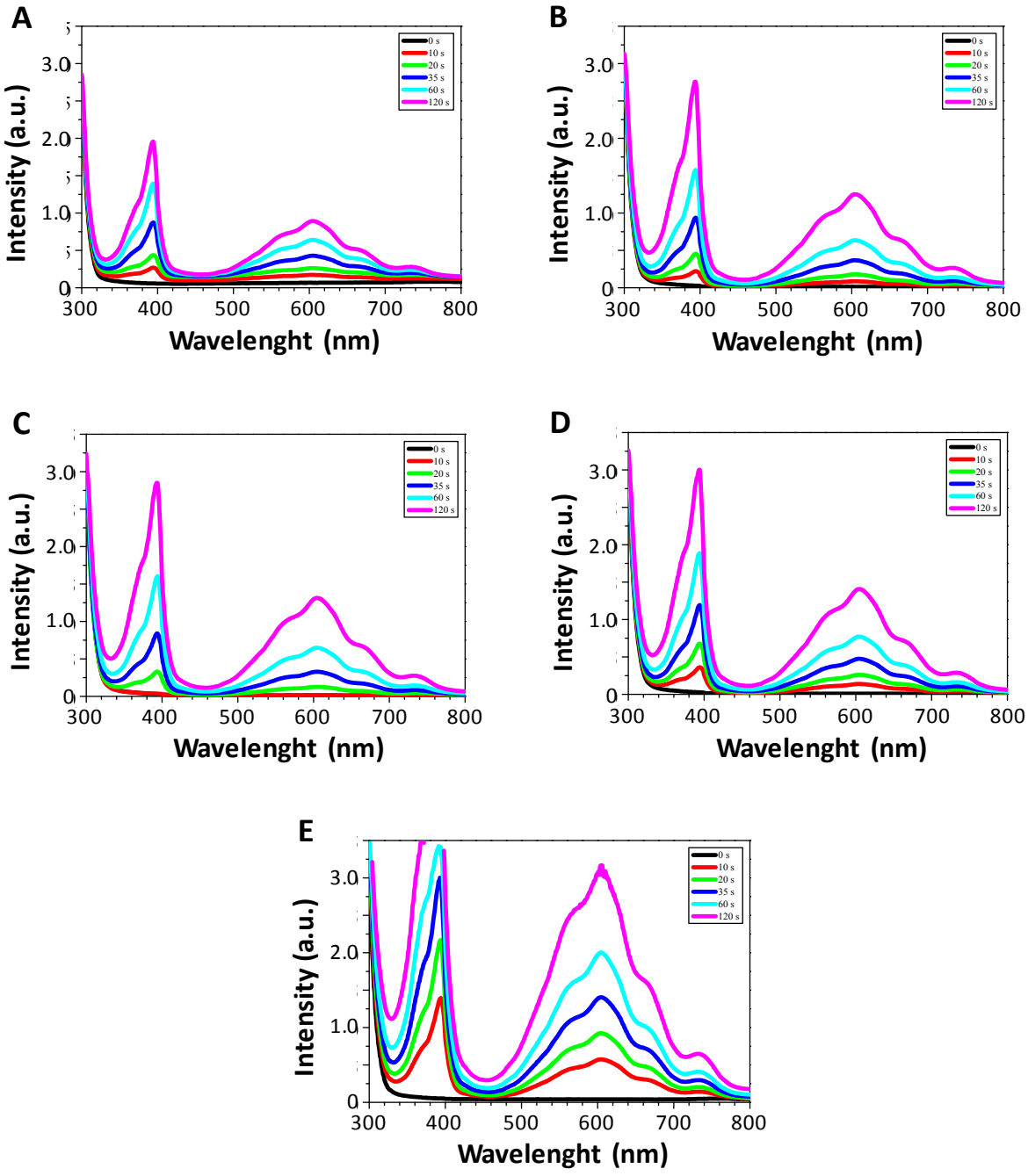


Fig. S3 XRD patterns of A) $LaTiO_2N$, B) $BaTaO_2N$, C) Ta_3N_5 and D) GaN:ZnO were performed using the powder diffraction method (RINT2500HLR+, Rigaku Corporation).

