特约评述

DOI: 10.12211/2096-8280.2024-022

氢化酶固定化研究进展

雷航彬[1]，何宁[1]，李斐煊[1]，董玲玲[1]，王世珍[1,2]
([1] 厦门大学化学化工学院化学工程与生物工程系，福建 厦门 361005；[2] 厦门大学厦门市合成生物学重点实验室，福建 厦门 361005)

摘要： 氢化酶催化氢气向质子和电子的可逆转化，具有广阔的工业应用前景。但游离的氢化酶存在着对氧气敏感、传递电子速率慢等缺点。本文综述了碳材料、金属及半导体、高分子和金属-有机框架材料（MOF）固定化氢化酶。碳材料具有价格低廉、比表面积大等优势。金属及半导体有着良好的导电性能和优异的催化性能。高分子材料具有良好的生物相容性和机械性能，可以提高氢化酶的稳定性和对氧气的耐受性。MOF比表面积大，可设计调控，为理化性质不同的氢化酶提供了广泛的载体选择。复合材料固定化氢化酶可以结合不同材料的优势，拓宽固定化氢化酶的应用场景。固定化氢化酶可用于氢气的高效生产与应用以及生物不对称加氢制备手性化合物，为转变能源结构、实现绿色转型、解决环境问题提供了可选方案。

关键词： 氢化酶固定化；生物电催化；碳材料；半导体材料；高分子材料；金属-有机框架（MOF）

中图分类号： Q814.2　　**文献标志码：** A

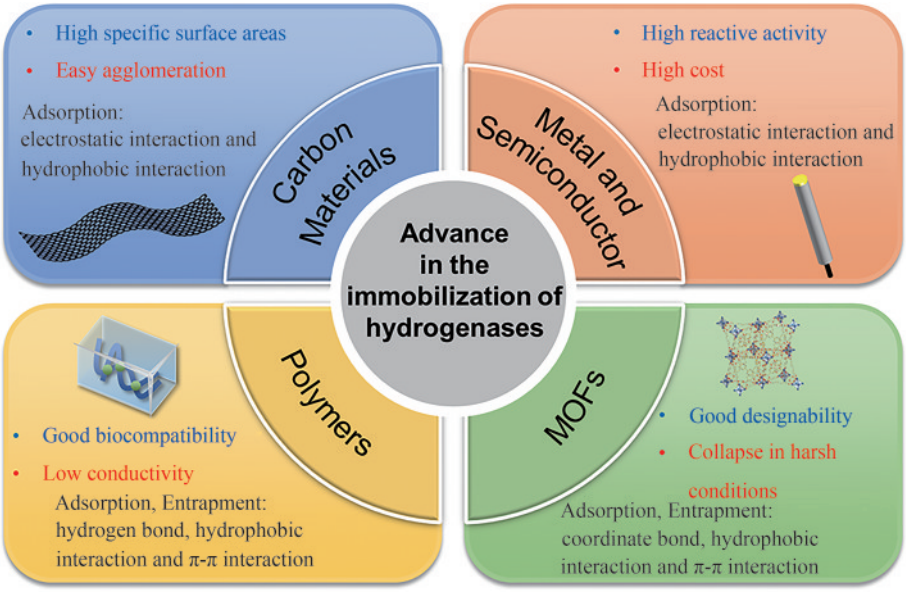
Advance in the immobilization of hydrogenases

LEI Hangbin[1], HE Ning[1], LI Feixuan[1], DONG Lingling[1], WANG Shizhen[1,2]
([1]*Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, Fujian, China*; [2]*The Key Lab for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen 361005, Fujian, China*)

Abstract: Hydrogenases catalyze the reversible conversion of hydrogen gas into protons and electrons which is promising for industrial application. However, free hydrogenases face challenges such as oxygen sensitivity and low electron transfer rates. This review summarized the immobilization of hydrogenases by carbon materials, metals, semiconductors, polymers and metal-organic-frameworks (MOFs). Carbon materials provide the advantages of low cost and large specific surface areas, while they tend to agglomerate. Hydrogenases are immobilizated on carbon materials through adsorption, usually involving electrostatic interactions and hydrophobic interactions, and are used in bioelectrocatalysis, biofuel cells and bioreactors. Metals and semiconductors, known for high conductivity and excellent reactive activity, are expensive and less stable. Through adsorption involving electrostatic interaction and

