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Ao Xia *et al.*

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# Engineering a semi-artificial photosynthetic biofilm for robust and high-efficiency CO<sub>2</sub>-to-methane conversion

Huize Chen,<sup>ab</sup> Ao Xia,<sup>ID \*ab</sup> Yun Huang,<sup>ID ab</sup> Junyi Ji,<sup>ID c</sup> Jingmiao Zhang,<sup>ab</sup> Xianqing Zhu,<sup>ID ab</sup> Xun Zhu<sup>ID ab</sup> and Qiang Liao<sup>ID ab</sup>

Hybrid semi-artificial photosynthetic systems, which integrate semiconductor nanomaterials with methanogens, offer an innovative strategy for the solar-driven conversion of CO<sub>2</sub> to CH<sub>4</sub> with high selectivity. However, these systems face challenges, including light harvesting losses, low quantum efficiency, and instability due to photodamage. To overcome the intrinsic limitations, we introduce a paradigm-shifting strategy: leveraging biofilms as a new platform for efficient solar-driven CO<sub>2</sub>-to-CH<sub>4</sub> conversion. The strategic modification of carbon nitride promoted the self-assembly of stable biofilms. This process formed an integrated, cross-linked network comprising the material, cells, and extracellular polymeric substances, which remarkably improved light utilization efficiency compared to traditional suspension systems. Furthermore, the extracellular polymeric substance matrix served as a biocompatible shield, effectively quenching reactive oxygen species and suppressing photodamage to the cells. To further enhance efficiency, *Methanosarcina barkeri* was decorated with silver nanoparticles. This modification rewires the electron transfer pathway, promoting a ferredoxin-independent mechanism and significantly enhancing cellular electron uptake. We achieved a state-of-the-art performance with a record 1.92% quantum yield and 97.1% methane selectivity by suppressing photodamage. This study pioneers the paradigm of integrating biofilms within hybrid systems. By elucidating its advantages and potential applications, our work provides a foundational blueprint for engineering the hybrid-biofilm microenvironment and designing practically viable reactors.

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## Introduction

The urgent need to mitigate atmospheric CO<sub>2</sub> levels and transition towards sustainable chemical production has spurred intense research into novel carbon-neutral technologies. Semi-artificial photosynthetic systems are novel platforms that couple microorganisms with light-absorbing synthetic materials, leveraging the photophysics of the synthetic materials and the selectivity and metabolic precision of microbial systems to realize highly selective and sustainable solar-to-chemical energy conversion under mild conditions.<sup>1,2</sup> This system demonstrates significant potential for energy production,<sup>3,4</sup> pollutant degradation,<sup>5,6</sup> and high-value chemical production.<sup>7,8</sup> Recent studies have demonstrated that methanogens can utilize photogenerated electrons from semiconductors through direct contact to enable highly selective CO<sub>2</sub>-to-CH<sub>4</sub> conversion.<sup>3,9,10</sup>

However, existing suspended hybrid systems are inherently limited by two critical challenges: light transfer limitations and photodamage. In such systems, the loose coupling between semiconductor materials and cells leads to significant light scattering, ineffective absorption, and shielding.<sup>3,11–14</sup> Intrinsic light absorption by the aqueous medium further exacerbates photon loss, collectively reducing the system's overall light utilization efficiency. Conversely, strategies aimed at enhancing performance by increasing incident light intensity inevitably cause microbial photodamage, thereby severely compromising long-term operational stability.<sup>15,16</sup> Such damage stems from direct radiation-induced damage to cellular components and the generation of light-induced reactive oxygen species (ROS).<sup>4,17–21</sup> Another critical issue is the mismatch between the electron generation efficiency of semiconductor nanomaterials (e.g.,  $3 \times 10^{-11}$  s for CdS) and the electron utilization efficiency of methanogen metabolism (e.g.,  $10^{-4}$  to  $10^{-1}$  s for cytochrome-mediated processes), which leads to byproduct accumulation and reduced selectivity.<sup>22</sup> The high cost and biotoxicity associated with metal-based semiconductors also necessitate the development of new systems based on non-toxic, earth-abundant materials.

<sup>a</sup>Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing University, Chongqing 400044, China. E-mail: aoxia@cqu.edu.cn

<sup>b</sup>School of Energy and Power Engineering, Chongqing University, Chongqing 400044, China

<sup>c</sup>School of Chemical Engineering, Sichuan University, Chengdu 610065, China



To address these limitations of suspended systems, we turned to nature for inspiration. In their natural habitats, microorganisms have evolved sophisticated strategies to cope with environmental stress and optimize resource exchange. Microorganisms predominantly exist in nature as structured communities known as biofilms, rather than as free-floating planktonic cells. Biofilms are three-dimensional structures embedded within a self-produced extracellular matrix.<sup>23</sup> This architecture provides internal cells with enhanced protection against environmental stresses and facilitates improved nutrient acquisition through metabolic cooperation.<sup>24,25</sup> Inspired by this natural organization, we propose a biofilm-based, semi-artificial photosynthetic system.

In the hybrid biofilm system, we employed a metal-free carbon nitride material as the photoelectric semiconductor. This material was modified by incorporating  $K^+$  ions and grafting terminal cyano groups ( $-C\equiv N$ ). This modification induced a positive zeta potential for cell attraction.<sup>26,27</sup> Moreover, this introduction of potassium ions serves as a Lewis acidic site, attracting extracellular polymeric substance (EPS) attachment through acid-base interactions.<sup>28</sup>  $^{NCN}CN_x$ , EPSs, and microbial cells self-assembled into a highly cross-linked network. Although not naturally formed, the self-assembled biofilm retains essential cells and key EPS components, consistent with the characterization of biofilms as microbial aggregates that typically accumulate at solid-liquid interfaces within a highly hydrated EPS matrix.<sup>24</sup> Furthermore, this system holds the potential to evolve into an organized multicellular structure with a complex three-dimensional architecture during subsequent growth. This structure enhanced material-cell interfacial binding, promoting the formation of a structurally robust biofilm.

The thin, dense biofilm allows for efficient light transmission and electron transfer, enabling superior performance under continuous illumination. Notably, the EPSs within the biofilm were also found to serve as scavengers of light-induced reactive oxygen species (ROS), thereby protecting the cells and retaining hybrid biofilm stability. The intrinsic properties of this biofilm significantly expand the hybrid system's capacity to utilize high-energy ultraviolet (UV) light, which accounts for approximately 5% of the solar spectrum. To address the mismatch between the electron uptake capacity of methanogens and the photoelectron generation rate of the semiconductor, we modified the cell surface with silver nanoparticles (Ag-NPs), achieving an exceptionally high quantum yield and methane production performance under continuous illumination. To the best of our knowledge, this study is the first to propose that it is the emergent properties of biofilms, arising from their collective multi-cellular assembly, that constitute an optimal framework for hybrid systems. This work offers inspiration for diverse semi-artificial photosynthesis configurations and opens up new avenues for designing practical hybrid biofilm reactors.

