Bio-inorganic Hybrid Materials for Photocatalytic Hydrogen Production

Heryn Wang Literature Seminar November 10, 2022

Fossil fuels remain our primary source of energy, supplying more than 80% of the global energy demand in 2021.[1] But fossil fuel use causes several negative environmental consequences including climate change and the destruction of natural habitats. For these reasons, there is a pressing need to develop clean, sustainable, and renewable energy sources to replace our current dependence on fossil fuels.

Hydrogen has attracted much attention as a promising source of alternative energy due to its high energy capacity, environmental friendliness, and high conversion efficiency. But today, 95% of global hydrogen is generated from fossil fuels, particularly by the steam-methane reforming process from natural gas.[2] In order to achieve a green hydrogen energy economy with near-zero emission of carbon dioxide, it is essential to develop sustainable means of producing hydrogen that do not rely on fossil fuels.

Photocatalytic water splitting, in which solar energy is used to break down water into hydrogen and oxygen, is an attractive means of producing hydrogen. A typical particulate photocatalyst comprises a semiconductor photocatalyst that absorbs solar light and catalyst(s) on which the water oxidation-reduction reaction is carried out (**Figure 1**). When the semiconductor is illuminated with light that has energy greater than its band gap, electrons are excited to the conduction band, leaving behind positive holes in the valence band. These photogenerated electrons and holes can be used to reduce water to hydrogen and oxidize water to oxygen, respectively. The near-infinite energy supply from the sun and the zero emission of carbon dioxide makes the photocatalytic approach a potential means of producing hydrogen. However, its commercialization is limited by its low efficiency and the cost associated with the synthesis of the co-catalysts, which are usually based on platinum metals.

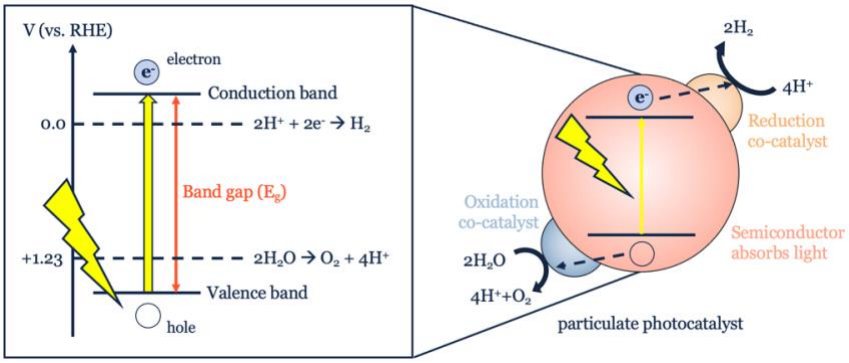

Figure 1. Schematic diagram of a particulate photocatalyst for photocatalytic water splitting.

Another promising means of producing hydrogen is the utilization of microorganisms and their natural biocatalytic processes to extract hydrogen from biomass. Bio-hydrogen production processes can be divided into two classes depending on the need for light: Photo-fermentation refers to the process in which bacteria use solar light as the energy source to perform photosynthesis. Dark fermentation, on the other hand, is the process in which bacteria break down complex carbohydrates to produce hydrogen and various organic acids as byproducts in the absence of light.[4] Bio-hydrogen production is desirable for its potential to use lignocellulosic biomass, a waste material, as its energy source. However, the low H_2 production yield and low substrate conversion efficiency remain as fundamental problems to be addressed.

In an effort to maximize the advantages and minimize the disadvantages of photocatalytic and biological approaches toward green hydrogen production, researchers have begun to develop

bio-inorganic hybrid systems. This novel technology integrates the light-harvesting capability of inorganic semiconductors with the catalytic efficiency of hydrogenase. Previous work has focused on using purified hydrogenase enzymes as the biological component; however, the practical use of the semiconductor-enzyme hybrid is greatly hindered by the poor stability of the isolated enzyme, the low yield of hydrogen, and the complicated purification process.[5] Thus, researchers began employing hydrogenase-containing whole-cell microorganisms as an alternative, which proved to be beneficial for both their high stability and the ease in obtaining them.

