ACS ES&T Engineering

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Perspective

Photobiocatalytic Solar Fuel and Solar Chemical Conversion: Sufficient Activity and Better Selectivity

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Cite This: *ACS EST Engg.* 2022, 2, 989−1000

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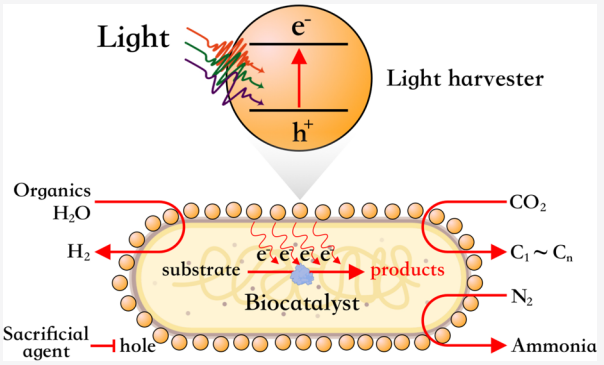
ABSTRACT: Converting renewable solar energy into fuel and value-added chemicals is a long-term objective of researchers and a promising solution for the energy crisis, environmental pollution, and global warming. Photosynthetic biohybrid systems (PBS) are receiving more and more attention, because they take advantage of both artificial semiconductor materials (high solar conversion efficiency) and living cells (high product selectivity) and, hence, enable the efficient capture and storage of solar energy in chemical bonds. In this perspective, we summarize findings on whole cell−semiconductor nanomaterial hybrid systems regarding solar driven H_2 evolution, CO_2 reduction, and N_2 fixation in the past years. First, hydrogen yield and duration of different H_2 evolution PBS are compared, and various assembly modes and electron transfer pathways are also introduced. Then, we evaluate the performance of CO_2 reduction PBS based on the type of multicarbon products, as well as the challenges encountered by researchers and corresponding tentative solutions. Finally, we focus on photobiocatalytic N_2 fixation, while introducing diverse N_2 fixing microorganisms. Overall, obvious achievements have been made in photobiocatalytic solar fuel and solar chemical conversion in recent years, as innovative PBSs are constructed, molecular mechanisms are explored, and tentative solutions are proposed for scale-up, but huge challenges still exist. In the future, we should focus on revealing the interfacial electron transfer mechanisms and cellular energy allocation in order to significantly promote the solar-to-chemical conversion efficiency and meet the practical requirements.



KEYWORDS: *photosynthetic biohybrid system, hydrogen evolution, CO_2 reduction, N_2 fixation*

1. INTRODUCTION

Solar energy is the most abundant clean energy source, which could meet our energy needs and reduce our dependence on fossil fuels, meanwhile solving problems of environmental pollution and global warming.[1] In order to harvest and store solar power, artificial photo(electro)catalytic materials and devices (e.g., semiconductor nanoparticles, photovoltaic cells, and photoelectrodes) have been developed for water splitting, CO_2 fixation, and N_2 fixation.[2−4] They have broadband light adsorption and diverse physical and chemical properties, which can achieve high (up to 20%) solar energy conversion efficiency,[5,6] but require complicated catalyst synthesis and costly metals, showing low product selectivity and stability. In contrast, nature photosynthetic organisms (plants and microbes) absorb solar energy to convert naturally abundant H_2O, CO_2, and N_2 into diverse valuable chemicals via specific enzymes and sophisticated metabolic pathways. Long-term evolution endows them with high product specificity and diversity (i.e., selectivity) and the capability of self-replication and self-repair, but they suffer from low solar-to-biomass conversion efficiency (<3%).[7]

The recently emerged photosynthetic biohybrid systems (PBSs, also called semiartificial photosynthetic systems) combine the advantages of both artificial and natural photosynthesis, achieving both high solar conversion efficiency and high product selectivity.[7,8] In PBSs, semiconductor materials harvest light energy and generate electrons or reducing equivalents, which could be received by biocatalysts (whole cells or isolated enzymes) to perform reductive reactions, synthesizing high value products such as hydrogen, acetic acid and ammonia, from organic substrates (or H_2O), CO_2, and N_2 (Figure 1). The first report on whole cell based PBS could date back to 1987. Krasnovsky and Nikandrov successfully combined *Clostridium butyricum* bacterial cells and TiO_2 for photobiocatalytic H_2 evolution[9] (Figure 2). However, little attention was paid to the study at that time. In 2016,

Special Issue: Photo-Energy Utilization for a Sustainable Environment: Photo(electro)catalysis

Received: November 16, 2021
Revised: February 8, 2022
Accepted: February 9, 2022
Published: February 21, 2022

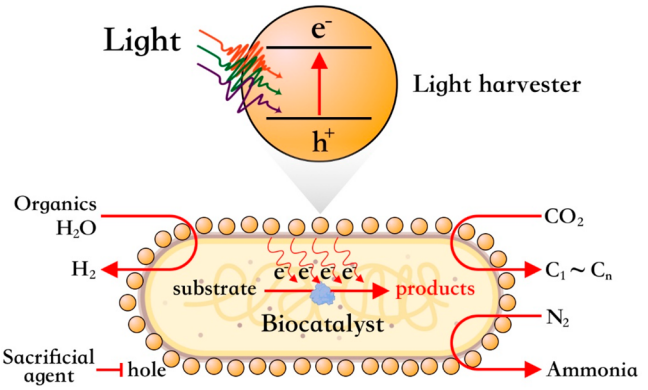


Figure 1. Schematic diagram of the whole cell photosynthetic biohybrid system for solar driven H_2 evolution, CO_2 reduction, and N_2 fixation.

Sakimoto et al. reported self-photosensitized *Moorella thermoacetica* to produce acetic acid from CO_2 using biologically precipitated CdS nanoparticles as light harvesters.[10] This study represents the beginning of a novel research field and incites following intensive explorations of PBS. Also in 2016, Honda et al. coupled *Escherichia coli* with TiO_2 for solar driven H_2 evolution.[11] Since then, *E. coli* is frequently employed for PBS construction and H_2 evolution, with diverse assembly strategies and improved performance. Both photoelectrode and nanomaterial could function as light harvesters. Compared to conventional photoelectrodes, semiconductor nanomaterials (e.g., nanoparticles and quantum dots) possess high specific surface area which increases the interface between particles and cells, thus benefiting electron transport.[12] In addition, they can be easily paired with the size of cells and show improved biocompatibility. Moreover, nanomaterial-based PBS are more cost-effective and simple to operate, as only a single bioreactor is required.

Both inorganic and organic materials can serve as light harvesters.[13] Inorganic nanoparticles have good optical and electronic properties, such as cadmium sulfide (CdS),[10] gold nanoclusters (AuNCs),[14] and indium phosphide (InP),[15] and thus usually function as light harvesters in semiartificial photosynthetic systems. They are also highly tunable. For example, the Au nanoclusters decorated with ligand glutathione show strong bioaffinity.[14] With a zinc sulfide (ZnS) shell, quantum dots are able to bind specifically to target enzymes.[16] Besides, some organic molecules could also play the role of photosensitizer,[13] such as eosin Y, graphitic carbon nitride (g-C_3N_4), and perylene diimide derivative (PDI)/poly(fluorene-*co*-phenylene) (PFP), which show excellent light harvesting ability and are environmentally friendly. The criteria for choosing photosensitizer nanomaterials have been summarized by Cestellos-Blanco et al.,[17] including tuning the bandgap (<3 eV) of nanomaterials to absorb visible light, matching the nanomaterial conduction potentials to potentials needed for biocatalysis, and selecting dimensions and surface charges of nanomaterials, as well as nanomaterial location and biocompatibility. Particularly, the biocompatibility of photosensitizers is very important and can be measured using colony-forming unit assays,[18] respiratory activity (2,3,5-triphenyltetrazolium chloride dehydrogenase activity), and cell membrane integrity (intracellular K^+ leakage).[19] The multiomic approaches were also used to explore the impact of nanomaterials to cells.[20] For example, CdS induced the upregulation of genes associated with oxidative stress, heavy metal resistance, and DNA repair in *Moorella thermoacetica* cells.

Besides light-harvesters, the other key component of PBSs is the biocatalyst. To produce diverse value-added chemicals, various of biocatalysts have been introduced in semiartificial photosynthetic systems. Generally, there are two types of biocatalysts, isolated enzymes, and whole cells. Enzymes have high catalytic activity and specificity and often exhibit inherent electrochemical activities which enable direct electron transfer from semiconductors.[21] In practice, enzymes, such as hydrogenases, carbon monoxide dehydrogenase, and nitrogenase,

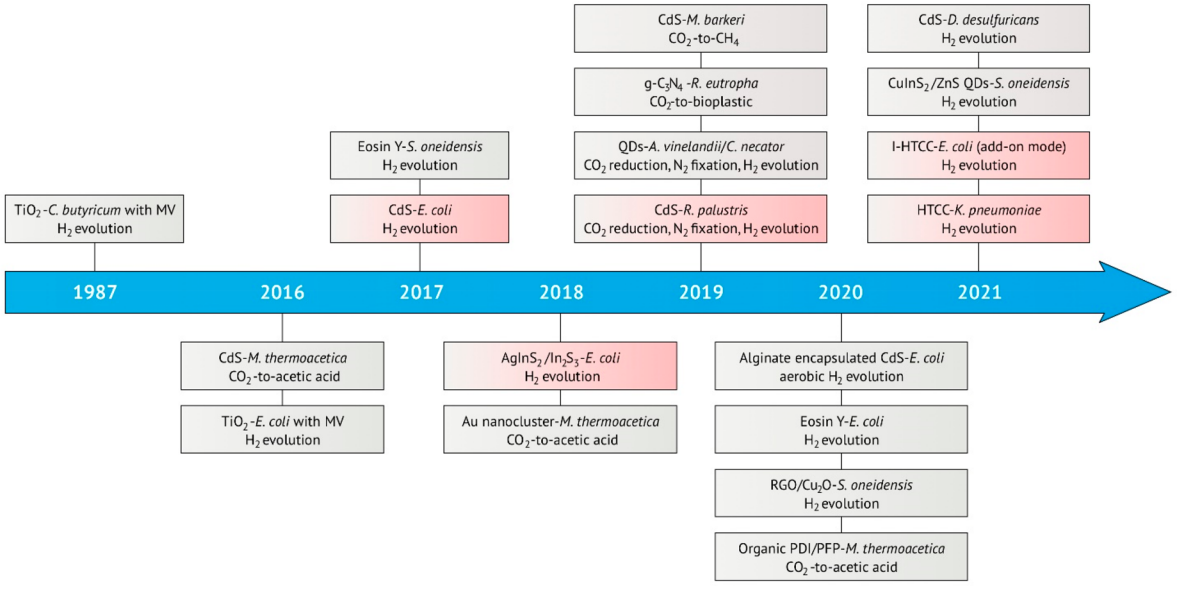


Figure 2. Brief development history of whole cell photosynthetic biohybrid systems. The works of our research group are marked red: MV, methyl viologen; RGO, reduced graphene oxide; PDI/PFP, perylene diimide derivative/poly(fluorene-*co*-phenylene); QD, quantum dot; I-HTCC, iodine-doped hydrothermally carbonized carbon.