## Results and discussion

### Assembly of the hybrid biofilm

The carbon nitride before and after modification was characterized using Fourier-transform infrared spectroscopy (FT-IR)

and X-ray photoelectron spectroscopy (XPS). In the FT-IR spectra (Fig. 1B), both materials exhibit an absorption peak at  $810\text{ cm}^{-1}$ , corresponding to the breathing mode of triazine units. An absorption band in the range of  $1150\text{--}1700\text{ cm}^{-1}$  is attributed to the stretching vibrations of C–N heterocycles. Compared with  $CN_x$ ,  $^{NCN}CN_x$  shows a new absorption band at  $2180\text{ cm}^{-1}$ , which corresponds to the stretching vibration of the cyano group. Meanwhile, the N–H stretching vibration peak of  $^{NCN}CN_x$  in the range of  $2900\text{--}3600\text{ cm}^{-1}$  is significantly reduced, indicating successful bonding between the cyano groups and terminal amine groups of  $CN_x$  following modification.

Regarding elemental composition,  $^{NCN}CN_x$  contains additional potassium (K) and trace amounts of sulfur (S) compared to  $CN_x$  (Fig. S1 and S2). In the C 1s spectrum of  $^{NCN}CN_x$  (Fig. 1C), three peaks are observed at 284.8, 285.9, and 288.1 eV, which are assigned to adventitious hydrocarbons, the cyano group, and the N–C=N bonds in the heptazine unit, respectively (Fig. 1C). In the N 1s spectrum (Fig. S3), three peaks appear at 398.5, 400.7, and 403.6 eV, corresponding to C–N=C, N–H bonds, and  $\pi$ -excitation, respectively. For the K 2p spectrum (Fig. 1C), the fitted peak can be indexed to metallic K (at 292.9 and 295.7 eV), which may be derived from  $K^+$  adsorption within the heptazine rings.<sup>29</sup> The incorporation of  $K^+$  leads to a more compact interlayer stacking, as evidenced by XRD analysis. Specifically, the diffraction angle of the (002) plane increases from the standard value of  $27.4^\circ$  (JCPDS 87-1526) to  $28.4^\circ$  (Fig. S4).<sup>30</sup> The synergistic effect of terminal cyano groups and incorporated  $K^+$  ions induces a notable positive shift in the zeta potential of the intrinsically negative  $CN_x$ . This is attributed to the cyano groups withdrawing electron density from the framework, a process further amplified by the introduction of the positively charged potassium ions<sup>31,32</sup> (Fig. 1D).

Isothermal Titration Calorimetry (ITC) analysis revealed that the interaction between  $^{NCN}CN_x$  and *M. barkeri* was a spontaneous binding process, yielding a Gibbs free energy change of binding ( $\Delta G$ ) of  $-27.20\text{ kJ mol}^{-1}$  and a binding constant ( $K_a$ ) of  $5.834 \times 10^4$ . This binding was attributed to electrostatic interactions between  $^{NCN}CN_x$  and the cell surface. Notably, the acid-treated  $^{NCN}CN_x$  underwent the leaching of  $K^+$  ions and the destruction of its cyano groups (Fig. S5),<sup>27</sup> and as a result, the acid-treated  $^{NCN}CN_x$  lost its binding affinity for the cells, precluding the reliable determination of  $\Delta G$  and  $K_a$  values (Fig. 1E). Furthermore, a significant spontaneous binding process was also observed between  $^{NCN}CN_x$  and the isolated extracellular polymeric substances, with  $\Delta G = -27.35\text{ kJ mol}^{-1}$  and  $K_a = 6.423 \times 10^4$  (Fig. S6). Scanning Electron Microscopy (SEM) images revealed that the  $^{NCN}CN_x$  nanoparticles adsorbed onto and almost completely covered the entire cell surface (Fig. 1F). Transmission electron microscopy (TEM) images further confirm the attachment of  $^{NCN}CN_x$  to *M. barkeri* cells, revealing small particles distributed around the cell surface (Fig. 1G). Under 365 nm light irradiation, aggregates composed of  $^{NCN}CN_x$  and *M. barkeri* cells emit blue fluorescence, originating from the intrinsic bright blue fluorescence of  $^{NCN}CN_x$  and the intracellular F420 enzyme (Fig. S7). FTIR analysis also confirms the coverage of cells by  $^{NCN}CN_x$  (Fig. S8). Due to the cross-linked network among *M. barkeri* cells, EPSs, and  $^{NCN}CN_x$