收稿日期：2024-03-11 修回日期：2024-05-17
基金项目：国家重点研发计划“合成生物学”重点专项“糖水氢电系统——体外多酶高效产氢及氢电装置的基础及工程研究”（2022YFA0912003）
引用本文：雷航彬，何宁，李斐煊，董玲玲，王世珍. 氢化酶固定化研究进展[J]. 合成生物学，2024，5(6)：1485-1497
Citation：LEI Hangbin，HE Ning，LI Feixuan，DONG Lingling，WANG Shizhen. Advance in the immobilization of hydrogenases[J]. Synthetic Biology Journal，2024，5(6)：1485-1497

hydrophobic interaction, immobilization of hydrogenases on metals and semiconductors are normally applied in bioelectrocatalysis, biofuel cells and photoelectrocatalysis. Polymers have good biocompatibility and mechanical strength but low conductivity. Immobilization of hydrogenases on polymers can improve the stability and oxygen tolerance of hydrogenases. Immobilization on polymers is realized through adsorption and entrapment, involving hydrogen bonds, hydrophobic interactions and π - π interactions, and is often used in bioelectrocatalysis and photoelectrocatalysis. MOFs are designable and have high specific surface areas, which provide wide choices for hydrogenases immobilization. However, MOFs tend to collapse in harsh conditions. Immobilization on MOFs through adsorption and entrapment involves coordinate bonds, hydrophobic interaction, and π-π interaction. Furthermore, the prospect of immobilization of hydrogenases by novel hybrid materials was proposed which can expand the applications of immobilized hydrogenases. Immobilization of hydrogenases facilitates the stability of hydrogenases, which can be applied in efficient production and application of hydrogen, as well as biological asymmetric hydrogenation for chiral medicine preparation. Immobilization of hydrogenases provide alternative options for transforming energy structures, realizing green manufacturing and solving environmental problems.



Keywords: immobilization of hydrogenases; bioelectrocatalysis; carbon materials; semiconductors; polymers; metal-organic frameworks

氢化酶（hydrogenases）能可逆地催化氢气转变为质子和电子，可以应用于合成化学[1]、生物催化[2]、生物燃料电池和绿色能源生产[3]等方面。氢气具有高热值、可循环再生、不产生污染等优点[4]，是一种替代化石能源的清洁能源，深受科研人员的关注[5]。制备氢气的方法可以分为热法、光催化法、电催化法和生物催化法[6]。氢化酶可将质子催化为氢气，是生物催化法制氢的关键酶。根据氢化酶活性位点金属离子的种类，将氢化酶分为镍铁氢化酶（[NiFe] hydrogenase）、铁铁氢化酶（[FeFe] hydrogenase）和唯铁氢化酶（[Fe] hydrogenase）（图1）[7]，镍铁硒氢化酶（[NiFeSe] hydrogenase）也属于镍铁氢化酶的一种。从催化的反应方向来看，大多数镍铁氢化酶是氢气摄取型的，偏向将氢气氧化为质子。铁铁氢化酶主要是产生分子氢，唯铁氢化酶通过氢气异裂催化特殊反应[8]，可以将甲烯基四氢甲基蝶呤不对称加氢还原为亚甲基四氢甲基蝶呤[9]，这一催化过程

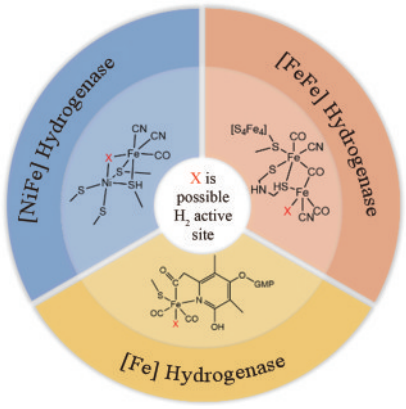


图1 镍铁氢化酶、铁铁氢化酶和唯铁氢化酶的活性位点和催化机理[7]