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000

have been employed for solar fuel and solar chemical conversion.[22,23] However, isolation and purification of enzymes are usually expensive, and it is difficult for enzymes to remain active *in vitro*, which increases the cost of enzyme-based PBSs and limits their application. As for whole cell biocatalysts, electroactive bacteria (e.g., *Moorella thermoacetica* and *Sporomusa ovata*) are capable of harvesting electrons by direct contact with electrodes or nanomaterials to support intracellular metabolism,[24] so they frequently serve as biocatalysts in semiartificial photosynthetic systems. Another group of whole cell biocatalysts are workhorse microbes (*Escherichia coli* and *Saccharomyces cerevisiae*), which could be easily subject to genetic engineering and generate diverse organic compounds.[15,25] In order to realize electron transfer from light harvesters to biocatalysts, many innovative assembly strategies have been developed, such as surface precipitation,[10] modular assembly,[15] and cell uptake.[14] The main electron transfer mechanisms include extracellular direct electron transfer, intracellular direct electron transfer, indirect electron transfer mediated by redox mediators,[26] such as methyl viologen, neutral red, and H_2. The long distance and multistep transport in last approach often causes loss of electrons, showing low electron transfer efficiency. For extracellular direct electron transfer, electron transfer directly from semiconductor materials to the surface of attached cells and then membrane-bound proteins of cells like bacteriorhodopsin, hydrogenases, quinols, and cytochrome c can mediate the interface electron transfer.[15] Ultrasmall gold nanoclusters and quantum dots can be absorbed by cells, thus achieving intracellular direct electron transfer. The approach is believed to be more effective than extracellular electron transfer, because electrons generated from nanomaterials can transfer to target enzymes or cytoplasmic mediators, without crossing the cell membrane.

Overall, various types of photobiocatalytic systems have been developed for solar fuel and solar chemical conversion in the past years. Given that the enzyme-based PBSs often show low stability and high cost, mainly whole cell-based PBSs are discussed in this perspective. As for the biohybrids driven by photovoltaic cells,[27] or photoelectrode-cell hybrid systems,[28,29] they have been extensively studied by researchers for decades and reviewed in recent papers regarding microbial electrosynthesis or microbial photoelectrosynthesis.[30,31] In this perspective, we mainly focus on publications of whole cell−nanomaterial hybrids which have emerged in recent years. Progress in the fields of photobiocatalytic hydrogen evolution, CO_2 reduction, and N_2 fixation is introduced. For each section, PBSs integrating different biocatalysts and photosensitizers are compared and innovative strategies to improve the performance of PBSs are highlighted. Eventually, we summarize problems often encountered by researchers and corresponding tentative solutions, as well as suggest future challenges including interfacial electron transfer mechanisms and cellular energy allocation.

2. PHOTOBIOCATALYTIC HYDROGEN EVOLUTION

As a clean and renewable energy source, hydrogen shows great potential to meet the increasing energy requirements of modern society instead of fossil fuel. More than 85% of the current hydrogen is produced from fossil fuels.[32] Hydrogen production by photo(electro)catalytic water splitting is investigated intensively in recent years, because it is sustainable and no harmful byproducts are generated in the process. A solar-to-hydrogen energy conversion efficiency of 30% has been achieved in a photovoltaic−electrolysis system,[2] which is close to the theoretical maximum efficiency (31.1%).[33] Nevertheless, solar to hydrogen conversion via photo(electro)-catalysis could be improved further, such as reducing the cost of the expensive photovoltaic cells and synthesis of semiconductors. Biological hydrogen production using microorganisms has been attracting increasing interest in recent years due to its mild reaction conditions and environmental friendliness. Because cheap organics, especially waste substrates, could be used as a feedstock for self-replication and sustenance of cells, the biological hydrogen production also shows potential to be profitable.[34] According to the type of microorganisms and substrates (water or organics), there are four biohydrogen production routes, including direct photolysis (green algae), indirect photolysis (cyanobacteria), photofermentation (purple nonsulfur bacteria), and dark fermentation (other anaerobic bacteria).[35] These hydrogen production microorganisms show no or poor solar energy utilization.

2.1. Performance of H_2 Evolution Biohybrids. The hydrogen production efficiency of microorganisms is significantly improved by combination with light-harvesting nanomaterials, which provide extra reducing power for cells. By now, various H_2 evolution PBSs have been constructed, in which microbes with hydrogen production routes of dark fermentation and photofermentation are involved while direct photolysis and indirect photolysis are almost unreported. For instance, the most studied bacterium *E. coli* has been applied as a biocatalyst in a biohybrid system, in which *E. coli* produce hydrogen from glucose via a dark fermentation pathway. Under illumination, photogenerated electrons are delivered from semiconductors into cells by membrane-bounded proteins or $NADH/NAD^+$, then participate in the glycolysis and increase the accumulation of pyruvate, and ultimately the production of H_2 is enhanced via the formate and NADH pathways (Figure 3).[36] As a model organism, *E. coli* could be easily genetically engineered. For example, it was engineered to express heavy metal-binding proteins PbrR, which could precipitate CdS nanoparticles on cell surface (Figure 3).[37] In other research, exogenous hydrogenase was introduced in to *E. coli* to enhance its H_2 production.[11] Our research group constructed a CdS−*E. coli* hybrid[36] and a $AgInS_2/In_2S_3$−*E. coli* hybrid[38] via cysteine desulfurase mediated surface precipitation for the first time in *E. coli*. Due to the differences in photosensitizer, substrate, and cell concentration, it is not easy to compare the absolute H_2 yield of different hybrids, so the relative H_2 production to bare cells is compared here. In regards to *E. coli* hybrids, it seems that the surface display hybrid[37] and eosin Y−*E. coli* hybrid[39] show large enhanced H_2 production, around or more than 10-fold (Table 1). In comparison, the ratio of H_2 yield of hybrids to bare cells is 1.3- to 2.8-fold that in other experiments.

Other microbes appear in biohybrid H_2 producing systems including *Shewanella oneidensis* and *Klebsiella pneumoniae*. The addition of both Cu_2O nanosheets[46] and $CuInS_2/ZnS$ quantum dots[18] largely increased the H_2 yield of *S. oneidensis*, but the H_2 production of the quantum dot based PBS lasted longer (45 h), owing to the better biocompatibility of $CuInS_2/ZnS$ quantum dots than Cu_2O nanosheets. Especially, screened *K. pneumoniae* were employed by our research group for PBS fabrication because they could utilize crude glycerol as precursors for H_2 production.[19] This is the first time to introduce hydrothermally carbonized carbon (HTCC) into PBS. The carbon based semiconductor material is obtained

https://doi.org/10.1021/acsestengg.1c00429
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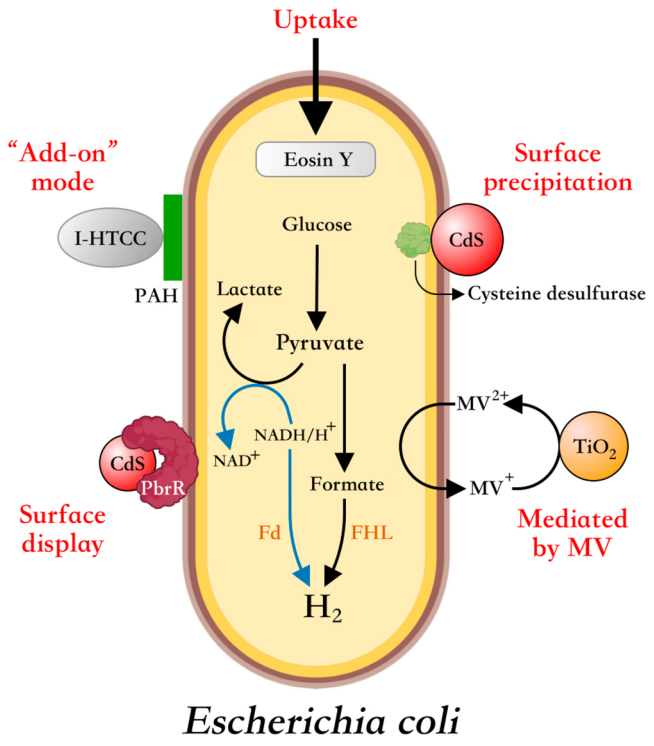

Figure 3. Schematic diagram of assembly strategies in *Escherichia coli*−photosensitizer hybrids, including surface precipitation, mediated by MV, surface display, "add-on" mode and uptake. PAH, poly(allylamine hydrochloride); FHL, formate hydrogen lyase; Fd, ferredoxin; I-HTCC, iodine-doped hydrothermally carbonized carbon; MV, methyl viologen.

easily through simple hydrothermal treatment of cheap carbohydrates, and also has a narrow bandgap and good biocompatibility. The system is both environmentally friendly and cost-effective, albeit only 35.3% enhancement in H_2 production after photosensitization is achieved.

Besides, a kind of photosynthetic bacteria are introduced into PBS by our research group, purple nonsulfur bacteria *Rhodopseudomonas palustris*, which produces H_2 with nitrogenase. Under 120 h of visible light irradiation, the H_2 yield of cells increased by around 100% and the photosynthetic efficiency increased by 186% after being coated with CdS nanoparticles,[43] which is superior to *R. palustris* coupled with conjugated polymers (oligofluorene and polythiophene) in duration and enhancement of H_2 yield.[44] Nevertheless, both of the two hybrid systems demonstrate the feasibility of semiconductor materials in enhancing the biological photofermentation process. Despite its complex metabolic pathways, the electron transduction mechanisms were investigated by our research group. We proposed that electrons generated from CdS took part in the photosynthetic electron transport chain in *R. palustris*, then transferred to ferredoxin and ferredoxin-$NADP^+$ oxidoreductase, and finally were received by nitrogenase and $NADP^+$. Increased reducing power in cells leads to improvement of the nitrogen fixation and release of more H_2. However, research on H_2 producing PBSs using green algae or cyanobacteria have not been reported yet, probably due to the low production efficiency. In 2020, it was reported that gold nanoparticles enhanced the photosynthesis of carotenoid in microalgae *Chlorella zofingiensis* by improving the electron transport in photosystem II.[47] The study indicated that H_2 production of microalgae could be promoted by a combination with semiconductor materials too.