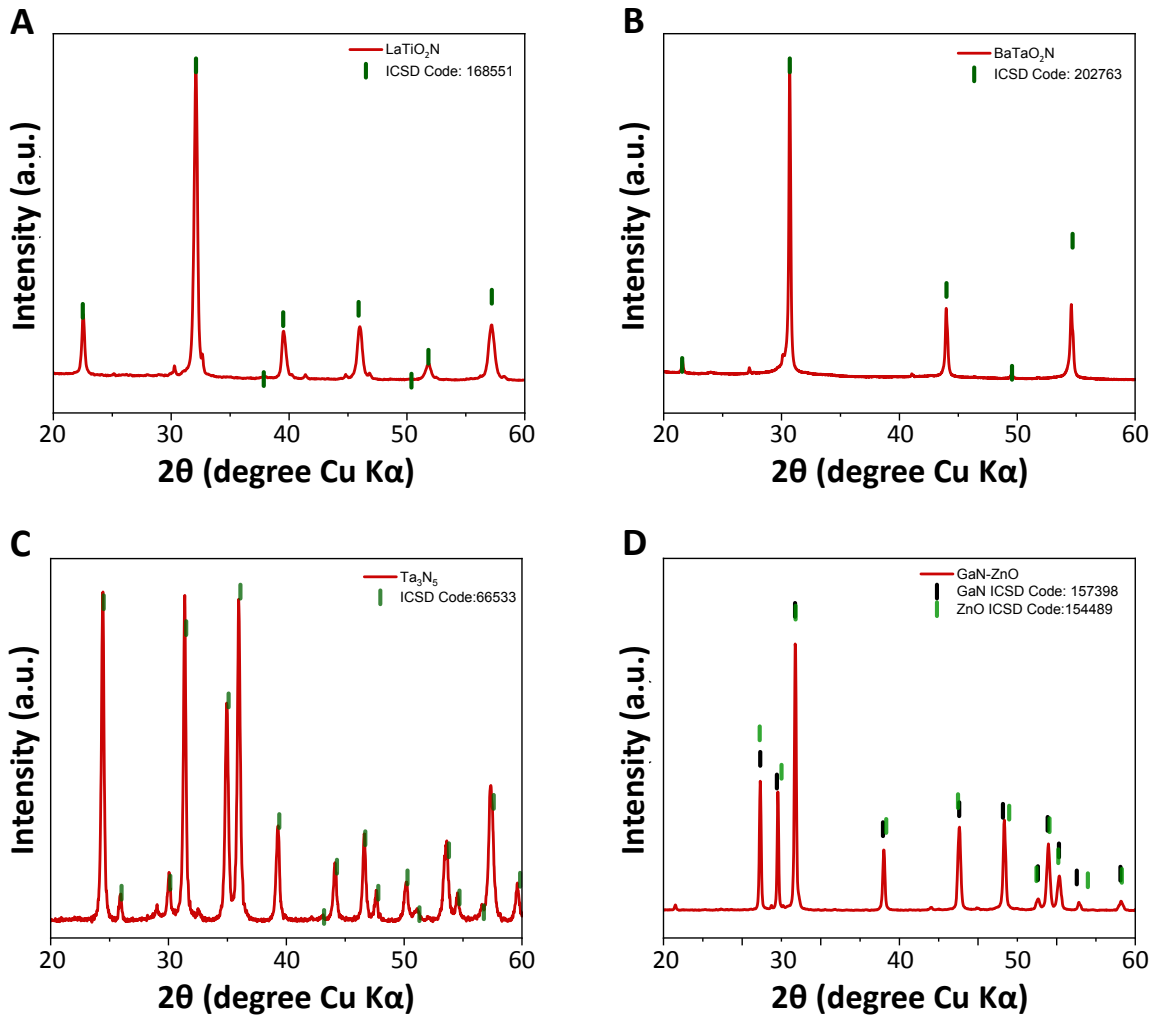
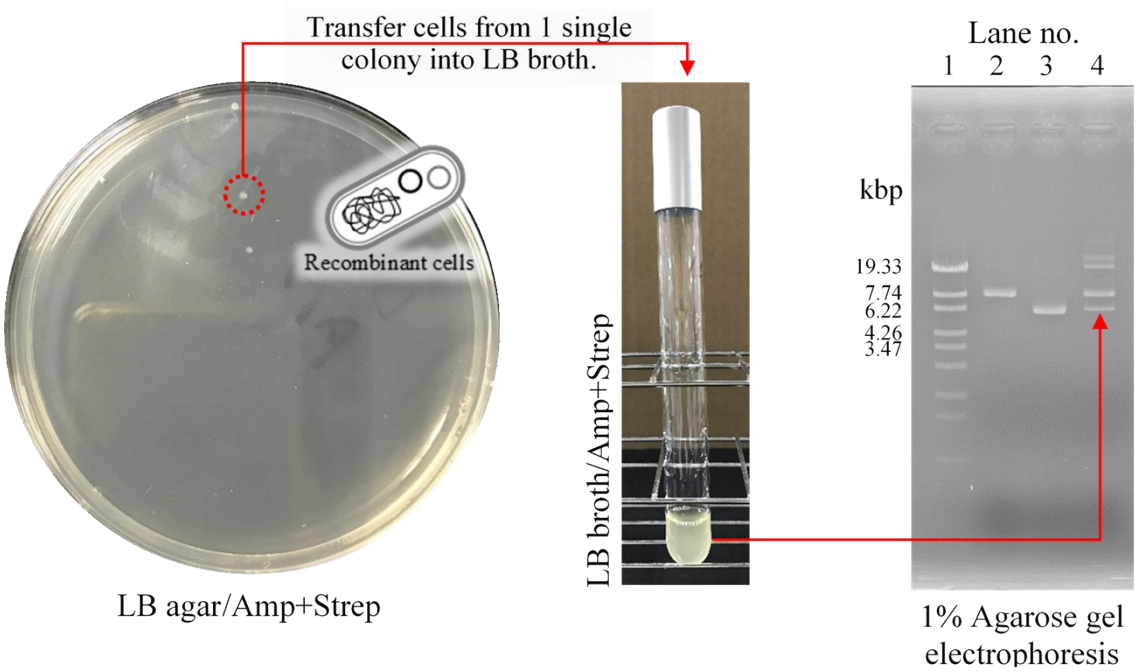