In 2016, Honda and co-workers were the first to employ a bio-inorganic hybrid system for the photocatalytic production of hydrogen. Recombinant *Escherichia coli (E. coli)* bacteria expressing genes coding [FeFe]-hydrogenase and maturases were prepared and incorporated in an aqueous suspension of anatase TiO_2. However, this hybrid system required the presence of an external electron mediator, such as methyl viologen (MV), to transport the electrons from the extracellular inorganic semiconductors to the cytoplasm of the bacteria where the biocatalysis takes place.[7] Because of the inevitable loss of photo-generated electrons in the transferring process as well as the cost and the toxicity of MV, it is highly desirable to develop bio-inorganic hybrid systems that can function in the absence of an electron mediator.

Sakimoto and co-workers reported an electron mediator-free bio-inorganic hybrid system in which CdS nanoparticles were bio-precipitated directly onto the surface of the acetogenic bacterium *Moorella thermoacetica*. The deposition of CdS nanoparticles on the cell surface allowed the photo-generated electrons to diffuse to the cytoplasm of bacteria through the membrane-bound redox proteins.[8] Since the discovery of this method, the bio-precipitation of CdS nanoparticles has been applied in several other bio-inorganic hybrid systems.[9,10] Recently, Martins and co-workers developed bacteria-CdS hybrid systems using the CdS bio-precipitation technique and three different bacteria (*D. desulfuricans*, *C. freundii* and *S. oneidensis*) that are known to express high levels of hydrogenase (**Figure 2**). Of the three hybrids, *D. desulfuricans*-CdS demonstrated highest activity toward photocatalytic hydrogen production. The *D. desulfuricans*-CdS hybrid with optimized Cd concentration and cell loading was able to reach a high apparent quantum yield (AQY) of 4 % under LED illumination with λ = 445 nm even in the absence of an external electron mediator. The enhanced catalytic performance can be explained by the high level of hydrogenase present in the periplasm of *D. desulfuricans*, as opposed to its cytoplasm. The three periplasmic hydrogenases ([FeFe] HydAB, [NiFe] HynAB and [NiFe] HynABC) and two membrane-bound hydrogenases ([NiFe] Ech and [NiFe] Coo) in *D. desulfuricans* allow a more efficient transfer of electrons from the extracellular CdS nanoparticles to the hydrogenases, thereby resulting in higher catalytic activity. The *D. desulfuricans*-CdS hybrid

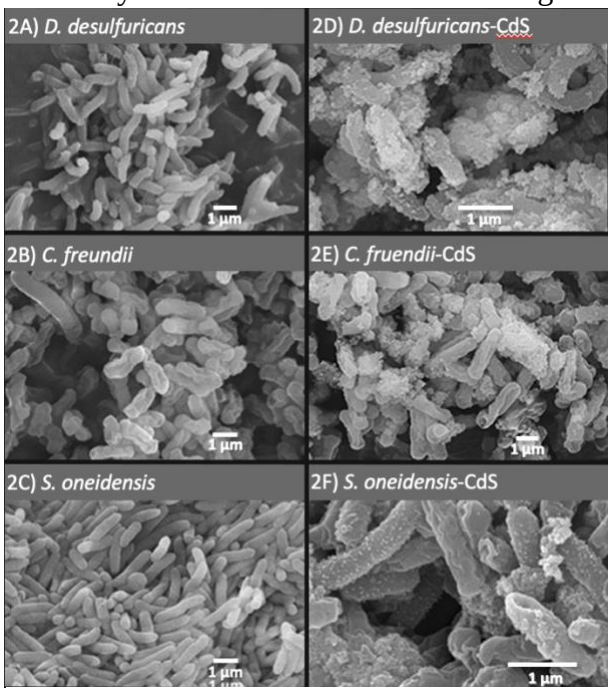

Figure 2. SEM images of pristine cells: A) *D. desulfuricans*, B) *C. freundii*, C) *S. oneidensis*, and CdS biohybrid systems: D) *D. desulfuricans*-CdS, E) *C. freundii*-CdS, F) *S. oneidensis*-CdS.

was also able to generate hydrogen continuously for more than 10 days in the absence of an electron mediator. The high catalytic activity of *D. desulfuricans*-CdS, coupled with its excellent stability, makes it an attractive photocatalyst for sustainable hydrogen production.[11]