Fig. 1 Active site and catalytic mechanism of [NiFe] hydrogenase, [FeFe] hydrogenase and [Fe] hydrogenase[7] (Y is methenyltetrahydromethanopterin)

是微生物将二氧化碳转化为甲烷的一个关键步骤。从对氧气的耐受性来看，镍铁氢化酶对氧气有一定的耐受能力[10]，在有氧的环境下失去活性，在具有合适的还原剂的情况下可以恢复活性[11]。铁铁氢化酶对氧气高度敏感，在有氧气的情况下会发生不可逆的失活[12]。唯铁氢化酶也对氧气敏感，在有氧的环境下失活。

固定化酶能够提高酶的活性和稳定性，常用的固定化技术包括物理吸附、包埋和共价结合[13]。氢化酶固定化可以提高其稳定性和对氧气的耐受性[14]，有利于将氢化酶应用在工业生产中。氢化酶固定化的研究还存在氢化酶对氧气敏感、传递电子速率慢等挑战。目前氢化酶固定化综述论文较少。本文梳理了氢化酶固定化的研究进展，总结了碳材料、金属及半导体、高分子以及金属-有机框架固定化氢化酶。本文重点讨论氢化酶固定化载体的选择以及氢化酶在制备氢气、生物电催化、生物燃料电池和电化学传感器等方面的应用展望。

1 碳材料固定化氢化酶

碳材料由于其价格低廉、比表面积大、电导率高等优点[15]，用于氢化酶固定化的载体可以提高氢化酶的稳定性[16]。碳材料固定化氢化酶可以应用在生物电催化、生物燃料电池、生物反应器等方面（表1）。

碳材料固定化氢化酶可以提高氢化酶的稳定性。Liu等[34]将聚(4-乙烯基吡啶)(P4VP)共价固定在多壁碳纳米管（MWNT）表面，再用碘甲烷（CH_3I）烷基化，生成MWNT-P4VPMe。MWNT-P4VPMe吸附固定化来源于*Thiocapsa roseopersicina*的氢化酶，在室温（25 ℃）下储存41天，用磷酸盐缓冲液（pH 7.0）测量酶活，仍能保持最初活性的62.26%。Mazurenko等[35]使用模板法制备多孔碳材料，固定来源于*Aquifex aeolicus*的氢化酶发现不同孔径分布的碳材料固定化效果差别显著，大于酶直径的大孔径有利于提高酶负载量来增强催化电流，与酶的大小接近的孔构成限域空间，可产生笼效应，降低酶的侧链热运动，从而抑制亚基的解离和肽链的解链，大幅提升酶在高温下的稳定性。Sun等[36]将吡啶硫基改性的多壁碳纳米管通过Langmuir-Blodgett方法转移到基底表面上并制备成膜，进而将氢化酶吸附到膜上，提高了氢化酶的稳定性和催化效率。

固定化氢化酶可以提高氢化酶生物电极和氢化酶燃料电池的催化效率和稳定性。碳材料由于其易于活化的性质，通过简单的活化、修饰即可作为电极。Wang等[37]将来源于*Pyrococcus furiosus*的氢化酶吸附在多壁碳纳米管修饰的玻碳电极上，构建了高效的酶-电极界面，提高了氢化酶的电子传递速率和生物电催化反应。Gentil等[38]报道了功能化碳纳米管固定来源于*Desulfomicrobium baculatum*的氢化酶，将其集成到传统的质子交换膜燃料电池中，防止空气中的氧气扩散到阳极，使氢化酶能够氧化氢气而不失活。该燃料电池最大功率密度