2.2. Electron Transfer and Assembly Strategy. Photoelectron transfer from semiconductors to enzymes in cells is a critical step that affects H_2 production efficiency. In 2016, a biohybrid integrating *E. coli* and TiO_2 was constructed to achieve solar driven H_2 production, in which methyl viologen (MV) served as organic electron mediator (Figure 3).[11] However, MV has toxicity to cells, and this extracellular indirect electron transfer approach also suffers from poor conductivity and high photoelectrons loss (0.3−0.6% quantum yield).[11,45] Thereafter, multiple electron transfer strategies were designed by researchers to improve electron transduction and eliminate the requirement for electron mediators. In a study, reduced graphene oxide (RGO) was adopted to integrate *S. oneidensis* cells and Cu_2O.[46] As a result, H_2

Table 1. Summary of the Performances of Hydrogen Evolution Photobiocatalytic Systems, Including Relative H_2 Yield (to Bare Cells) and Duration

species	photosensitizer	efficiency/yield[a]	relative H_2 yield to bare cells	duration
Azotobacter vinelandii	CdS-ZnS quantum dots	AQE[a] 13%	NA	2 h[16]
Desulfovibrio desulfuricans	CdS, methyl viologen	AQE 23%	NA	50 h[40]
Desulfovibrio desulfuricans	CdS	AQE 4%	NA	10 d[40]
Escherichia coli	TiO_2	AQE 0.31%	NA	15 h[11]
Escherichia coli	CdS	AQE 7.93%	1.3 fold	3 h[36]
Escherichia coli	$AgInS_2/In_2S_3$	AQE 3.3%	1.3 fold	3 h[38]
Escherichia coli	CdS	yield 81 μmol/10^8 cells	10 fold	24 h[37]
Escherichia coli	TiO_2	yield 3.6 mmol/mmol glucose	2.8 fold	15 h[41]
Escherichia coli	Eosin Y	AQE > 10%	>10 fold	24 h[39]
Escherichia coli	iodine-doped hydrothermally carbonized carbon	AQE 9.11%	1.57 fold	3 h[42]
Klebsiella pneumoniae	hydrothermal carbonation carbon	yield 1020 μmol	1.35 fold	3 h[19]
Rhodopseudomonas palustris	CdS nanoparticles	photosynthetic efficiency 6.73%	2 fold	120 h[43]
Rhodopseudomonas palustris	oligofluorene, polythiophene	yield 1250 nmol	~1.67 fold	2 h[44]
Shewanella oneidensis	Eosin Y	AQE 0.6%	>10 fold	24 h[45]
Shewanella oneidensis	Cu_2O, reduced graphene oxide	yield 322 μmol/g Cu_2O	>10 fold	4 h[46]
Shewanella oneidensis	$CuInS_2$/ZnS quantum dots	AQE 15.02%	10 fold	45 h[18]

[a]The apparent quantum efficiency (AQE) of light driven H_2 production system is the number of additional evolved H_2 molecules multiplied by 2 and divided by the number of incident photons.

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000

production was enhanced by 11−38-fold compared to a biohybrid system without RGO or with an organic electron mediator, as RGO can effectively collect and transfer electrons which is superior to soluble electron mediators. Specifically, RGO transfers electrons to membrane-bound redox proteins of cells and, then, to hydrogenase located in periplasmic space. Light harvesting nanoparticles could also be added on cells via electrostatic interactions, namely "add-on" mode (Figure 3).[42] Cell membranes are usually negatively charged. In the research conducted by our group, the cell surface of *E. coli* was modified by positively charged poly(allylamine hydrochloride), enabling adhesion of negatively charged iodine-doped hydrothermally carbonized carbon (I-HTCC).[42] Under visible light irradiation, electrons generated from semiconductors were transferred to the cell membrane directly, contributing to the enhanced hydrogen production. Notably, the "add-on" mode could be versatilely applied to easy assembly of various cells and negatively charged materials (e.g., mesoporous carbon, acid-treated pollen and graphene flakes) based on practical requirements.

Moreover, a periplasmic photosensitized biohybrid system, integrating $CuInS_2$/ZnS quantum dots and *S. oneidensis*, was introduced by Luo et al.[18] Because *S. oneidensis* expresses periplasmic hydrogenases, and there is chemical binding affinity between Fe−S clusters in hydrogenase and zinc in quantum dots (QDs), $CuInS_2$/ZnS QDs were anchored into the periplasm of cells successfully. The hydrogen production of *S. oneidensis* increased more than 10-fold and lasted for 45 h, which was ascribed to direct electron transfer between QDs and hydrogenase. There is another example of intracellular photosensitization. A biohybrid system forms by simple addition of QDs to culture medium, due to the affinity binding of zinc-rich QDs to his-tagged molybdenum−iron nitrogenase in *Azotobacter vinelandii*, resulting in enhanced H_2 production and high quantum yields (13%).[16] This is explained by the direct contact between semiconducting materials and enzymes, which avoids transmembrane electron loss.

The physical and chemical methods that prepare nanomaterials typically require harsh conditions (e.g., high temperatures and high purity reagents) and often involve toxic metals. In contrast, not only does the *in situ* biosynthesis of nanoparticles reduce the cost of semiconductor synthesis, but also their inherent high biocompatibility helps to improve the sustainability and stability of the solar driven hydrogen production system. Also, the close connection between these nanoparticles and cells benefits electron transduction. Another potential strength of the biosynthetic strategy is that nanoparticles can be regenerated during cell proliferation, which may solve the problem of nanoparticle loss resulting from photodegradation and decrease of loading ratio after cell proliferation.[10] In this way, the sustainability of the PBS is enhanced greatly. It is reported that *R. palustris* and *E. coli* could precipitate Cd^{2+} via cysteine desulfurase and thus synthesize CdS nanoparticles spontaneously,[36,43] which is a mechanism to protect cells from toxic metal ions. These CdS nanoparticles significantly improved the H_2 production of microbes under illumination. Particularly, after self-photosensitization with CdS, *Desulfovibrio desulfuricans* continually produces H_2 for 10 d,[40] which seems to be the most sustainable solar driven H_2 evolution PBS reported. The high biocompatibility of biosynthesized nanoparticles likely contributes to their high durability, but a detailed explanation of this is not provided by the authors.

Except for CdS nanoparticles, various semiconducting nanoparticles synthesized by microorganisms, such as In_2S_3,[38] ZnS, SnO_2, and TiO_2,[48] also show potential to be employed in solar driven H_2 production. Other inorganic nanomaterial synthesis mechanisms involve redox enzymes and components in electron transport pathways, such as nitrate and nitrite reductase, c-type cytochrome, and metallothionein. More importantly, for microorganisms that cannot synthesize nanoparticles intrinsically, they could be genetically engineered to acquire the metal ion binding and reduction ability, which widen the application of this low-cost, environmentally friendly biosynthesis technique. For instance, the surface-display technique enables *E. coli* to synthesize CdS nanoparticles, by expressing exogenous heavy metal-binding proteins PbrR, which could precipitate CdS nanoparticles on the cell surface (Figure 3).[37]

2.3. Other Considerations to Improve PBSs. Hydrogenase and/or nitrogenase are the key enzymes in biological hydrogen production process, but they are inhibited by oxygen, limiting the practical application of solar driven H_2 producing hybrid systems. To address the issue, a biomimetic silica encapsulation strategy is employed in a *E. coli*−CdS hybrid system to enable the continuously H_2 production (96 h) under aerobic conditions.[37] Cells in the outer layer of the aggregate consume oxygen through aerobic respiration, thus creating an anaerobic microenvironment for cells in the core. In another study, solar driven H_2 production under aerobic condition was achieved via sodium alginate encapsulation strategy.[41] Encapsulated hybrid cell beads were constructed by mixing TiO_2−*E. coli* hybrids and sodium alginate. The alginate gel in the outer layer could prevent the diffusion of O_2 into the beads. Bead size is delicately controlled to avoid decreasing the penetration of light. The encapsulated hybrid system produced more hydrogen than that without encapsulation, although the H_2 production is still lower than that under anaerobic conditions. In summary, the feasibility of sustainable H_2 evolution under natural conditions has been confirmed experimentally, and the technology shows great promise.

Usually, various organics are adopted as substrates, providing energy source and/or protons for H_2 evolution. Therefore, the cost of substrates is an important factor to evaluate practical application of the biohybrid system. In this context, cost-effective substrates are selected for solar driven H_2 production, such as wastewater and crude glycerol. For instance, wastewater was utilized for solar-driven hydrogen production, realizing both hydrogen production and wastewater treatment at low cost.[36] In another case, a hybrid system comprising HTCC and *K. pneumoniae* was established for solar driven H_2 production from crude glycerol,[19] which is a kind of industrial byproduct. Purification of crude glycerol is expensive and energy-intensive, so the hybrid system is capable of both bioremediation and efficient clean energy production. In the future, other microbial species that are capable of metabolizing cheap organic wastes could be introduced into inorganic−microbe hybrid systems to achieve cost-effective solar-driven hydrogen production. In addition, more excellent semiconducting materials (e.g., Al-doped $SrTiO_3$ particulate,[49] $SrTiO_3$:Rh particle[50,51]) could also be applied to PBS in order to improve its performance, as the apparent quantum efficiency of existing PBSs for H_2 evolution is still lower than photo(electro)catalysis (30%).[52]

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ACS EST Engg. 2022, 2, 989−1000

3. PHOTOBIOCATALYTIC CO_2 REDUCTION AND CONVERSION

To realize carbon neutrality, measures have been taken to decrease the emissions of greenhouse gas CO_2 and promote carbon recycling. Converting CO_2 into value-added chemicals using renewable solar energy as a driving force has appeared as a promising technology to solve climate issues and store solar energy. Moreover, conversion of solar energy to organic chemicals possesses the advantages of easy storage and transportation, compared to solar to hydrogen conversion. Up to now, photocatalysis, electrocatalysis, and biocatalysis are three main approaches to achieve CO_2 reduction. The photo(electro)catalysis approach uses synthetic semiconducting materials to harvest light energy and chemical catalysts for CO_2 reduction, in which significant breakthroughs have been made in the past decades.[3] Specially, Cu based materials are of great promise because of their exclusive C−C coupling ability. The selectivity to C_2 is improved further by strategies such as surface modification of semiconductors, improving charge separation efficiency, and stabilizing reaction intermediates.[53] For instance, it is reported that the faradaic efficiency of CO_2-to-ethanol reached 41% by modifying a copper surface with porphyrin-based metallic complexes.[54] Photoelectrochemical CO_2 conversion to acetic acid could achieve carbon selectivity of 91.5% using Zn-doped Cu_2O as a catalyst.[55] Nevertheless, these technologies usually require complicated catalyst synthesis, their products are scarcely C_{2+} compounds, and the mechanism of reaction processes remains unclear.[3,53,56] The low product selectivity as well as low stability of catalysts impede the large-scale production of multicarbons with higher energy densities and wider industrial application.