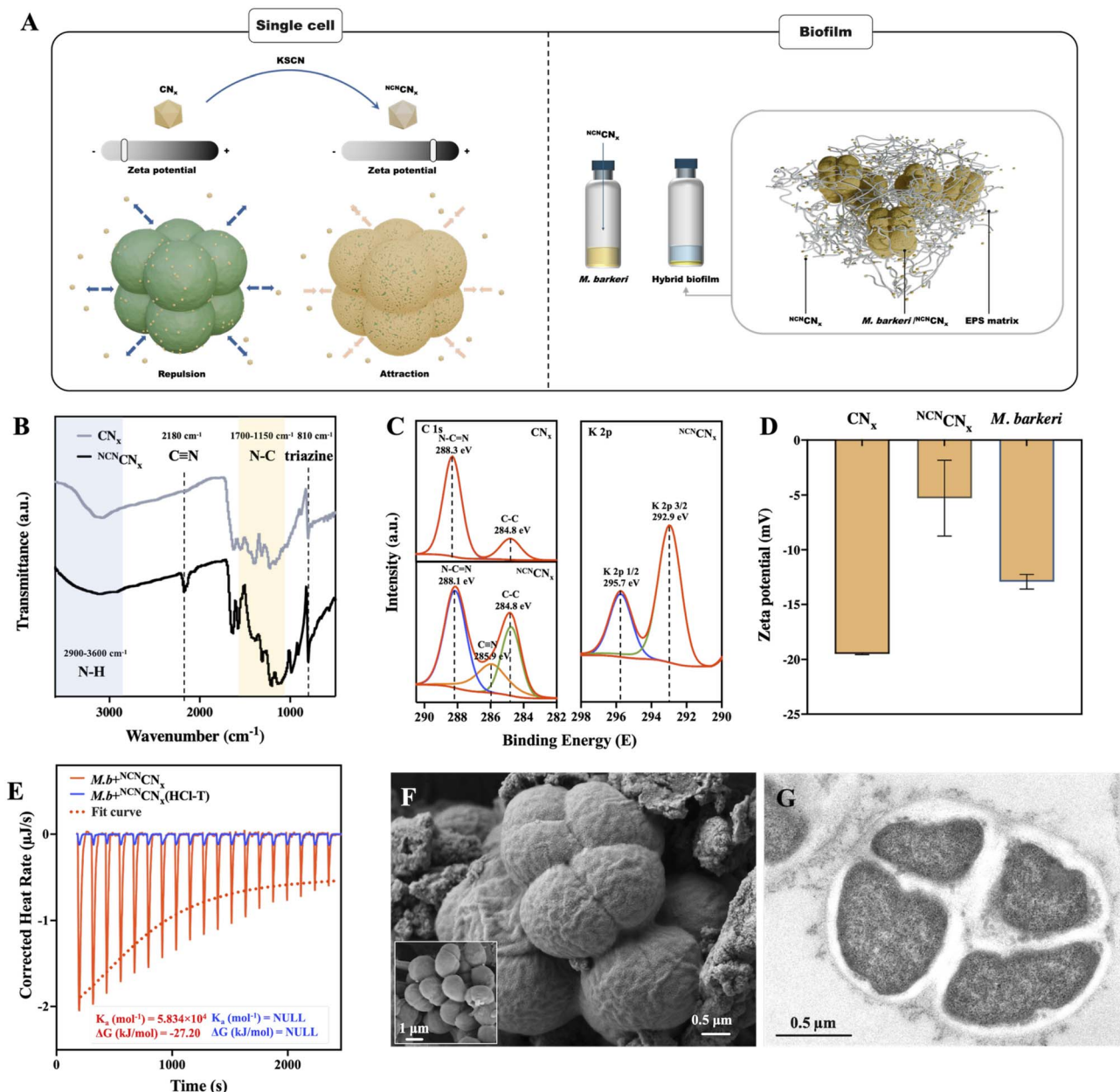


Fig. 1 Formation of hybrid biofilms. (A) Schematic illustration of hybrid single cells and hybrid biofilms. (B) FTIR spectra of  $\text{CN}_x$  and  $\text{N}^{\text{CN}}\text{CN}_x$ . (C) C 1s and K 2p XPS spectra of  $\text{CN}_x$  and  $\text{N}^{\text{CN}}\text{CN}_x$ . (D) Zeta potential of  $\text{CN}_x$ ,  $\text{N}^{\text{CN}}\text{CN}_x$ , and *M. barkeri*. (E) Real-time ITC thermograms for the interactions of *M. barkeri* with  $\text{N}^{\text{CN}}\text{CN}_x$  and with acid-treated  $\text{N}^{\text{CN}}\text{CN}_x$ . (F) SEM image of the  $\text{N}^{\text{CN}}\text{CN}_x/\text{M. barkeri}$  surface (the inset image shows bare *M. barkeri*). (G) TEM image of  $\text{N}^{\text{CN}}\text{CN}_x/\text{M. barkeri}$ .

(Fig. S9), the bio-hybrid was macroscopically observed as aggregated biofilm deposits at the bottom of the bottle (Fig. 1A) (Video S1).

### Methane generation under illumination

The photocatalytic  $\text{CO}_2$  reduction system for  $\text{CH}_4$  production using hybrid biofilms was constructed and operated in a light incubator at a controlled temperature ( $35\text{ }^\circ\text{C}$ ) and a specific wavelength ( $395 \pm 5\text{ nm}$ ) (Fig. S10), to investigate the effects of light intensity on methane production. The results reveal that the methane production rate is positively correlated with light

intensity (Fig. 2A and B), whereas the quantum yield shows a negative correlation, reaching its maximum value (1.5%) at the lowest light intensity tested ( $0.1\text{ mW cm}^{-2}$ ) (Fig. 2E). Under illumination, a portion of the photogenerated electrons from  $\text{N}^{\text{CN}}\text{CN}_x$  enter the intracellular metabolic pathway via membrane-integrated cytochromes of *M. barkeri* cells, while the other electrons reduce water at the  $\text{N}^{\text{CN}}\text{CN}_x$  surface, producing hydrogen as a byproduct.<sup>3,11</sup> Under high-light intensity conditions, limited by the electron uptake capacity of *M. barkeri* cells and potential metabolic inhibition induced by photodamage, the proportion of electrons diverted to hydrogen production



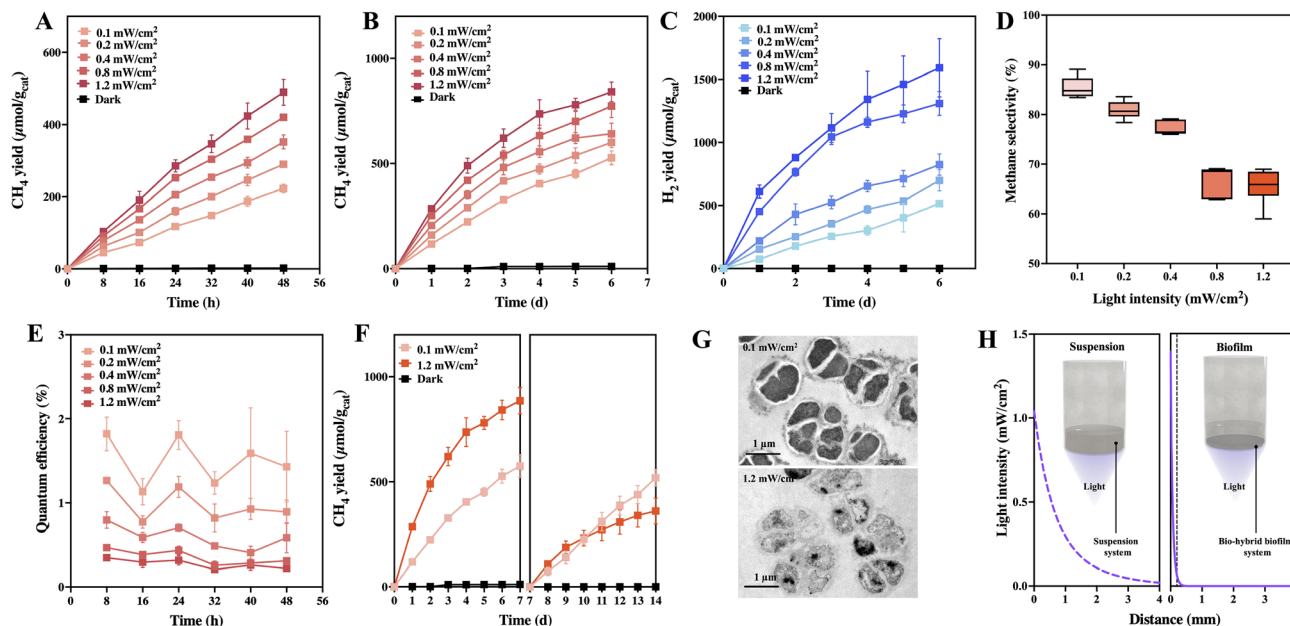


Fig. 2 Performance and characterization of hybrid biofilms. (A) Methane production within 48 hours. (B) Methane production within 6 days. (C) Hydrogen production within 6 days. (D) Methane selectivity over six days under continuous illumination (with one data point measured per day). (E) Methane quantum yield within 48 hours. (F) Comparison of methane production at high and low light intensity over 14 days. (G) TEM image of *M. barkeri* cells on day 14. (H) Light intensity distribution along the direction of illumination in suspension and biofilm systems.