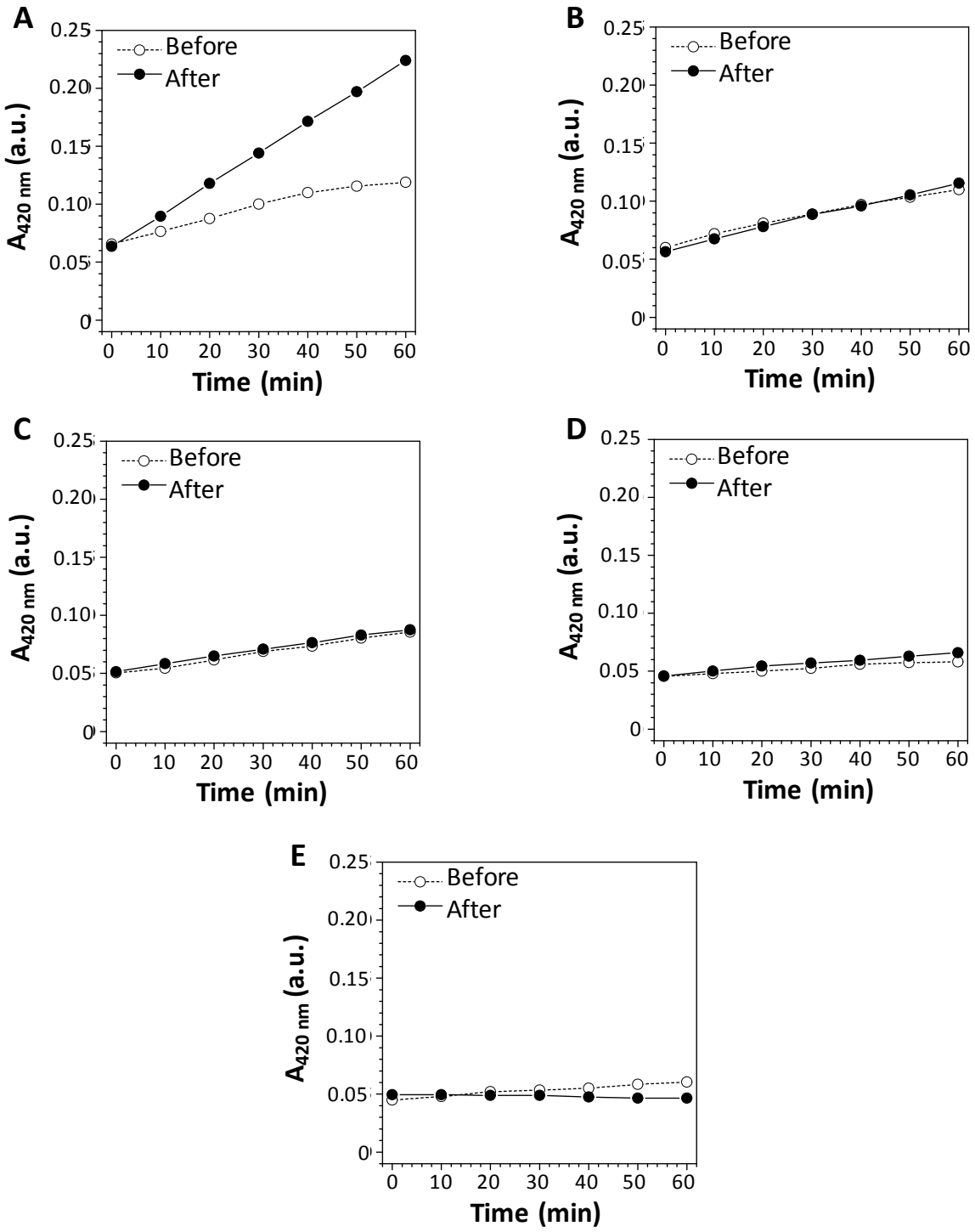