To further shorten the electron transfer pathway between the inorganic semiconductor and the biocatalytic site in the microorganism, Zhang and co-workers translocated ultra-small gold nanoclusters (Au NC) into the intracellular cytoplasm of non-photosynthetic bacterium *M. thermoacetica.* The direct transfer of electrons from the intracellular gold nanoclusters to the metabolic pathway of the bacterium resulted in the photogeneration acetic acid from carbon dioxide.[12]

By applying this same concept, Wu and co-workers developed a hybrid system in which light-harvesting graphitic-C_3N_4 quantum dots (QDs), ranging 2-4 nm in size, were distributed over both the periplasm and the cytoplasm of the non-photosynthetic bacterium *E. coli* (**Figure 3a**). Under simulated solar light, the g-C_3N_4 QDs /*E. coli* hybrid reached a state-of-the-art photocatalytic hydrogen generation rate of 7,800 $\pm$ 12 μmol g^{-1} h^{-1}. The researchers attributed the enhanced biomass conversion rate to the formation of a unique junction between the g-C_3N_4 QD and NAD^+, a photo-sensitive species that is reduced to NADH upon irradiation (**Figure 3b**). In pristine *E. coli*, the conversion of NAD^+ to NADH via solar light irradiation facilitates hydrogen production by promoting glycolysis while inhibiting pyruvate-to-lactate conversion (**Figure 3c**). In the g-C_3N_4 QDs/*E. coli* hybrid system, g-C_3N_4 QDs form a unique junction with NAD^+ through a π-π interaction. This junction allows a direct and efficient transfer of photo-generated electrons from g-C_3N_4 QDs to NAD^+, thereby facilitating its conversion to NADH and promoting the production of hydrogen.[13]

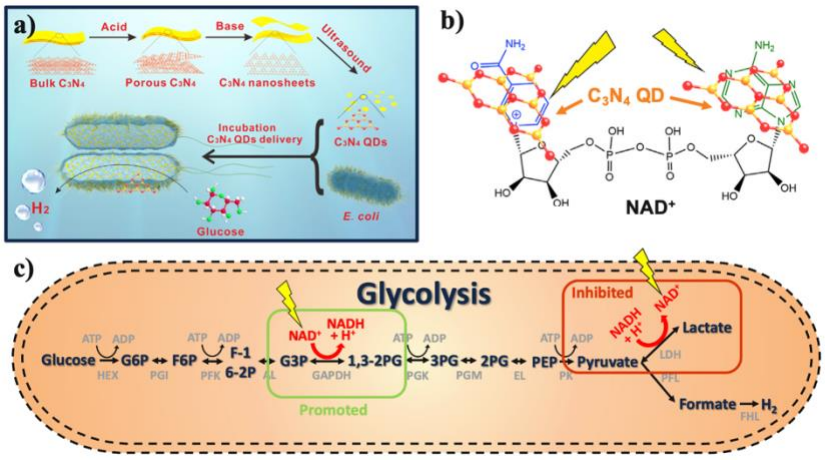

Figure 3. a) Schematic illustration of the fabrication of C_3N_4 QDs/*E. coli* hybrid, b) Schematic diagram of C_3N_4 QDs/NAD^+ junction, c) Diagram of proposed hydrogen production pathway in C_3N_4 QDs/*E. coli* hybrid

Bio-inorganic hybrids for photocatalytic hydrogen production are undoubtedly still in their infancy. Since the first report of a catalytic hybrid system in 2016 by Honda and co-workers, several different semiconductor-microorganism composites have been developed. Despite the advancements, the quantum yields of the hybrids calculated in the absence of extracellular electron mediator still remain under 10 %. The pathway that the photo-generated electron travels from the semiconductor to the biocatalytic site within the bacteria remains largely unknown. The impacts that the semiconductors have on cell metabolisms, and whether they can be precisely tuned to facilitate the catalytic activity as desired, are some of the important questions to be answered by further study. An in-depth understanding of both materials chemistry and synthetic biology is required to develop semiconductor-microorganism hybrid systems with enhanced charge transfer efficiency, improved energy consumption efficiency, and higher stability.

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