increases from 14.55% ( $0.1 \text{ mW cm}^{-2}$ ) to 34.46% ( $1.0 \text{ mW cm}^{-2}$ ) (Fig. 2C and D). Although hybrid biofilms produce more methane at high light intensity, this condition adversely affects stability and selectivity, making it unsuitable for reliable long-term operation.

During the 48-hour illumination experiment, the quantum yield remained stable and CH<sub>4</sub> concentration increased linearly (Fig. S11). However, under six days of continuous illumination, the CH<sub>4</sub> production rate in the high-light-intensity group ( $1.0 \text{ mW cm}^{-2}$ ) exhibited a gradual decline (Fig. 2B and F), which can be attributed to a dual mechanism: mass transfer limitations arising from sacrificial reagent depletion in the bulk region and photodamage to cells induced by UV radiation. Notably, the decay in quantum yield observed in the low-light-intensity group ( $0.1 \text{ mW cm}^{-2}$ ) is primarily due to sacrificial reagent depletion rather than photodamage, which was validated by a 14-day long-term stability experiment on methane production (Fig. 2G). After replenishing the sacrificial reagent on day 7, the methane production performance of the high-light-intensity group in the second cycle dropped to only 40% of that in the first cycle. In contrast, the low-light-intensity group retained over 90% of its initial performance in the second cycle after the mass transfer limitations caused by sacrificial reagent depletion were alleviated. TEM imaging revealed marked differences in cellular morphology at different light intensities (Fig. 2H): cells exposed to low light intensity retained intact and well-defined structures, whereas those at high light intensity exhibited significant structural damage. The structure of the heteropolysaccharides on the outermost layer of the cell wall became indistinct, likely due to protein cross-linking or denaturation induced by UV exposure and reactive

oxygen species (ROS)-mediated damage.<sup>33–35</sup> Loss of intracellular contents further led to the disappearance of the characteristic sarcina-like morphology. Unlike the optimal light intensity of  $0.8\text{--}1.0 \text{ mW cm}^{-2}$  reported in previous studies,<sup>3,9,13</sup> the hybrid biofilm in this study achieved excellent methane production performance at a much lower light intensity ( $0.1 \text{ mW cm}^{-2}$ ), attributed to more efficient light transmission.

Conventional hybrid systems typically employ suspended configurations, wherein microbial cells and solid photocatalyst particles may lack stable associations and remain dispersed throughout the liquid phase. This arrangement inherently hinders light penetration and distribution, often necessitating high incident light intensities to achieve adequate performance. However, such high intensities may lead to a non-uniform light field within the reactor, resulting in photodamage to cells near the light source while leaving cells deeper within the suspension light-limited.<sup>36–38</sup> In contrast, the *M. barkeri*/<sup>NCN</sup>CN<sub>x</sub> combination developed in this study formed a dense, thin biofilm immobilized at the bottom of the vessel. This biofilm architecture presents distinct advantages for light delivery compared to suspended systems, achieving a 111.9% increase in methanogenesis performance under equivalent illumination conditions ( $11.9 \mu\text{mol g}_{\text{cat}}^{-1} \text{ h}^{-1}$  vs.  $5.6 \mu\text{mol g}_{\text{cat}}^{-1} \text{ h}^{-1}$ ) (Fig. S12). The biofilm configuration minimizes light scattering and absorption losses within the bulk liquid. Modeling simulations indicated that within the 4 mm-deep suspension, light intensity was attenuated by 90% at the vertical midpoint (a depth of 2 mm). However, the biofilm was concentrated at the bottom, a location that placed it within a region of relatively high irradiance and thereby enhanced its overall light utilization efficiency (Fig. 2H). While dense



biofilms may inherently impose mass transport resistance, potentially causing apoptosis of inner cells due to substrate depletion, this process is integral to the biofilm's lifecycle. The dynamic balance between gradient-driven apoptosis and the recruitment of new cells is crucial for sustaining the biofilm's long-term stability. Moreover, for small molecules like  $\text{CO}_2$ , the diffusion barrier is limited. The observed linear methane production rate under continuous illumination can serve as direct evidence.

### Protection of EPSs against photodamage

Under continuous irradiation, the hybrid biofilm exhibited pronounced color changes: the initial white biofilm progressively turned blue with increasing light intensity (Fig. 3A). This color change originates from the intrinsic photophysical response of  $^{\text{NCN}}\text{CN}_x$ , rather than from the formation of organic radicals.<sup>3</sup> The cyano group and potassium ions within  $^{\text{NCN}}\text{CN}_x$  modify its electronic structure, altering the HOMO-LUMO distribution, narrowing the bandgap, and suppressing the radiative decay of photoelectrons. Consequently, this promotes the accumulation of long-lived photoelectrons within the  $^{\text{NCN}}\text{CN}_x$  conduction band, leading to the emergence of surface plasmon resonance (SPR).<sup>39,40</sup> This SPR results in broad optical absorption across the 500–800 nm spectral range, imparting the material's characteristic blue color observable macroscopically (Fig. S13). Notably, the color transition is reversible—upon termination of irradiation, the material gradually returns to its original white state through an electron release process, with

the fading synchronized with the dissipation of accumulated electrons (Fig. S14) (Video S2). The experimental results indicate that deeper blue shades correspond to higher stored electron densities, which are still capable of driving biomethane production under dark conditions. However, the efficiency of methane synthesis in the dark is limited: photoelectron accumulation over 12 hours results in only a 20% increase in methane production during the subsequent 12-hour dark period (Fig. 3B). Although this additional dark-phase methane production can mathematically enhance the calculated quantum yield, it remains an inefficient strategy for methane synthesis under natural day/night cycles. In contrast, retaining stable and highly efficient photo-biocatalytic activity at constant low light intensity represents a more viable and effective approach.