Fig. S4 Strategies for [FeFe]-hydrogenase gene transformation from *C. acetobutylicum* into host cells *E. coli* BL21(DE3) (Hyd$^+$ *E. coli*). Recombinant cells were cultivated in selective LB medium including ampicillin (100 µg/mL) and streptomycin (40 µg/mL). After plasmid extraction process by illustra^{TM} plasmidPrep Mini Spin Kit (GE Healthcare), 1% agarose gel electrophoresis confirms the presence of transformed plasmids for [FeFe]-hydrogenase expression as shown on the following Lane no.: 1) λ/Sty I digest Marker, 2) pEHydEA standard, 3) pCHydFG standard, and 4) both pEHydEA and pCHydFG plasmids extracted from Hyd$^+$ *E. coli*.



After co-transformation of pEHydEA and pCHydFG plasmids in host cells, Hyd$^+$ *E. coli* was cultivated and used as biocatalyst in the experiments as shown in **Fig. 6**, **Fig. 7** and **Fig. 8B**.

Fig. S5 Cytotoxicity of each component on bacterial cell integrity: A) 10% (v/v) TEOA pH 8, B) 5 mg GaN:ZnO, C) 5 mM MV^{2+}, D) Water and E) all components under light irradiation. Bacterial cell lysis was determined by β-galactosidase (β-gal) assay based on the activity of extracellular β-galactosidase released as an indicator of cell membrane damage.[1]



1. K. L. Griffith and R. E. Wolf, Jr., *Biochem. Biophys. Res. Commun.*, 2002, **290**, 397–402.

The influence of each component of the photobiocatalytic system on the bacterial cell integrity was determined by β-gal assay. The different components, GaN:ZnO (Fig. S5 B), MV^{2+} (Fig. S5 C), water (Fig. S5 D) and all components under irradiation (Fig. S5 E) have no effects on bacterial cells as evidenced by the same absorbance level detected before and after 1 h exposure. It was found that only 10% TEOA pH 8 (Fig. S5 A) showed an increase at the 420-nm absorbance determination after 1 h exposure indicating the release of β-galactosidase caused by cell membrane damage. However, the complete system of photobiocatalytic H_2 production remains continued for up to 12 h as shown in **Fig. 7B**, indicating that [FeFe]-hydrogenase still functions efficiently. This finding highlights the effect of each component, including GaN:ZnO, MV^{2+} and water, on recombinant *E. coli*, in order to extend hydrogenase activity and improve H_2 production efficiency for long-term experiments.