In contrast, the advantages of natural biocatalytic CO_2 fixation systems in plants and microbes are mild reaction conditions and high production selectivity (specificity and diversity),[57] but their light absorption and energy conversion efficiency is poor. Both photosynthetic and nonphotosynthetic microorganisms are able to assimilate CO_2. For photosynthetic CO_2 fixing microorganisms, cyanobacteria and microalgae produce O_2 while purple nonsulfur bacteria do not.[57,58] Nonphotosynthetic microorganisms can perform CO_2 fixing both aerobically and anaerobically. Overall, six biological CO_2 fixation pathways have been proposed including the Calvin−Benson−Bassham cycle, the reductive tricarboxylic acid (rTCA) cycle, the Wood−Ljungdahl pathway, the 3-hydroxypropionate bicycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, and the dicarboxylate/4-hydroxybutyrate cycle.[57] Among them, the Wood−Ljungdahl pathway in nonphotosynthesis is the most energy-saving CO_2 fixation pathway.[57]

In order to achieve efficient CO_2 reduction using renewable solar energy, semiconducting materials are integrated with living cells. For PBSs, CO_2 could cross the cell membrane and participate into metabolism via diffusion and does not require energy consumption or carriers, thus the dissolution of CO_2 is usually not a constraint for PBSs, which may be an advantage of PBSs. In contrast, poor CO_2 solubility is a key factor limiting photocatalyzed CO_2 conversion.[59,60] Also, due to the specificity of cells, the competition with the hydrogen evolution reaction, which is a major consideration in photoelectrosynthesis,[59] exerts a minor affect on PBSs. Up to now, semiartificial photosynthetic systems have been employed successfully for the production of various compounds from CO_2, such as acetic acid, methane, formic acid, butyrate, and poly-β-hydroxybutyrate (PHB), depending on the type of biocatalysts (Figure 4, Table 2). Correspondingly, different CO_2 reduction pathways are involved, including the Wood−Ljungdahl pathway, Calvin cycle, and half-Wood−Ljungdahl−formolase pathway.

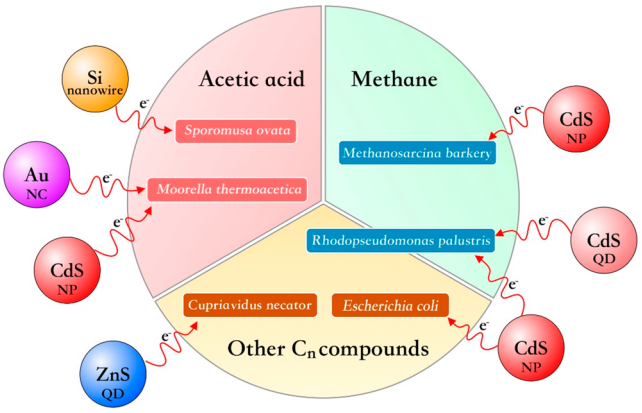

Figure 4. Representative microorganisms involved in photobiocatalytic CO_2 reduction and conversion: PDI, perylene diimide derivative; PFP, poly(fluorene-*co*-phenylene); NP, nanoparticle; NC, nanocluster; QD, quantum dot.

3.1. Acetic Acid. The most reported multicarbon compound derived from PBS is acetic acid (Table 2), whereas acetogens are utilized as biocatalysts. For example, acetogen *M. thermoacetica* are able to synthesize acetic acid from CO_2 through the Wood−Ljungdahl pathway. Inorganic semiconductor CdS nanoparticles,[10] gold nanoclusters,[14] and organic semiconductor PDI/PFP[61] are able to provide additional reducing equivalent to *M. thermoacetica* for enhancing acetic acid production. Of them, the gold nanoparticle based PBS shows the highest quantum yield and long-term duration, due to the good biocompatibility and low toxicity of the Au nanocluster.[14] In addition, during photoactivation of nanoparticles, reactive oxygen species (ROS) are created, which are toxic to cells. Certain conformations of Au nanoclusters are reported to be able to quench reactive oxygen species, which makes Au nanoclusters superior to CdS. Another innovative way to protect cells from oxidative stress is metal organic framework (MOF) wrapping. Cestellos-Blanco and colleagues reported that the MOF wrapped *M. thermoacetica* PBS produced 3-fold more acetate than its bare counterpart,[62] because the zirconium cluster in the MOF can decompose ROS.

The other microbial species employed for solar driven CO_2-acetate conversion is *S. ovata*,[63,64] which also fixes CO_2 via the Wood−Ljungdahl pathway. The high surface area of the Si nanowire increased its interface with cells. Although *S. ovata* is strictly anaerobic, the PBS produced acetic acid under aerobic condition for 200 h, because an oxygen reduction reaction electrocatalyst was designed to create an anaerobic microenvironment. Remarkably, the product specificity is extremely high, with 90% of the electrons were directed toward acetate.[10] Also, the Wood−Ljungdahl pathway is the most energy-saving CO_2 fixation pathway. These two advantages make the acetogenic bacteria prime candidates for light-driven chemical production. Furthermore, acetic acid could be converted to other high-value chemicals (e.g., *n*-butanol and PHB) by *E.*

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000

Table 2. Summary of the Performances of Photobiocatalytic CO_2 Conversion Systems

species	materials	products	efficiency/yield	duration
Azotobacter vinelandii	CdS/ZnS QDs	formic acid	turnover number 10^6	NA[16]
Cupriavidus necator	CdS/ZnS QDs	C_2H_4, isopropanol, 2,3-butanediol, C_{11}−C_{15} methyl ketones, polyhydroxybutyrate	turnover number 10^6−10^8	NA[16]
Escherichia coli	CdS	L-malate and butyrate	L-malate yield = 1.48 mol/mol glucose butyrate yield = 0.79 mol/mol glucose	4 d[25]
Methanosarcina barkeri	CdS	CH_4	AQE[a] = 0.34%	5 d[67]
Methanosarcina barkeri	Ni:CdS	CH_4	AQE = 2.08%	6 d[68]
Moorella thermoacetica	PDI/PFP	acetic acid	AQE = 1.6%	3 d[61]
Moorella thermoacetica	CdS	acetic acid	AQE = 2.44%	4 d[10]
Moorella thermoacetica	CdS NPs, TiO_2−Mn phthalocyanine	acetic acid	yield ∼ 1.2 mM	3.5 d[65]
Moorella thermoacetica	Au NC	acetic acid	AQE = 2.86%	7 d[14]
Moorella thermoacetica	Au NC, alginate	acetic acid	yield ∼ 1.4 mM	3 d[62]
Rhodopseudomonas palustris	CdS QDs	CH_4	yield ∼ 171 nmol/mg total protein	4 d[69]
Rhodopseudomonas palustris	CdS NPs	carotenoid, PHB	photosynthetic efficiency 5.98%	8 d[70]
Sporomusa ovata	silicon nanowire	acetate	AQE = 3.6%	7 d[63]

[a]Apparent quantum efficiency (AQE) refers to the ratio of electrons used to convert substrate to product and the incident photons.

coli,[64] which widens the product spectrum of solar-acetogen PBSs.

In PBSs, sacrificial reductant thiol amino acid cysteine (Cys) is consumed continuously to quench h^+ during photocatalysis, and the depletion of Cys leads to photodamage or oxidative stress to cells, which limits the sustainable CO_2 fixation. In order to improve the acetic acid yield and achieve longer sustained acetic acid photosynthesis, a "Z-scheme" approach is proposed, in which Cys was regenerated from the oxidized disulfide form cystine (CySS) through water oxidation with TiO_2−Mn(II) phthalocyanine.[65] The shortage of the approach is that the CySS reduction rate decreases with the accumulation of O_2. The oxygen sensitivity of CdS and *M. thermoacetica* also contradicts the increase of oxygen. Therefore, more creative solutions are called for to improve the system.

Furthermore, proteomic and metabolic investigations provide insights into the molecular mechanisms underlying *M. thermoacetica*−CdS quantum dots hybrid system.[66] Except for the Wood−Ljungdahl pathway, the tricarboxylic acid cycle and a part of glycolysis were also activated by CdS with illumination, which was believed to be related to energy conservation. Especially, several electron transfer proteins showed significant upregulation, such as ferredoxin, flavoprotein, and NADH dehydrogenase, demonstrating their important roles in operation of the PBS. Increased expression of proteins associated with heavy metal resistance and reactive oxidative species (ROS) stress were also observed, suggesting that protecting cells from oxidative damage and heavy metal toxicity are key factors to optimize the hybrid system. Moreover, using transient absorption and time-resolved infrared spectroscopic techniques, Kornienko et al. reported that charge transfer between CdS and *M. thermoacetica* is mediated by membrane-bound hydrogenase or other electron acceptors (ferredoxin, flavoproteins, cytochrome, and menaquinones) whereas the former pathway displays higher quantum efficiency.[66]

3.2. Methane. As an essential energy source, methane also gains much attention, and several biohybrid systems have been constructed for solar methane conversion (Table 2). *Methanosarcina barkeri* is a kind of nonphototrophic methanogen which is electroactive and converts CO_2 to clean energy CH_4. Coated with CdS nanoparticles, the CH_4 production of *M. barkeri* was largely higher than bare cells under light irradiation, but the quantum efficiency (0.34%) was low.[67] Furthermore, doping Ni into CdS nanoparticles promoted stability of CdS nanoparticles and interface electron transfer, thus improving the performance of the PBS significantly, with a quantum yield of 2.08%.[68] The electron transfer and CO_2-to-CH_4 conversion pathway were also revealed by the authors, involving a series of enzymes, such as [NiFe] hydrogenase, ferredoxin, and methyl-S-CoM reductase. In contrast to the complex CH_4 production pathway, the photosynthetic bacterium *R. palustris* can directly transform CO_2 to methane which is catalyzed by mutated nitrogenase only. However, *R. palustris* mainly absorbs infrared light and has a poorly visible light response. Coated with CdS quantum dots of excellent visible light response significantly enhanced its production of methane (79% more).[69] A methane yield of 171 nmol/mg total protein was achieved by the biohybrids, which is comparable to the yield of a photothermal−magnetic reaction system[71] and superior to many photocatalytic systems.[72] Furthermore, the authors proposed that photoelectrons generated from CdS might be injected into nitrogenase directly or facilitated by ATP generation.