During the experiment, a significant color change was also observed in the bulk solution: as the illumination time and light intensity increased, the solution gradually turned transparent orange-yellow (Fig. 3A). This phenomenon may be attributed to the release of the intracellular F420 cofactor. Three-dimensional fluorescence excitation-emission matrix (3D-EEM) analysis of the liquid phase after six days of exposure at varying light intensities revealed an increase in the characteristic peak intensity with increasing light intensity (Fig. 3C). Notably, the signal region ( $E_x \approx 420$  nm and  $E_m \approx 480$  nm) closely matched the spectral signature of the oxidized form of F420, a 5-deazaflavin F420.<sup>41</sup> UV-vis absorption spectroscopy further showed that the supernatant exhibited strong

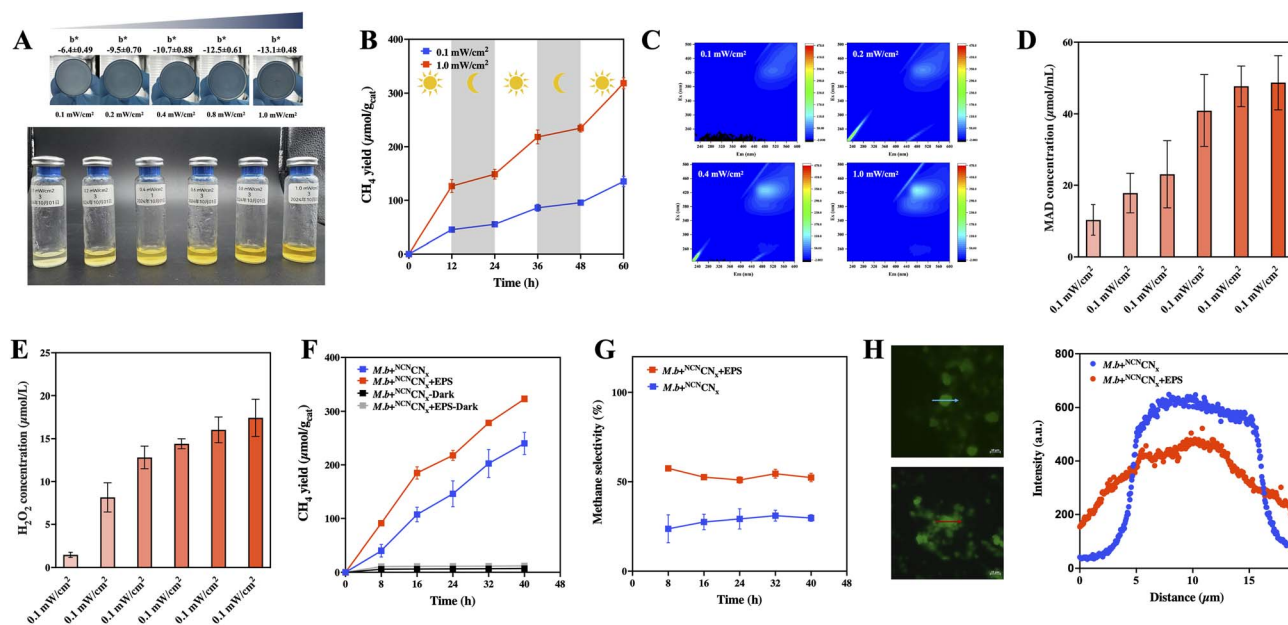


Fig. 3 Optical properties of hybrid biofilm systems under illumination, photodamage of biofilms, and the protective role of EPSs in hybrid biofilms. (A) Top: the biofilm at the bottom of the bottle turns blue under light (the inset numbers represent the mean CIE  $b^*$  values derived from digital colorimetric analysis, quantifying the intensification of the blue color). Bottom: the supernatant of the hybrid system turns yellow under light. (B) Methane production under light/dark cycles. (C) 3D-EEM analysis of the supernatant at varying light intensities. (D) MDA concentration at different light intensities. (E)  $\text{H}_2\text{O}_2$  concentration at different light intensities. (F) Methane production under EPS addition. (G) Methane selectivity under EPS addition. (H) Left: fluorescence microscopy image of DCFDA-labeled cells. Right: fluorescence intensity at the marked positions on the fluorescent image.



absorption in the ultraviolet range (300–320 nm) across all tested intensities, likely corresponding to the reduced form of F420, which does not exhibit notable fluorescence (Fig. S15).<sup>42,43</sup> The prominent F420 signal in the high-light intensity group may indicate extensive cell lysis. Malondialdehyde (MDA), a key byproduct of lipid peroxidation and a well-established biomarker of oxidative cellular damage,<sup>46</sup> was found to increase with increasing light intensity (from 10.2 to 48.6  $\mu\text{mol mL}^{-1}$ ) (Fig. 3D). Additionally, the concentration of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), a representative reactive oxygen species (ROS),<sup>44</sup> also showed a positive correlation with light intensity (ranging from 1.5 to 17.4  $\mu\text{mol mL}^{-1}$ ) (Fig. 3E). These findings collectively indicate that high-intensity UV-induced ROS are the primary drivers of cellular damage, causing membrane disruption and cell lysis and ultimately leading to the failure of the hybrid biofilm system.

The interaction between ROS and EPSs in biofilms was also investigated. When EPSs extracted from *M. barkeri* were co-incubated with  $\text{H}_2\text{O}_2$  for 6 hours, the concentrations of extracellular proteins and polysaccharides decreased by 27.5% and 60.9%, respectively (Fig. S16). These findings indicate that the EPS matrix of hybrid biofilms may act as a sacrificial shield, degrading upon assault by ROS to preserve cellular viability.<sup>45</sup> Furthermore, 3D-EEM analysis was conducted to monitor the compositional evolution of EPS extracts both in isolation and in the presence of  $^{\text{NCN}}\text{CN}_x$  under light irradiation (Fig. S17). The standalone EPS control exhibited no discernible changes in its fluorescence profile, suggesting that the incident light energy was insufficient to trigger direct photolysis. Conversely, the EPS- $^{\text{NCN}}\text{CN}_x$  mixture displayed a significant reduction in fluorescence intensity within the regions associated with fulvic acid-like and humic acid-like substances on days 3 and 6.<sup>46</sup> These findings underscore the critical role of photocatalytically generated ROS in mediating the oxidative degradation of EPS components. To evaluate the protective effects of EPSs under high light stress, hybrid biofilms supplemented with exogenous EPSs were subjected to high-intensity irradiation. Compared to control biofilms (lacking EPS supplementation), the EPS-supplemented systems exhibited a 34.6% increase in methane production (Fig. 3F) and a simultaneous 48.2% decrease in hydrogen byproduct production. Consequently, methane selectivity improved to 53.6%, substantially higher than the 28.2% recorded for the control group (Fig. 3G). Furthermore, assays using the DCFDA fluorescence probe indicated that intracellular ROS levels were 27.4% lower in cells within EPS-supplemented biofilms relative to the control (Fig. 3H).<sup>47</sup> These findings support the idea that EPSs provide cellular protection under light, likely by acting as a physical barrier and/or effectively scavenging ROS. The electrically conductive bio-interface formed by EPSs may also have contributed.<sup>48</sup> Although metal cocatalysts and heterojunctions can alleviate photocorrosion, applying these to semi-artificial systems is hindered by strict biocompatibility requirements and the difficulty of balancing interfacial charge transfer with ROS tolerance.<sup>16</sup> Intrinsic EPS components provide a more advantageous solution, effectively enabling the hybrid biofilm to harness the full solar spectrum.