Table S1 Analysis on the rate of incident photon irradiated through different band pass filters for AQY analysis.

Bandpass filters	Light power	Area	[a] E_{Total}	[b] E_P	[c] $N_P = E/E_P$	[d] Rate of incident photon
350 nm	0.0034 $W.cm^{-2}$	2.7 cm^2	0.0092 $J.s^{-1}$	5.679×10^{-19} J	0.16×10^{17} s^{-1}	0.0266×10^{-6} $mol.s^{-1}$
390 nm	0.0040 $W.cm^{-2}$	2.7 cm^2	0.0107 $J.s^{-1}$	5.097×10^{-19} J	0.21×10^{17} s^{-1}	0.0349×10^{-6} $mol.s^{-1}$
420 nm	0.0023 $W.cm^{-2}$	2.7 cm^2	0.0061 $J.s^{-1}$	4.733×10^{-19} J	0.13×10^{17} s^{-1}	0.0214×10^{-6} $mol.s^{-1}$
470 nm	0.0051 $W.cm^{-2}$	2.7 cm^2	0.0138 $J.s^{-1}$	4.229×10^{-19} J	0.33×10^{17} s^{-1}	0.0540×10^{-6} $mol.s^{-1}$
500 nm	0.0034 $W.cm^{-2}$	2.7 cm^2	0.0092 $J.s^{-1}$	3.975×10^{-19} J	0.23×10^{17} s^{-1}	0.0384×10^{-6} $mol.s^{-1}$

[a] E_{Total} indicates the total energy of incident photon, E_{Total} (W or J s^{-1}) = Power (W cm^{-2}) × Area (cm^2);

[b] E_P indicates the energy of one photon, E_P (J) = hc/λ, where h is the Planks constant = 6.63×10^{-34} J s, c is the speed of light = 2.998×10^8 m s^{-1} and λ is wavelength (m);

[c] N_P is the number of incident photon, N_P (s^{-1}) = E_{Total} (J s^{-1}) / E_P (J);

[d] Rate of incident photon (mol s^{-1}) = N_P / Avogadro number = N_P (s^{-1}) / 6.022×10^{23} mol^{-1}.

Table S2 MV^{2+} reduction rate and apparent quantum yield (AQY) analysis of GaN:ZnO and P-25.

Bandpass filters	GaN:ZnO		P-25	
	Rate of MV reduction	[a] **AQY%**	Rate of MV reduction	[a] **AQY%**
350 nm	0.5×10^{-3} mol s^{-1}	**1.88 %**	12.0×10^{-3} mol s^{-1}	**45.11 %**
390 nm	0.4×10^{-3} mol s^{-1}	**1.15 %**	3.5×10^{-3} mol s^{-1}	**10.03 %**
420 nm	0.2×10^{-3} mol s^{-1}	**0.93 %**	0.04×10^{-3} mol s^{-1}	**0.19 %**
470 nm	0.2×10^{-3} mol s^{-1}	**0.37 %**	0.02×10^{-3} mol s^{-1}	**0.04 %**
500 nm	0.1×10^{-3} mol s^{-1}	**0.26 %**	0.03×10^{-3} mol s^{-1}	**0.08 %**

$$^{a}\text{AQY\%} = 100 \times \frac{\text{Rate of reduced MV}\bullet + \text{ (mol s - 1)}}{\text{Rate of incident photons (mol s - 1)}}$$

The results in Table S1 and S2 were obtained from the experiments under monochromatic light source of desired wavelengths and used to support data in **Fig. 5**. To avoid thermochromic effect, the reaction tubes were illuminated by using a heatless xenon lamp (MAX-303, Asahi Spectra) equipped with narrow bandpass filters (350, 390, 420, 470 and 500 nm) for AQY analysis.