3.3. Other Multicarbon Compounds. In addition to acetic acid and methane, there are also other multicarbon compounds generated from photobiocatalytic CO_2 reduction, such as carotenoids, PHB, C_2H_4, and L-malate. Our research group found that production of crucial nutrient carotenoids and biodegradable thermoplastic PHB in *R. palustris* was increased by CdS nanoparticles.[70] In the process, photoelectrons were transferred into cellular NADPH, stimulating the Calvin cycle to fix more CO_2 for valuable chemical synthesis. In addition, ZnS coated quantum dots-*Cupriavidus necator* hybrids are reported for solar driven CO_2 reduction to C_2H_4, isopropanol, 2,3-butanediol, C_{11}−C_{15} methyl ketones, and PHB.[16] Particularly, for heterotrophic microorganisms that cannot reduce CO_2 naturally, the CO_2 fixation ability can be acquired via genetic manipulations, and CO_2 fixation efficiency

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000

could then be improved further by photosensitization. For example, it has been demonstrated that theoretical yield of L-malate and butyrate were achieved in *E. coli* through light driven CO_2 sequestration.[25] In this case, CdS nanoparticles increase the intracellular NADH and ATP content under illumination, which improves the efficiency of the half-Wood–Ljungdahl–formolase pathway and CO_2 mitigation switch (to reduce CO_2 emissions). In summary, photobiocatalytic techniques show versatile applications in light driven CO_2 reduction and solar chemical conversion, especially combined with genetic manipulations. Photoelectrochemical CO_2 conversion to acetic acid could achieve carbon selectivity of 91.5% and Faradaic efficiency of 58.1% using Zn-doped Cu_2O as catalyst,[55] which seems comparable to PBS (Table 2), but PBS is still advantageous in C_{2+} products, such as carotenoid, PHB, L-malate, and butyrate.

4. PHOTOBIOCATALYTIC N_2 FIXATION

Ammonia derived from N_2 fixation is critical for the survival of all forms of life and supports the production of important chemicals including fertilizers. Also, it is an important energy carrier and key component in the global nitrogen cycle. However, only a very small proportion of N_2 fixation occurs naturally by lightning or ultraviolet rays. Because the molecular structure of N_2 is ultrastable, high temperature and pressure are required for traditionally industrial N_2 reduction approach, Haber–Bosch process, which also cause a large quantity of CO_2 emission. Other artificial nitrogen reduction approaches, such as photocatalysis[73] and photoelectrochemical catalysis, usually suffer from low efficiency, low selectivity, and ambiguous mechanism.[4] For example, it is reported that apparent quantum efficiency of photocatalytic nitrogen fixation could reach 4.32%,[74] and a Faradaic efficiency of an electrocatalytic nitrogen reduction system is 30.2%.[75]

Microbial N_2 fixation catalyzed by nitrogenase can be performed at room temperature and ambient pressure, which accounts for around 2/3 of the fixed nitrogen on earth,[76] but is far from meeting the increasing agricultural and industrial demand. All nitrogen fixing microorganisms, also called diazotrophs, are prokaryotes, including bacteria (e.g., species of *Rhizobium*, *Azotobacter*, and *Bacillus*) and archaea (e.g., Methanosarcinales and Methanobacteriales).[77] Nitrogenase is sensitive to oxygen, so diazotrophs evolve various adaptive mechanisms to protect nitrogenase from oxygen. According to their tolerance to oxygen, diazotrophs can be classified into three groups: aerobic (e.g., cyanobacteria),[78] microaerobic (e.g., *Xanthobacter* sp.), and anaerobic (e.g., Methanosarcinales) nitrogen fixing microorganisms. Free-living diazotrophs (some species of *Cupriavidus* and *Azospirillum*) produce ammonium for their nitrogen supply, while ammonia produced by symbiotic (some species of *Rhizobium*, *Frankia*, and *Anabaena*) diazotrophs is excreted and absorbed by plants for the synthesis of various biomolecules. Of them, root nodule symbiosis is the most effective in nitrogen fixation.[79] Moreover, some diazotroph strains (e.g., species of *Rhizobium*, *Azotobacter*, and *Azospirillum*) have been made to commercial biofertilizer to provide nitrogen nutrient for crops,[79] but the efficiency of biofertilizer remains to be improved.

In order to enhance biological nitrogen fixation efficiency with sustainable solar energy, a CdS–nitrogenase MoFe protein biohybrid was designed by Brown et al. in 2016.[80] In the process, photoelectrons generated from CdS nanorods were transferred to the MoFe protein directly. However, the N_2 reduction persisted for only 5 h and its reaction rate was 63% of the reaction rate for the natural nitrogenase. The system shows low stability, short duration, and high cost, similar to nitrogenase bioelectrocatalysis[23] and other semiconductor–enzyme hybrid systems. Later, semiconductor–whole cell hybrid systems were successfully constructed, using anaerobic or aerobic nitrogen fixing bacteria. As mentioned earlier, photosynthetic *R. palustris* are able to precipitate Cd^{2+} through cysteine. In research conducted by our group, CdS nanoparticles were coated on *R. palustris* and improved their nitrogen fixation under visible light irradiation and anaerobic conditions, with solid biomass and intracellular ammonia and L-amino acids increased (Figure 5A).[43] Similarly, increased

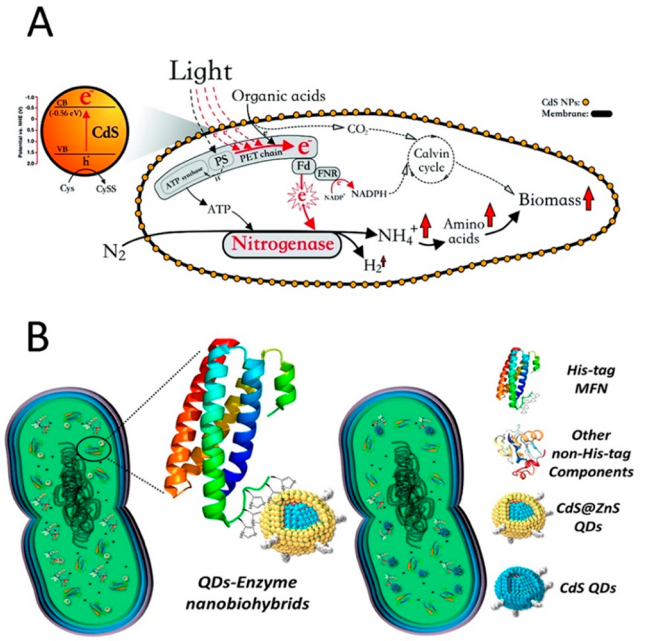

Figure 5. Nitrogen fixing photosynthetic biohybrid systems, including CdS–*R. palustris* (A), CdS-ZnS quantum dots–*A. vinelandii* (B) [Reprinted with permission from refs 43 (Copyright 2019 Royal Society of Chemistry) and 16 (Copyright 2019 American Chemical Society)].

NH_4^+ was observed in another CdS–*R. palustris* hybrid.[81] Mo–Fe nitrogenase gene (*nif*H) and V–Fe nitrogenase gene (*vnf*G) were upregulated during the experiment, demonstrating that nitrogenases participate in solar driven N_2 fixation. Overall, these experiments verify the feasibility of enhancing photosynthetic efficiency of photosynthetic microorganisms with semiconductor materials, which provide extra reducing equivalent and broaden light absorption for cells. Although photosynthetic microorganisms often have complex metabolism, there are also advantages of employing them for PBS construction, such as high tolerance to oxidative stress and to high light intensity resulting from million years of evolution. However, quantum yield of NH_4^+ is not provided by authors in these studies, resulting in difficulty to compare their performance to photo(electro)catalysis.

In another case, aerobic NH_3 production of *A. vinelandii* is improved by CdS-ZnS quantum dots (Figure 5B), although the duration of the hybrid remains to be improved.[16] Zinc has binding affinity with histidine-tagged MoFe nitrogenase in *A. vinelandii*, so the biohybrids can form by self-assembly.

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989–1000

Although *A. vinelandii* are aerobic nitrogen fixation organisms, they develop various strategies to protect their nitrogenase from oxygen, such as enhanced respiration, reversible conformational inactivation, and superoxide dismutase.[82] Significantly, their genetic pliability and natural competence endow the bacteria potential application in photocatalyzed nitrogen fixation through combination with various semiconducting materials. In the future, more research should be performed on *A. vinelandii*.

In addition to direct electron transfer from semiconductors to cells, using H_2 as a redox mediator is also a feasible approach to collect exogenous electrons for cellular nitrogen fixation. For example, chemoautotrophic bacterium *Xanthobacter autotrophicus* used H_2 generated from photoelectrocatalysis via CoPi/Co−P to fix nitrogen.[83] Accumulation of cellular total nitrogen and high nitrogenase activity were detected. Furthermore, plant growth was promoted significantly while *X. autotrophicus* cells were applied as biofertilizers. It is noteworthy that N_2-fixing nitrogenase enzymes are inhibited by O_2, but some diazotrophs including *X. autotrophicus* need O_2 for terminal electron acceptors, thus microaerobic conditions are required. Perfluorocarbon nanoemulsions can satisfy the O_2 demand of bacteria without affecting the activity of nitrogenase, therefore they were applied to create a microaerobic environment for an electrode−*X. autotrophicus* hybrid.[84] As a result, the overall electron efficiency for N_2 fixation increased 250% with a 120 h duration. However, biocompatibility of the perfluorocarbon needs to be improved and the kinetics of feedstock consumption and product formation remain out of control in the system.