### Silver nanoparticles facilitate transmembrane electron transfer

Low quantum yields and byproduct accumulation in semi-artificial photosynthetic systems are often attributed to a kinetic mismatch between the rate of photogenerated electron supply from the semiconductor and the electron uptake capacity of the microbial cells. To mitigate this limitation, we investigated  $\text{CO}_2$ -to- $\text{CH}_4$  conversion performance in hybrid biofilms featuring *M. barkeri* cells surface-modified with silver nanoparticles (Ag-NPs). Cell viability was shown to be unaffected by the deposition of Ag-NPs, as confirmed by control experiments (Fig. S18). Energy-dispersive X-ray spectroscopy (EDS) imaging verified the successful anchoring of Ag-NPs onto the cell surface (Fig. 4A). In the hybrid system, the Ag-NPs shielded by carbon nitride exhibited a weaker EDS signal. Positioned between the cells and the  $^{\text{NCN}}\text{CN}_x$ , these Ag-NPs could serve as pathways for electron transfer, thereby facilitating the electron transfer process (Fig. 4B). Under illumination at 0.1  $\text{mW cm}^{-2}$ , the Ag-NP-modified biofilm exhibited a 30% higher methane production rate and a 31% greater apparent quantum yield compared to unmodified controls (Fig. 4C and D). Concurrently,  $\text{H}_2$  byproduct formation was substantially suppressed, leading to an 8% increase in methane selectivity (Fig. 4E). Cyclic voltammetry (CV) analysis revealed an enhanced cathodic current response from the Ag-NP-modified system near the potential associated with  $\text{CO}_2$  reduction ( $-0.3 \text{ V vs. SCE}$ ) (Fig. S19). These electrochemical data, combined with conductivity measurements (Fig. S20), indicate that Ag-NPs facilitate extracellular electron transfer within the biofilm. A comparative analysis with existing methanogen-based hybrids suggests that achieving a high quantum yield and production yield at low light intensities is a prerequisite for the long-term, stable, and efficient operation of an ideal system (Fig. 4F). Most reported *Methanosarcina*-based hybrid systems rely on UV irradiation with intensities ranging from 0.8 to 5  $\text{mW cm}^{-2}$ , typically achieving apparent quantum yields between 0.34% and 1.26% (with one study utilizing AM 1.5G simulated sunlight). In contrast, this work employs a hybrid biofilm architecture to significantly enhance photon utilization efficiency and improve robustness against ROS. The performance of hybrid systems under illumination is typically monitored over a period of 5–20 days; however, the ultimate stability at varying light intensities remains an unexplored gap in the field (Table S4). The system exhibited a stable methane production rate of 141.9  $\mu\text{mol g}_{\text{cat}}^{-1} \text{d}^{-1}$ . Notably, this performance was achieved under continuous illumination at an unprecedentedly low intensity of 0.1  $\text{mW cm}^{-2}$ , resulting in a quantum yield of 1.92%. A significant gap in current research is the lack of data on the long-term stability of hybrid systems under continuous illumination. Addressing this knowledge gap is paramount for assessing their viability for practical implementation.

The proteomes of *M. barkeri* in the hybrid biofilms of *M. barkeri*- $^{\text{NCN}}\text{CN}_x$  and Ag@*M. barkeri*- $^{\text{NCN}}\text{CN}_x$  were further analyzed using untargeted proteomics. A total of 2901 proteins were identified. Principal component analysis (PCA) revealed two distinct clusters, indicating that Ag-NP modification altered