表1 碳材料固定化氢化酶的应用

Table 1 Applications of carbon materials for hydrogenases immobilization

材料	具体固定化载体	氢化酶来源	分类	应用	参考文献
碳材料	石墨	*Escherichia coli* ([NiFe])	[NiFe]	生物电催化	[17]
	石墨	*Aquifex aeolicus*	[NiFe]	生物电催化	[18]
	石墨	*Clostridium acetobutylicum*	[FeFe]	生物电催化	[19]
	石墨	*Ralstonia eutropha*	[NiFe]	生物电催化	[20]
	石墨	*Desulfovibrio gigas*	[NiFe]	生物电催化	[21]
	石墨	*Ralstonia metallidurans*	[NiFe]	生物燃料电池	[22]
	石墨	*Allochromatium vinosum*	[NiFe]	动力学研究	[23]
	碳黑	*Ralstonia eutropha*	[NiFe]	光谱电化学研究	[24]
	碳丝	*Thiocapsa roseopersicina*	[NiFe]	生物燃料电池	[25]
	碳丝	*Thiocapsa roseopersicina*	[NiFe]	生物反应器	[26]
	碳纸	*Pyrococcus furiosus*	[NiFe]	生物燃料电池	[27]
	碳毡	*Clostridium acetobutylicum*	[NiFe]	生物燃料电池	[28]
	单壁碳纳米管	*Clostridium acetobutylicum*	[FeFe]	生物电催化	[29]
	单壁碳纳米管	*Clostridium acetobutylicum*	[FeFe]	生物电催化	[30]
	单壁碳纳米管	*Allochromatium vinosum*	[FeFe]	生物电催化	[31]
	多壁碳纳米管	*Aquifex aeolicus*	[NiFe]	电化学传感器	[32]
	多壁碳纳米管	*Ralstonia eutropha*	[NiFe]	生物燃料电池	[33]
		Aquifex aeolicus			

为0.89 mW/cm^2，连续运行1 h后输出下降20%。Tsygankov等[39]报道了疏水性碳电极固定来源于*Thiocapsa bogorovii*的氢化酶。氢化酶电极和铂基氧电极组成的燃料电池，可达到2 mW/cm^{2}的功率密度和6 mA/cm^{2}的电流密度。该燃料电池在初始功率密度为1.78 mW/cm^{2}测试49天，依然保持电化学活性。

氢化酶固定在电极上的取向显著影响酶电极的电化学性能，需要根据氢化酶的性质选取合适的固定化载体。Ruiz-Rodríguez等[40]使用软件PyGBe来计算模拟镍铁氢化酶（PDB：1e3d）与石墨电极之间相互作用的静电分量，考察pH、盐浓度和电势对酶定向固定化的影响。pH影响氢化酶表面电荷从而影响氢化酶在石墨电极上的取向，盐浓度通过影响离子强度调整氢化酶取向。电势会影响石墨电极表面电荷，而对氢化酶取向的影响相对较小。Wang等[41]将来源于*Pyrococcus furiosus*的氢化酶吸附在功能化多壁碳纳米管上修饰到玻碳电极上。发现带正电的和带负电的多壁碳纳米管上氢化酶的取向相反，说明静电相互作用影响显著。使用改性后亲水的多壁碳纳米管研究疏水相互作用对氢化酶的影响，发现随着亲水性增加，电化学催化的电流也随之增加。因此，静电相互作用和疏水相互作用的协同作用影响了氢化酶在电极上的取向。

2 金属及半导体固定化氢化酶

金属及半导体有着良好的导电能力，是固定化酶的良好载体。金属及半导体固定化氢化酶可以应用于生物电催化、生物燃料电池、光电催化等方面（表2）。

2.1 金属固定化氢化酶

金属电极有着良好的导电能力，金属电极固定化氢化酶常用于生物电催化、生物燃料电池等。与碳材料电极不同，金属电极固定化酶通常是使用自组装单分子层来修饰金属电极。氢化酶在裸露的金属电极表面固定效果差，使用自组装单分子层修饰金属电极有助于氢化酶的固定化。

金属电极表面的性质对氢化酶固定化影响显著。金属电极表面亲水性越强，越有利于氢化酶电子转移。金属电极表面电场强度越高，氢化酶

表2 金属及半导体材料固定化氢化酶的应用

Table 2 Applications of metals and semiconductors for hydrogenases immobilization