Semiartificial photosynthesizing systems show great potential in achieving highly efficient, environmentally friendly, and low cost nitrogen fixation. However, compared to light driven H_2 evolution and CO_2 reduction based on an inorganic−biological hybrid system, studies on photobiocatalytic nitrogen fixation are rare, and many different kinds of nitrogen-fixing microorganisms and their possible integration with semiconductor materials remain largely unexplored. We suggest several criteria for choosing N_2 fixing microorganisms to fabricate PBS, including high nitrogen fixing efficiency, oxygen demand, ability to combine with nanomaterials and utilize exogenous electrons, and being liable to genetic manipulation as well as low cost for culture and maintenance.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Tremendous progress has been made in past five years in the field of whole-cell photosynthetic biohybrid systems, especially in enhancing biocatalytic solar fuel and solar chemical conversion with photosensitizers, involving H_2 evolution, CO_2 reduction, and N_2 fixation. Of them, H_2 evolution PBSs have been extensively studied but show no significant superiority to photo(electro)catalysis. Future works on PBSs may focus on CO_2-to-C_{2+} conversion and nitrogen fixation. Electron transfer from semiconductors to cells is a key factor affecting the activity of PBSs, and it largely depends on assembly method. Given that the indirect electron transfer is less efficient and most microbes are not capable of precipitating nanomaterials, developing a universal method to assemble light harvesters and cells with efficient electron transfer is an urgent issue in the development of PBS. In 2021, an "add-on" mode via electrostatic interactions is proposed by our research group to assemble various negatively charged carbon-based semiconductors with positively charged cells,[42] which extends the application scope of PBS largely without the limitation of material and cell types. In addition, a tannic acid (TA) based modular-assembly strategy was developed by our group.[85] The material-loading efficiency and biocompatibility can be optimized via adjusting TA concentration. As a result, InP nanoparticles, Bi nanospheres, and g-C_3N_4 functionalized by TA-Fe^{3+} nanofilms were successfully assembled onto the surface of yeast (*S. cerevisiae*), demonstrating that the strategy can be applied versatilely for fabricating PBSs. Besides, there are many other difficulties in the way of constructing highly efficient and sustainable hybrid systems, but corresponding solutions have been proposed and verified experimentally by researchers (Table 3). Expensive electron sacrificial agent Cys was regenerated by water oxidation with TiO_2−Mn(II) phthalocyanine. Carbon based semiconductor HTCC was introduced into PBS to avoid production of cytotoxic metal ions induced by photocorrosion. In order to protect cells from oxidative stress, metal organic framework wrapping and biomimetic silica encapsulation strategies are adopted. Another solution is to utilize ROS tolerant microorganism strains. In contrast to the enzyme−nanomaterial systems, in whole-cell−nanomaterial hybrids electrons are introduced to all the pathways in cells without specificity, which limits the conversion efficiency of solar to target chemicals. Ding et al. designed quantum dots which could enter into cells and specifically bind to target enzymes. In this way, the selectivity of products in PBSs is improved significantly.

Table 3. Problems that Limit Large Scale Application of Photosynthetic Biohybrid Systems and Tentative Solutions

problems	solutions	refs
consume expensive electron sacrificial agent	tandem "Z-scheme" to regenerate Cys	65
electrons are introduced to all the pathways in cells without specificity	specific binding to target enzymes	16, 18
	genetic engineering of cells	
oxidative stress from reactive oxygen species and oxygen	metal organic framework wrapping	62, 37, 64, 86
	AuNCs photosensitizer	
	biomimetic silica encapsulation	
	Si nanowire	
	ROS tolerant strain	
photocorrosion induced toxic metal ions	carbon based semiconductor	42, 19, 45
	metal resistant strains	
	organic photosensitizer	
expensive photosensitizer	earth-abundant semiconductor minerals	17

However, these solutions are not perfect and have their own limitations. For instance, regeneration of Cys results in the problem of oxidative stress. Gold nanoclusters solve the oxidative stress problem but increase cost. Using organic semiconductors avoids the release of toxic metal ions, but the light-harvesting efficiency remains to be improved. Intracellular photosensitization promotes electron transfer from semiconductors to cells, but cells may hamper light absorption of semiconductors. Moreover, there are other obstacles limiting the scaling up of PBSs. The abiotic−biotic interfacial electron transfer mechanism is fundamental to improve the performance of PBS but remains largely unexplored. Given the low

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000

sample concentration and cell sensitivity to interference, the *in vivo* time-resolved profile of electron flux still remains a big challenge. Transient absorption and time-resolved infrared spectroscopic techniques,[66] X-ray absorption spectroscopy, and electrochemical impedance spectroscopy are promising technologies to reveal the interfacial electron transfer. Moreover, natural cells have evolved to consume energy for reproducing cells or synthesizing other organic macromolecules necessary for survival; hence, the electrons are not always utilized for producing the target compound. In order to improve quantum yield, investigating the mechanism of cellular energy allocation and regulating metabolism pathways are necessary for further research.

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Funding

This work was supported by the National Key R&D Program of China (grant no. 2021YFA0910800), National Natural Science Foundation of China (grant no. 22008252 and 92062109), the Natural Science Foundation of Guangdong Province (no. 2018A0303130210), and National Natural Science Foundation of China (grant no. 42002303).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Shih, C. F.; Zhang, T.; Li, J.; Bai, C. Powering the future with liquid sunshine. *Joule* **2018**, *2* (10), 1925−1949.

(2) Jia, J.; Seitz, L. C.; Benck, J. D.; Huo, Y.; Chen, Y.; Ng, J. W. D.; Bilir, T.; Harris, J. S.; Jaramillo, T. F. Solar water splitting by photovoltaic-electrolysis with a solar-to-hydrogen efficiency over 30%. *Nat. Commun.* **2016**, *7* (1), 13237.

(3) Du, C.; Wang, X.; Chen, W.; Feng, S.; Wen, J.; Wu, Y. A. CO_2 transformation to multicarbon products by photocatalysis and electrocatalysis. *Materials Today Advances* **2020**, *6*, 100071.

(4) Xue, X.; Chen, R.; Yan, C.; Zhao, P.; Hu, Y.; Zhang, W.; Yang, S.; Jin, Z. Review on photocatalytic and electrocatalytic artificial nitrogen fixation for ammonia synthesis at mild conditions: advances, challenges and perspectives. *Nano Research* **2019**, *12* (6), 1229−1249.

(5) Yin, H.; Ho, J. K. W.; Cheung, S. H.; Yan, R. J.; Chiu, K. L.; Hao, X.; So, S. K. Designing a ternary photovoltaic cell for indoor light harvesting with a power conversion efficiency exceeding 20%. *Journal of Materials Chemistry A* **2018**, *6* (18), 8579−8585.

(6) Yoshikawa, K.; Kawasaki, H.; Yoshida, W.; Irie, T.; Konishi, K.; Nakano, K.; Uto, T.; Adachi, D.; Kanematsu, M.; Uzu, H.; Yamamoto, K. Silicon heterojunction solar cell with interdigitated back contacts for a photoconversion efficiency over 26%. *Nature Energy* **2017**, *2* (5), 17032.

(7) Cestellos-Blanco, S.; Zhang, H.; Kim, J. M.; Shen, Y.-x.; Yang, P. Photosynthetic semiconductor biohybrids for solar-driven biocatalysis. *Nature Catalysis* **2020**, *3* (3), 245−255.

(8) Kornienko, N.; Zhang, J. Z.; Sakimoto, K. K.; Yang, P.; Reisner, E. Interfacing nature's catalytic machinery with synthetic materials for semi-artificial photosynthesis. *Nat. Nanotechnol.* **2018**, *13* (10), 890−899.

(9) Krasnovsky, A. A.; Nikandrov, V. V. The photobiocatalytic system: Inorganic semiconductors coupled to bacterial cells. *FEBS Lett.* **1987**, *219* (1), 93−96.

(10) Sakimoto, K. K.; Wong, A. B.; Yang, P. Self-photosensitization of nonphotosynthetic bacteria for solar-to-chemical production. *Science* **2016**, *351* (6268), 74−77.

(11) Honda, Y.; Hagiwara, H.; Ida, S.; Ishihara, T. Application to photocatalytic H_2 production of a whole-cell reaction by recombinant *Escherichia coli* cells expressing [FeFe]-hydrogenase and maturases genes. *Angew. Chem.* **2016**, *128* (28), 8177−8180.

(12) Gupta, P.; Noori, M.; Núñez, A.; Verma, N. An insight into the bioelectrochemical photoreduction of CO_2 to value-added chemicals. *iScience* **2021**, *24* (4), 102294.

(13) Sahoo, P. C.; Pant, D.; Kumar, M.; Puri, S. K.; Ramakumar, S. S. V. Material−microbe interfaces for solar-driven CO_2 bioelectrosynthesis. *Trends Biotechnol.* **2020**, *38* (11), 1245−1261.

(14) Zhang, H.; Liu, H.; Tian, Z.; Lu, D.; Yu, Y.; Cestellos-Blanco, S.; Sakimoto, K. K.; Yang, P. Bacteria photosensitized by intracellular gold nanoclusters for solar fuel production. *Nat. Nanotechnol.* **2018**, *13* (10), 900−905.

(15) Guo, J.; Suástegui, M.; Sakimoto, K. K.; Moody, V. M.; Xiao, G.; Nocera, D. G.; Joshi, N. S. Light-driven fine chemical production in yeast biohybrids. *Science* **2018**, *362* (6416), 813−816.

(16) Ding, Y.; Bertram, J. R.; Eckert, C.; Bommareddy, R. R.; Patel, R.; Conradie, A.; Bryan, S.; Nagpal, P. Nanorg microbial factories: light-driven renewable biochemical synthesis using quantum dot-bacteria nanobiohybrids. *J. Am. Chem. Soc.* **2019**, *141* (26), 10272−10282.

(17) Cestellos-Blanco, S.; Kim, J. M.; Watanabe, N. G.; Chan, R. R.; Yang, P. Molecular insights and future frontiers in cell photosensitization for solar-driven CO_2 conversion. *iScience* **2021**, *24* (9), 102952.