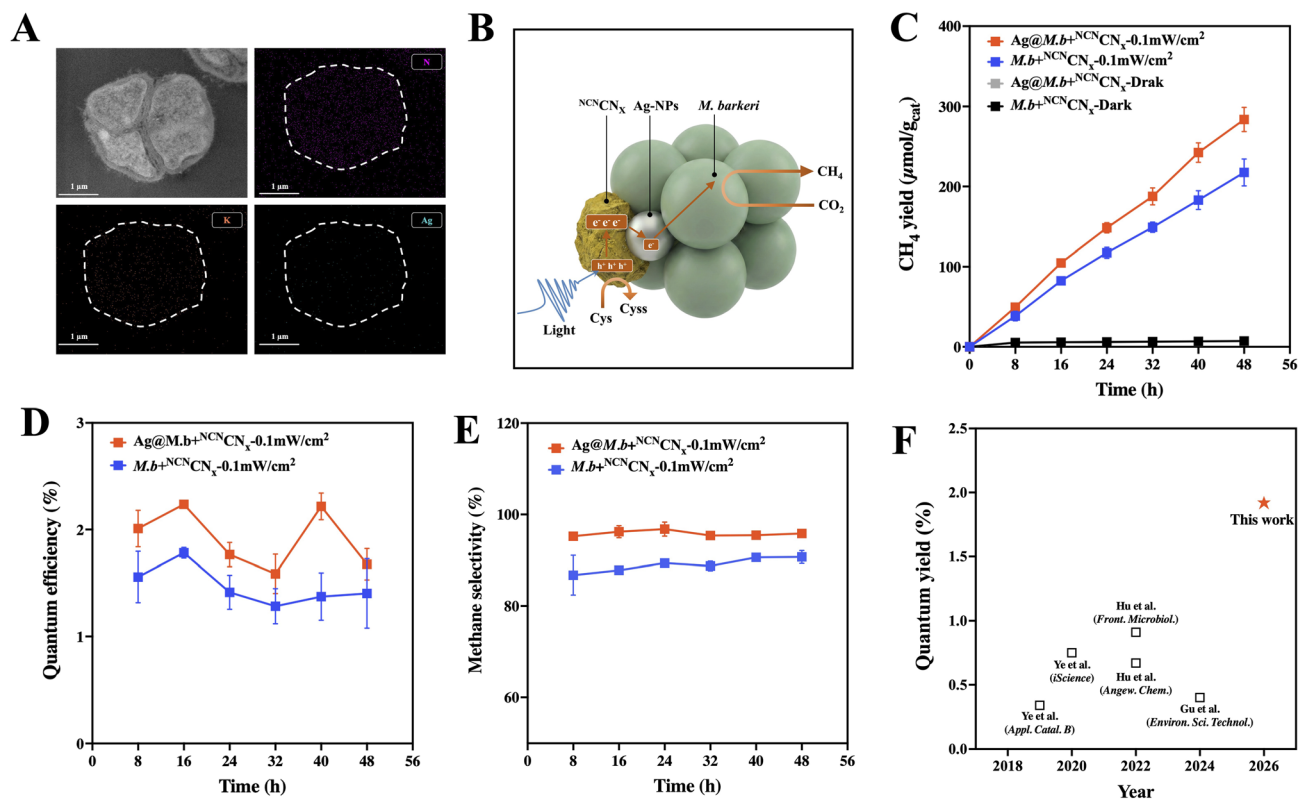


Fig. 4 Bioconversion characteristics of hybrid biofilms modified with Ag-NPs. (A) EDS mapping of *M. barkeri* cells modified with Ag-NPs. (B) Schematic illustration of electron transfer enhancement by Ag-NPs in the *M. barkeri*-<sup>NCN</sup>CN<sub>x</sub> hybrid system. (C) Methane production using hybrid biofilms modified with Ag-NPs. (D) Quantum yield of hybrid biofilms modified with Ag-NPs. (E) Methane selectivity of hybrid biofilms modified with Ag-NPs. (F) Quantum yields reported in other relevant literature in recent years (under continuous illumination).

the physiological state of *M. barkeri* in the hybrid biofilms under light irradiation (Fig. 5A). In the Ag@*M. barkeri*-<sup>NCN</sup>CN<sub>x</sub> system, 50 proteins were significantly upregulated (fold change >1.20 or  $p < 0.05$ ) and 63 proteins were significantly downregulated (fold change <0.80 or  $p < 0.05$ ) (Fig. 5B). Further functional analysis of differentially expressed proteins was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. In addition to categories related to energy production and conversion (C), amino acid transport and metabolism (E), and secondary metabolite biosynthesis, transport, and catabolism (Q), multiple proteins involved in other diverse biological functions were also found to be upregulated.

To gain deeper insight into the underlying mechanisms, proteins involved in electron transfer, CO<sub>2</sub> conversion, and energy metabolism in *M. barkeri* were analyzed (Fig. 5D). Specifically, the enzymatic steps involved in the conversion of CO<sub>2</sub> to CH<sub>4</sub> are catalyzed by distinct protein complexes. Untargeted proteomics data revealed that formylmethanofuran dehydrogenase (Fmd) and methenyltetrahydromethanopterin cyclohydrolase (Mch) were significantly upregulated in Ag@*M. barkeri*-<sup>NCN</sup>CN<sub>x</sub> hybrid biofilms compared to the control. These protein complexes catalyze the fixation of CO<sub>2</sub> onto methanofuran (MFR) and mediate the interconversion of formyl and methenyl oxidation states during C1 metabolism, playing essential roles in the early stages of methane formation.<sup>49</sup> The membrane-associated methyltransferase (Mtr), which catalyzes

the transfer of the methyl group from methyltetrahydromethanopterin (CH<sub>3</sub>-H<sub>4</sub>MPT) to coenzyme M (CoM-SH),<sup>50</sup> was also significantly upregulated. Moreover, CobN-like chelatase BtuS—an essential corrinoid cofactor donor in the Mtr complex—showed increased expression.<sup>51</sup> Additionally, the biosynthesis pathway of coenzyme B, which is indispensable for the final methanogenesis step catalyzed by methyl-coenzyme M reductase (Mcr), was significantly enhanced. In this step, Mcr catalyzes the transfer of a methyl group from coenzyme M (CoM-SH) and reduces it to methane, utilizing coenzyme B (CoB-SH) as an electron donor.<sup>52</sup> In contrast, the downregulation of MtaA (involved in methanol-dependent methanogenesis)<sup>53</sup> and MtmB (associated with dimethylamine-dependent methanogenesis)<sup>54</sup> suggests a metabolic shift in *M. barkeri*, where the CO<sub>2</sub> reduction pathway is prioritized over alternative substrate utilization pathways. Regarding energy metabolism, the upregulation of V-type ATP synthase subunits C and alpha, K<sup>+</sup>-insensitive pyrophosphate-energized proton pump, and putative manganese-dependent inorganic pyrophosphatase indicates an increased demand for energy (in the form of ATP or proton gradient),<sup>55</sup> which is consistent with the observed enhancement in methane production.

Notably, under conditions that enhance methane production, ferredoxin—a key low-potential electron carrier—was significantly downregulated.<sup>49</sup> This reduction may indicate that