材料	具体固定化载体	氢化酶来源	分类	应用	参考文献
金属	金电极	*Chlamydomonas reinhardtii*	[FeFe]	生物电催化	[42]
	金电极	*Desulfovibrio vulgaris*	[NiFe]	电化学研究	[43]
	金电极	*Desulfovibrio vulgaris*	[NiFe]	电化学分析	[44]
	金电极	*Ralstonia eutropha*	[NiFe]	电化学研究	[45]
	金电极	*Desulfovibrio vulgaris*	[NiFe]	生物电催化	[46]
	金电极	*Ralstonia eutropha*	[NiFe]	生物电催化	[47]
	硫醇修饰金电极	*Aquifex aeolicus*	[NiFe]	生物电催化	[48]
	紫精修饰金电极	*Desulfovibrio desulfuricans*	[FeFe]	生物燃料电池	[49]
	碳纳米管修饰金电极	*Desulfovibrio gigas*	[NiFe]	生物燃料电池	[50]
	碳纳米管修饰金电极	*Desulfovibrio fructosovorans*	[NiFe]	生物燃料电池	[51]
	纳米金电极	*Aquifex aeolicus*	[NiFe]	生物燃料电池	[52]
	纳米金电极	*Allochromatium vinosum*	[NiFe]	单酶分子电化学	[53]
	银纳米团簇	*Escherichia coli*	[NiFe]	光电催化	[54]
半导体	TiO_2	*Thiocapsa roseopersicina*	[NiFe]	光电催化	[55]
	TiO_2	*Desulfomicrobium baculatum*	[NiFeSe]	光电催化	[56]
	TiO_2	*Desulfomicrobium baculatum*	[NiFeSe]	光电催化	[57]
	TiO_2	*Clostridium acetobutylicum*	[FeFe]	生物电催化	[58]
	PVK|IO-TiO_2	*Desulfovibrio vulgaris*	[NiFeSe]	光电化学集成系统	[59]
	ITO	*Desulfovibrio vulgaris*	[NiFe]	光电催化	[60]
	ITO	*Desulfomicrobium baculatum*	[NiFeSe]	光电催化	[61]
	ITO	*Ralstonia eutropha*	[NiFe]	生物电子设备	[62]
	CN_x（氮化碳）	*Desulfomicrobium baculatum*	[NiFeSe]	光电催化	[63]
	CdS	*Clostridium acetobutylicum*	[FeFe]	光电催化	[64]
	CdS	*Clostridium acetobutylicum*	[FeFe]	电子转移动力学研究	[65]
	CdTe	*Clostridium acetobutylicum*	[FeFe]	光电催化	[66]
	CdTe	*Thiocapsa roseopersicina*	[NiFe]	光电催化	[67]
	In_2S_3	*Desulfovibrio vulgaris*	[NiFeSe]	光电催化	[68]
	FTO-NiO-In_2S_3	*Desulfovibrio vulgaris*	[NiFeSe]	光电催化	[69]

与金属电极表面结合得越紧密。Ciaccafava等[70]将1-丁硫醇自组装单分子层修饰的金电极吸附来源于*Aquifex aeolicus*的膜结合镍铁氢化酶。研究发现氢化酶固定在亲水表面上无特定构象，酶催化氢气氧化过程包含直接电子转移（direct electron transfer，DET）和间接电子转移（mediated electron transfer，MET）。由于疏水作用，氢化酶固定在疏水界面上有特定构象，氢化酶的活性位点倾向于远离疏水表面，酶催化氢气氧化过程仅包含间接电子转移（MET）（图2）。Gutiérrez-Sánchez等[71]使用4-ATP自组装单分子层修饰的金电极固定来源于*Desulfovibrio vulgaris*的膜结合氢化酶，电子传递的方式与氢化酶位点和电极表面的距离有关。当氢化酶固定化取向为活性位点靠近电极，电极与氢化酶之间的电子传递是直接电子转移（DET）。而当活性位点远离电极，电极与氢化酶之间的电子传递为间接电子转移（MET）。Utesch等[72]利用烷硫醇自组装单分子层修饰的金电极吸附固定化来源于*Desulfovibrio gigas*的镍铁氢化酶。基于分子动力学模拟并集合表面增强红外吸收实验验证，发现氢化酶的固定化与修饰金电极表面电场强度具有强相关性。修饰电极表面电场强度越高，吸附效果越好。推测电场强度影响氢化酶的构象，从而影响固定化的效果。