(18) Luo, B.; Wang, Y.-Z.; Li, D.; Shen, H.; Xu, L.-X.; Fang, Z.; Xia, Z.; Ren, J.; Shi, W.; Yong, Y.-C. A periplasmic photosensitized biohybrid system for solar hydrogen production. *Adv. Energy Mater.* **2021**, *11* (19), 2100256.

(19) Chan, H. S.; Xiao, K.; Tsang, T. H.; Zeng, C.; Wang, B.; Peng, X.; Wong, P. K. Bioremediation of crude glycerol by a sustainable organic−microbe hybrid system. *Frontiers in Microbiology* **2021**, *12*, 654033.

(20) Zhang, R.; He, Y.; Yi, J.; Zhang, L.; Shen, C.; Liu, S.; Liu, L.; Liu, B.; Qiao, L. Proteomic and metabolic elucidation of solar-powered biomanufacturing by bio-abiotic hybrid system. *Chem.* **2020**, *6* (1), 234−249.

(21) Lee, S. H.; Choi, D. S.; Kuk, S. K.; Park, C. B. Photobiocatalysis: activating redox enzymes by direct or indirect transfer of photoinduced electrons. *Angew. Chem., Int. Ed.* **2018**, *57* (27), 7958−7985.
(22) Armstrong, F. A.; Hirst, J. Reversibility and efficiency in electrocatalytic energy conversion and lessons from enzymes. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (34), 14049.
(23) Rapson, T. D.; Gregg, C. M.; Allen, R. S.; Ju, H.; Doherty, C. M.; Mulet, X.; Giddey, S.; Wood, C. C. Insights into nitrogenase bioelectrocatalysis for green ammonia production. *ChemSusChem* **2020**, *13* (18), 4856−4865.
(24) Nevin, K. P.; Hensley, S. A.; Franks, A. E.; Summers, Z. M.; Ou, J.; Woodard, T. L.; Snoeyenbos-West, O. L.; Lovley, D. R. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Appl. Environ. Microbiol.* **2011**, *77* (9), 2882.
(25) Hu, G.; Li, Z.; Ma, D.; Ye, C.; Zhang, L.; Gao, C.; Liu, L.; Chen, X. Light-driven CO_2 sequestration in *Escherichia coli* to achieve theoretical yield of chemicals. *Nature Catalysis* **2021**, *4* (5), 395−406.
(26) Pan, Q.; Tian, X.; Li, J.; Wu, X.; Zhao, F. Interfacial electron transfer for carbon dioxide valorization in hybrid inorganic-microbial systems. *Applied Energy* **2021**, *292*, 116885.
(27) Nevin, K.; Woodard, T.; Franks, A.; Summers, Z.; Lovley, D. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *Mbio* **2010**, *1* (2), 20714445.
(28) Xiao, S.; Li, Z.; Fu, Q.; Li, Y.; Li, J.; Zhang, L.; Liao, Q.; Zhu, X. Hybrid microbial photoelectrochemical system reduces CO_2 to CH_4 with 1.28% solar energy conversion efficiency. *Chemical Engineering Journal* **2020**, *390*, 124530.
(29) Fu, Q.; Xiao, S.; Li, Z.; Li, Y.; Kobayashi, H.; Li, J.; Yang, Y.; Liao, Q.; Zhu, X.; He, X.; Ye, D.; Zhang, L.; Zhong, M. Hybrid solar-to-methane conversion system with a Faradaic efficiency of up to 96%. *Nano Energy* **2018**, *53*, 232−239.
(30) Aryal, N.; Ammam, F.; Patil, S. A.; Pant, D. An overview of cathode materials for microbial electrosynthesis of chemicals from carbon dioxide. *Green Chem.* **2017**, *19* (24), 5748−5760.
(31) Fang, X.; Kalathil, S.; Reisner, E. Semi-biological approaches to solar-to-chemical conversion. *Chem. Soc. Rev.* **2020**, *49* (14), 4926−4952.
(32) Hosseini, S. E.; Wahid, M. A. Hydrogen production from renewable and sustainable energy resources: Promising green energy carrier for clean development. *Renewable and Sustainable Energy Reviews* **2016**, *57*, 850−866.
(33) Hu, S.; Xiang, C.; Haussener, S.; Berger, A. D.; Lewis, N. S. An analysis of the optimal band gaps of light absorbers in integrated tandem photoelectrochemical water-splitting systems. *Energy Environ. Sci.* **2013**, *6* (10), 2984−2993.
(34) Sivaramakrishnan, R.; Shanmugam, S.; Sekar, M.; Mathimani, T.; Incharoensakdi, A.; Kim, S.-H.; Parthiban, A.; Edwin Geo, V.; Brindhadevi, K.; Pugazhendhi, A. Insights on biological hydrogen production routes and potential microorganisms for high hydrogen yield. *Fuel* **2021**, *291*, 120136.
(35) Bolatkhan, K.; Kossalbayev, B. D.; Zayadan, B. K.; Tomo, T.; Veziroglu, T. N.; Allakhverdiev, S. I. Hydrogen production from phototrophic microorganisms: Reality and perspectives. *Int. J. Hydrogen Energy* **2019**, *44* (12), 5799−5811.
(36) Wang, B.; Zeng, C.; Chu, K. H.; Wu, D.; Yip, H. Y.; Ye, L.; Wong, P. K. Enhanced biological hydrogen production from *Escherichia coli* with surface precipitated cadmium sulfide nanoparticles. *Adv. Energy Mater.* **2017**, *7* (20), 1700611.
(37) Wei, W.; Sun, P.; Li, Z.; Song, K.; Su, W.; Wang, B.; Liu, Y.; Zhao, J. A surface-display biohybrid approach to light-driven hydrogen production in air. *Science Advances* **2018**, *4* (2), No. eaap9253.
(38) Jiang, Z.; Wang, B.; Yu, J. C.; Wang, J.; An, T.; Zhao, H.; Li, H.; Yuan, S.; Wong, P. K. $AgInS_2/In_2S_3$ heterostructure sensitization of *Escherichia coli* for sustainable hydrogen production. *Nano Energy* **2018**, *46*, 234−240.
(39) Honda, Y.; Shinohara, Y.; Fujii, H. Visible light-driven, external mediator-free H_2 production by a combination of a photosensitizer and a whole-cell biocatalyst: *Escherichia coli* expressing [FeFe]-hydrogenase and maturase genes. *Catalysis Science & Technology* **2020**, *10* (17), 6006−6012.
(40) Martins, M.; Toste, C.; Pereira, I. A. C. Enhanced light-driven hydrogen production by self-photosensitized biohybrid systems. *Angew. Chem., Int. Ed.* **2021**, *60* (16), 9055−9062.
(41) Ramprakash, B.; Incharoensakdi, A. Encapsulated titanium dioxide nanoparticle-*Escherichia coli* hybrid system improves light driven hydrogen production under aerobic condition. *Bioresour. Technol.* **2020**, *318*, 124057.
(42) Xiao, K.; Tsang, T. H.; Sun, D.; Liang, J.; Zhao, H.; Jiang, Z.; Wang, B.; Yu, J. C.; Wong, P. K. Interfacing iodine-doped hydrothermally carbonized carbon with *Escherichia coli* through an "Add-on" mode for enhanced light-driven hydrogen production. *Adv. Energy Mater.* **2021**, *11* (21), 2100291.
(43) Wang, B.; Xiao, K.; Jiang, Z.; Wang, J.; Yu, J. C.; Wong, P. K. Biohybrid photoheterotrophic metabolism for significant enhancement of biological nitrogen fixation in pure microbial cultures. *Energy Environ. Sci.* **2019**, *12* (7), 2185−2191.
(44) Wang, Z.; Gao, D.; Geng, H.; Xing, C. Enhancing hydrogen production by photobiocatalysis through *Rhodopseudomonas palustris* coupled with conjugated polymers. *Journal of Materials Chemistry A* **2021**, *9* (35), 19788−19795.
(45) Rowe, S. F.; Le Gall, G. n. l.; Ainsworth, E. V.; Davies, J. A.; Lockwood, C. W.; Shi, L.; Elliston, A.; Roberts, I. N.; Waldron, K. W.; Richardson, D.; et al. Light-driven H_2 evolution and C = C or C = O bond hydrogenation by *Shewanella oneidensis*: A versatile strategy for photocatalysis by nonphotosynthetic microorganisms. *ACS Catal.* **2017**, *7* (11), 7558−7566.
(46) Shen, H.; Wang, Y.-Z.; Liu, G.; Li, L.; Xia, R.; Luo, B.; Wang, J.; Suo, D.; Shi, W.; Yong, Y.-C. A whole-cell inorganic-biohybrid system integrated by reduced graphene oxide for boosting solar hydrogen production. *ACS Catal.* **2020**, *10* (22), 13290−13295.
(47) Li, X.; Sun, H.; Mao, X.; Lao, Y.; Chen, F. Enhanced photosynthesis of carotenoids in microalgae driven by light-harvesting gold nanoparticles. *ACS Sustainable Chem. Eng.* **2020**, *8* (20), 7600−7608.
(48) Choi, Y.; Lee, S. Y. Biosynthesis of inorganic nanomaterials using microbial cells and bacteriophages. *Nature Reviews Chemistry* **2020**, *4* (12), 638−656.
(49) Nishiyama, H.; Yamada, T.; Nakabayashi, M.; Maehara, Y.; Yamaguchi, M.; Kuromiya, Y.; Nagatsuma, Y.; Tokudome, H.; Akiyama, S.; Watanabe, T.; Narushima, R.; Okunaka, S.; Shibata, N.; Takata, T.; Hisatomi, T.; Domen, K. Photocatalytic solar hydrogen production from water on a 100-m2 scale. *Nature* **2021**, *598* (7880), 304−307.
(50) Bala Chandran, R.; Breen, S.; Shao, Y.; Ardo, S.; Weber, A. Z. Evaluating particle-suspension reactor designs for Z-scheme solar water splitting via transport and kinetic modeling. *Energy Environ. Sci.* **2018**, *11* (1), 115−135.
(51) Fabian, D. M.; Hu, S.; Singh, N.; Houle, F. A.; Hisatomi, T.; Domen, K.; Osterloh, F. E.; Ardo, S. Particle suspension reactors and materials for solar-driven water splitting. *Energy Environ. Sci.* **2015**, *8* (10), 2825−2850.
(52) Wang, Z.; Li, C.; Domen, K. Recent developments in heterogeneous photocatalysts for solar-driven overall water splitting. *Chem. Soc. Rev.* **2019**, *48* (7), 2109−2125.
(53) Xie, H.; Wang, J.; Ithisuphalap, K.; Wu, G.; Li, Q. Recent advances in Cu-based nanocomposite photocatalysts for CO_2 conversion to solar fuels. *Journal of Energy Chemistry* **2017**, *26* (6), 1039−1049.
(54) Li, F.; Li, Y. C.; Wang, Z.; Li, J.; Nam, D.-H.; Lum, Y.; Luo, M.; Wang, X.; Ozden, A.; Hung, S.-F.; Chen, B.; Wang, Y.; Wicks, J.; Xu, Y.; Li, Y.; Gabardo, C. M.; Dinh, C.-T.; Wang, Y.; Zhuang, T.-T.; Sinton, D.; Sargent, E. H. Cooperative CO_2-to-ethanol conversion via enriched intermediates at molecule−metal catalyst interfaces. *Nature Catalysis* **2020**, *3* (1), 75−82.