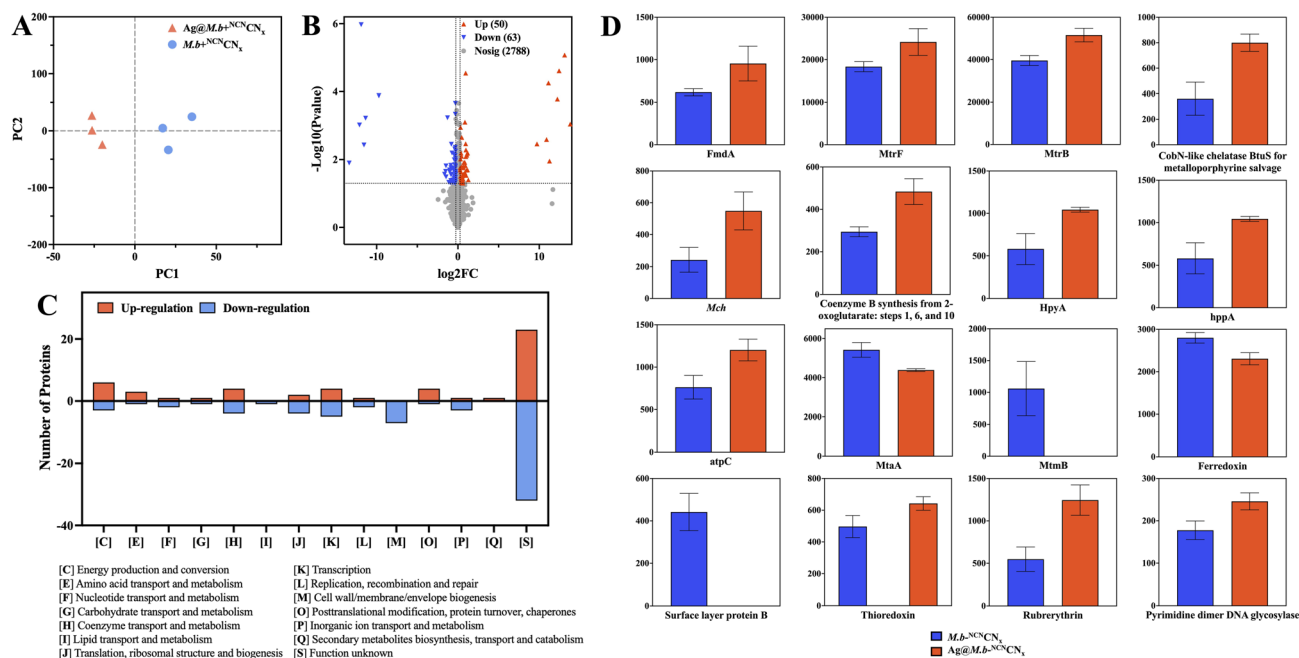


Fig. 5 Proteomic analysis of Ag@*M. barkeri*-<sup>NCN</sup> and *M. barkeri*-<sup>NCN</sup>. (A) PCA plot of Ag@*M. barkeri*-<sup>NCN</sup> and *M. barkeri*-<sup>NCN</sup>. (B) Volcano plot of Ag@*M. barkeri*-<sup>NCN</sup> and *M. barkeri*-<sup>NCN</sup>. (C) KEGG functional annotation for proteins with significant variations in Ag@*M. barkeri*-<sup>NCN</sup> compared with *M. barkeri*-<sup>NCN</sup>. (D) Relative concentration of proteins for electron transfer, energy conversion, CO<sub>2</sub> fixation, and oxidative stress response in Ag@*M. barkeri*-<sup>NCN</sup> compared with *M. barkeri*-<sup>NCN</sup>.

the Ag-NPs attached to the cell surface provided an alternative electron transfer pathway, compensating for the decreased availability of ferredoxin (Fig. 6). The hydrogenase maturation factor HypA, which plays a critical role in the final assembly of [NiFe] active sites in the membrane-associated Vht and Frh hydrogenases,<sup>56,57</sup> was remarkably upregulated. This may suggest an increased flux of Fd-independent electrons from Vht to methyl-coenzyme M reductase (Mcr), partially compensating for the reduced ferredoxin levels.<sup>49</sup> Meanwhile, the Vht and Hdr complexes in *M. barkeri* contain membrane-bound cytochrome *b*, which is capable of directly accepting photoelectrons from

the semiconductor.<sup>58,59</sup> The enhanced activity of these pathways indicates that the addition of Ag-NPs promotes cytochrome-mediated electron transfer, rather than relying on hydrogenase-mediated pathways such as those involving Ech. Additionally, a significant downregulation of surface layer protein B was observed in *M. barkeri* following Ag-NP modification, suggesting the integration of the nanoparticles into the cell membrane.<sup>60,61</sup> This incorporation may facilitate interactions with Vht and Hdr, leading to the formation of a novel electron transport pathway that enhances extracellular electron uptake. Moreover, the upregulation of rubrerythrin and thioredoxin (oxidative stress response)<sup>62,63</sup> and TldE (DNA repair-associated)<sup>64</sup> collectively indicates heightened oxidative stress in Ag@*M. barkeri*-<sup>NCN</sup> compared to the control.

## Conclusions

In summary, a semi-artificial photosynthetic biofilm system was constructed, which achieved the efficient conversion of CO<sub>2</sub> to methane under continuous illumination. The tailored carbon nitride materials not only retain the excellent biocompatibility of metal-free photocatalysts, but the incorporation of K<sup>+</sup> ions and cyano groups also modulates their surface potential. This modification facilitates the efficient self-assembly of the materials with cells and extracellular polymeric substances, thereby constructing a mechanically robust biofilm featuring a cross-linked network. This architecture not only optimizes light absorption efficiency to enable high-performance conversion but also establishes an EPS-rich matrix as a natural barrier, attenuating the oxidative damage elicited by reactive oxygen

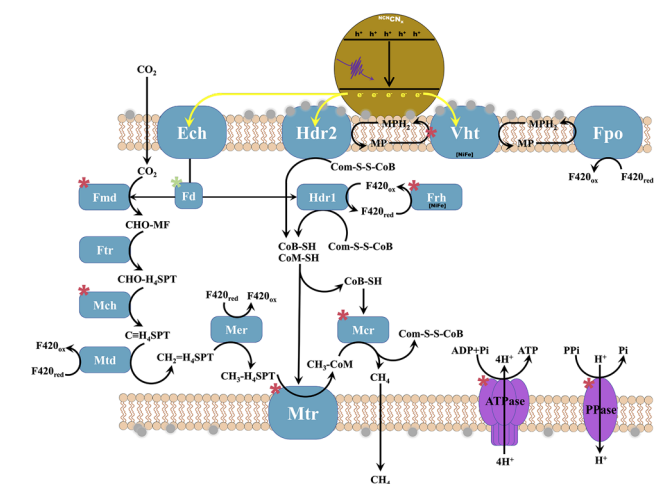


Fig. 6 Pathway of electron transfer and CO<sub>2</sub> reduction in Ag@*M. barkeri*-<sup>NCN</sup> (red\*: upregulation and green\*: downregulation).



species. Furthermore, single-cell-level silver nanostructure modification further augments the intracellular ferredoxin-independent pathway, enabling the system to achieve unprecedented performance breakthroughs in the realm of semi-artificial photosynthesis: the final apparent quantum yield and product selectivity reached 1.92% and 97.1%, respectively.

While semi-artificial photosynthesis has achieved functional verification for various chemicals, low light utilization and photo-instability remain key engineering bottlenecks. Transitioning from suspended to biofilm systems surmounts these challenges, enabling versatile reactor designs through biofilm conformability. Rather than mere biomass accumulation, biofilms possess emergent properties driven by their internal heterogeneity. Future research targeting this heterogeneity will enable precise modulation of solute transport and electron transfer networks. Exploiting these features to engineer multi-species consortia promises a qualitative leap in catalytic efficiency for complex artificial photosynthetic systems.

## Author contributions

H. C.: conceptualization, methodology, software, investigation, writing – original draft. A. X.: conceptualization, supervision, funding acquisition, writing – review & editing. Y. H.: software, validation, writing – review & editing. J. J.: methodology, writing – review & editing. J. Z.: methodology, visualization. X. Z.: software, visualization. X. Z.: resources, project administration. Q. L.: resources, supervision.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

Additional data are available from the corresponding author upon reasonable request.

Supplementary information (SI): the experimental methods included in this study are described in detail in the SI. References 3, 9–12, 16, 65–71 are cited in the SI. See DOI: <https://doi.org/10.1039/d6sc00459h>.

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