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000

(55) Wang, X.; Gao, C.; Low, J.; Mao, K.; Duan, D.; Chen, S.; Ye, R.; Qiu, Y.; Ma, J.; Zheng, X.; Long, R.; Wu, X.; Song, L.; Zhu, J.; Xiong, Y. Efficient photoelectrochemical CO2 conversion for selective acetic acid production. *Science Bulletin* **2021**, *66* (13), 1296−1304.
(56) Ross, M. B.; De Luna, P.; Li, Y.; Dinh, C.-T.; Kim, D.; Yang, P.; Sargent, E. H. Designing materials for electrochemical carbon dioxide recycling. *Nature Catalysis* **2019**, *2* (8), 648−658.
(57) Salehizadeh, H.; Yan, N.; Farnood, R. Recent advances in microbial CO_2 fixation and conversion to value-added products. *Chemical Engineering Journal* **2020**, *390* (15), 124584.
(58) Rudolf, C.; Grammel, H. Fructose metabolism of the purple non-sulfur bacterium *Rhodospirillum rubrum*: effect of carbon dioxide on growth, and production of bacteriochlorophyll and organic acids. *Enzyme Microb. Technol.* **2012**, *50* (4−5), 238−246.
(59) Wang, Y.; Liu, J.; Wang, Y.; Al-Enizi, A. M.; Zheng, G. Tuning of CO_2 reduction selectivity on metal electrocatalysts. *Small* **2017**, *13* (43), 1701809.
(60) Christoforidis, K. C.; Fornasiero, P. Photocatalysis for hydrogen production and CO_2 reduction: the case of copper-catalysts. *ChemCatChem.* **2019**, *11* (1), 368−382.
(61) Gai, P.; Yu, W.; Zhao, H.; Qi, R.; Li, F.; Liu, L.; Lv, F.; Wang, S. Solar-powered organic semiconductor−bacteria biohybrids for CO_2 reduction into acetic acid. *Angew. Chem., Int. Ed.* **2020**, *59* (18), 7224−7229.
(62) Cestellos-Blanco, S.; Zhang, H.; Yang, P. Solar-driven carbon dioxide fixation using photosynthetic semiconductor bio-hybrids. *Faraday Discuss.* **2019**, *215* (0), 54−65.
(63) Su, Y.; Cestellos-Blanco, S.; Kim, J. M.; Shen, Y.-x.; Kong, Q.; Lu, D.; Liu, C.; Zhang, H.; Cao, Y.; Yang, P. Close-packed nanowire-bacteria hybrids for efficient solar-driven CO_2 fixation. *Joule* **2020**, *4* (4), 800−811.
(64) Liu, C.; Gallagher, J. J.; Sakimoto, K. K.; Nichols, E. M.; Chang, C. J.; Chang, M. C. Y.; Yang, P. Nanowire−bacteria hybrids for unassisted solar carbon dioxide fixation to value-added chemicals. *Nano Lett.* **2015**, *15* (5), 3634−3639.
(65) Sakimoto, K. K.; Zhang, S. J.; Yang, P. Cysteine−cystine photoregeneration for oxygenic photosynthesis of acetic acid from CO_2 by a tandem inorganic−biological hybrid system. *Nano Lett.* **2016**, *16* (9), 5883−5887.
(66) Kornienko, N.; Sakimoto, K. K.; Herlihy, D. M.; Nguyen, S. C.; Alivisatos, A. P.; Harris, C. B.; Schwartzberg, A.; Yang, P. Spectroscopic elucidation of energy transfer in hybrid inorganic−biological organisms for solar-to-chemical production. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (42), 11750.
(67) Ye, J.; Yu, J.; Zhang, Y.; Chen, M.; Liu, X.; Zhou, S.; He, Z. Light-driven carbon dioxide reduction to methane by *Methanosarcina barkeri*-CdS biohybrid. *Applied Catalysis B: Environmental* **2019**, *257* (15), 117916.
(68) Ye, J.; Ren, G.; Kang, L.; Zhang, Y.; Liu, X.; Zhou, S.; He, Z. Efficient photoelectron capture by Ni decoration in *Methanosarcina barkeri*-CdS biohybrids for enhanced photocatalytic CO_2-to-CH_4 conversion. *iScience* **2020**, *23* (7), 101287.
(69) Chen, M.-Y.; Fang, Z.; Xu, L.-X.; Zhou, D.; Yang, X.-J.; Zhu, H.-J.; Yong, Y.-C. Enhancement of photo-driven biomethanation under visible light by nano-engineering of *Rhodopseudomonas palustris*. *Bioresources and Bioprocessing* **2021**, *8*, 30.
(70) Wang, B.; Jiang, Z.; Yu, J. C.; Wang, J.; Wong, P. K. Enhanced CO_2 reduction and valuable C_{2+} chemical production by a CdS-photosynthetic hybrid system. *Nanoscale* **2019**, *11* (19), 9296−9301.
(71) Li, N.; Tu, Y.; Wang, K.; Huang, D.; Shen, Q.; Chen, W.; Zhou, J.; Ma, Q.; Liu, M. Construction of a photo-thermal-magnetic coupling reaction system for enhanced CO_2 reduction to CH_4. *Chemical Engineering Journal* **2021**, *421*, 129940.
(72) Moradi, M.; Khorasheh, F.; Larimi, A. Pt nanoparticles decorated Bi-doped TiO_2 as an efficient photocatalyst for CO_2 photo-reduction into CH_4. *Sol. Energy* **2020**, *211* (15), 100−110.
(73) Meng, S.-L.; Li, X.-B.; Tung, C.-H.; Wu, L.-Z. Nitrogenase inspired artificial photosynthetic nitrogen fixation. *Chem.* **2021**, *7* (6), 1431−1450.
(74) Ye, L.; Han, C.; Ma, Z.; Leng, Y.; Li, J.; Ji, X.; Bi, D.; Xie, H.; Huang, Z. Ni_2P loading on $Cd_{0.5}Zn_{0.5}S$ solid solution for exceptional photocatalytic nitrogen fixation under visible light. *Chemical Engineering Journal* **2017**, *307*, 311−318.
(75) Nazemi, M.; Panikkanvalappil, S. R.; El-Sayed, M. A. Enhancing the rate of electrochemical nitrogen reduction reaction for ammonia synthesis under ambient conditions using hollow gold nanocages. *Nano Energy* **2018**, *49*, 316−323.
(76) Mus, F.; Alleman, A. B.; Pence, N.; Seefeldt, L. C.; Peters, J. W. Exploring the alternatives of biological nitrogen fixation. *Metallomics* **2018**, *10* (4), 523−538.
(77) Sun, W.; Shahrajabian, M. H.; Cheng, Q. Nitrogen fixation and diazotrophs−a review. *Rom. Biotechnol. Lett.* **2021**, *26*, 2834−2845.
(78) Berman-Frank, I.; Lundgren, P.; Falkowski, P. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology* **2003**, *154* (3), 157−164.
(79) Soumare, A.; Diedhiou, A. G.; Thuita, M.; Hafidi, M.; Ouhdouch, Y.; Gopalakrishnan, S.; Kouisni, L. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. *Plants* **2020**, *9* (8), 1011.
(80) Brown, K. A.; Harris, D. F.; Wilker, M. B.; Rasmussen, A.; Khadka, N.; Hamby, H.; Keable, S.; Dukovic, G.; Peters, J. W.; Seefeldt, L. C.; King, P. W. Light-driven dinitrogen reduction catalyzed by a CdS: nitrogenase MoFe protein biohybrid. *Science* **2016**, *352* (6284), 448−450.
(81) Sakpirom, J.; Kantachote, D.; Siripattanakul-Ratpukdi, S.; McEvoy, J.; Khan, E. Simultaneous bioprecipitation of cadmium to cadmium sulfide nanoparticles and nitrogen fixation by *Rhodopseudomonas palustris* TN110. *Chemosphere* **2019**, *223*, 455−464.
(82) Noar, J. D.; Bruno-Bárcena, J. M. *Azotobacter vinelandii*: the source of 100 years of discoveries and many more to come. *Microbiology* **2018**, *164* (4), 421−436.
(83) Liu, C.; Sakimoto, K.; Colón, B.; Silver, P.; Nocera, D. Ambient nitrogen reduction cycle using a hybrid inorganic−biological system. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (25), 6450−6455.
(84) Lu, S.; Rodrigues, R. M.; Huang, S.; Estabrook, D. A.; Chapman, J. O.; Guan, X.; Sletten, E. M.; Liu, C. Perfluorocarbon nanoemulsions create a beneficial O_2 microenvironment in N_2-fixing biological | inorganic hybrid. *Chem. Catalysis* **2021**, *1* (3), 704−720.
(85) Ren, X.; Yin, P.; Liang, J.; Liu, X.; Zhan, W.; Niu, J.; Si, T.; Yu, T.; Wu, D.; Wang, B. Insight into the tannic acid-based modular-assembly strategy based on inorganic−biological hybrid systems: a material suitability, loading effect, and biocompatibility study. *Materials Chemistry Frontiers* **2021**, *5* (10), 3867−3876.
(86) Liu, C.; Colón, B. C.; Ziesack, M.; Silver, P. A.; Nocera, D. G. Water splitting-biosynthetic system with CO_2 reduction efficiencies exceeding photosynthesis. *Science* **2016**, *352* (6290), 1210−1213.

https://doi.org/10.1021/acsestengg.1c00429
ACS EST Engg. 2022, 2, 989−1000