金属电极固定化氢化酶可以降低析氢过电位，稳定氢化酶。Krassen等[73]将来源于*Chlamydomonas*

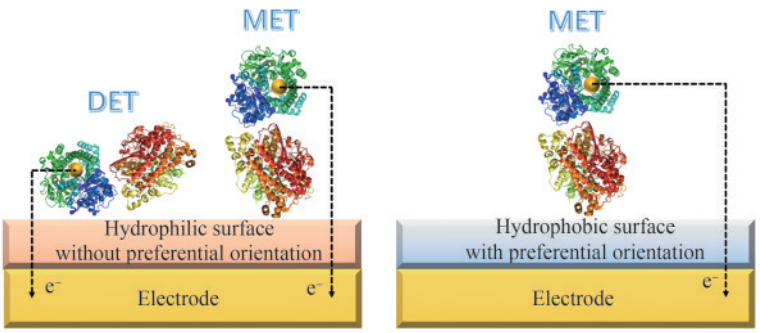


图2 电化学界面疏水性对氢化酶电子传递的影响[70]

Fig. 2 The effect of hydrophobicity of the electrode interface on hydrogenases electron transfer[70]

*reinhardtii*的氢化酶固定在用巯基丙酸自组装单分子层修饰的金电极表面上，固定化氢化酶产氢的比活为1.3 U/mg。以甲基紫精为中介体的裸金电极析氢过电位为−460 mV *vs.* NHE，使用氢化酶固定在用巯基丙酸自组装单分子层修饰的金电极表面析氢过电位为−290 mV *vs.* NHE，降低了析氢过电位。Harris等[74]将来源于*Ralstonia eutropha*的膜结合耐氧镍铁氢化酶直接吸附在金电极上，并利用表面增强红外吸收验证氢化酶与金电极之间的直接电子转移。Sezer等[75]将由HoxG、HoxK和HoxZ三个亚基组成的来源于*Ralstonia eutropha*的镍铁氢化酶固定在银电极上，发现当氢化酶固定在电极上时，HoxZ亚基主要是起到稳定氢化酶的作用，而对电子传递影响较小。

2.2 半导体固定化氢化酶

半导体可以促进氢化酶的电子传递，是固定化氢化酶的优良载体。Liu等[76]将铁氧还蛋白标记的铁铁氢化酶（*Chlamydomonas reinhardtti*）固定在黑色TiO_2纳米管上，实现直接电子传递。Davis等[77]将来源于*Cupriavidus necator*的镍铁氢化酶固定在富含锡的ITO电极上，在电位为−339 mV *vs.* SHE的条件下质子还原电流密度为−10.1 μA/cm^2，远高于相同条件下将镍铁氢化酶固定在玻碳电极上的−1.0 μA/cm^2。

半导体具有良好的光电化学性质，可以作为光电化学阴极分解水产生氢气[78]。氢化酶作为光电阴极的析氢催化剂，可以替换铂等贵金属催化剂，降低析氢反应（hydrogen evolution reaction，HER）的电位势垒。Tian等[79]将来源于*CrHydA1*的氢化酶固定在Cu_2O-ZnO电极上，在电位为0 V *vs.* RHE的条件下100 mW/cm^{2}光照200 s，产生了35.5 mC的电荷和0.68 nmol氢气。可能是由于非生产性副反应的发生，法拉第效率仅为1%。Lee等[80]将来源于*Desulfomicrobium baculatum*的氢化酶固定在有TiO_2涂层的p-Si光电阴极上。在电位为0 V *vs.* RHE、10 mW/cm^{2}光照1 h，产生了1 mC±0.2 mC的电荷和25 nmol±2 nmol的氢气，法拉第效率达到95%±6%。Luna-López等[81]将来源于*Desulfovibrio vulgaris*的氢化酶固定在FTO-$CuGaS_2$电极上，在电位为0.42 V *vs.* RHE。可见光照射135 min的条件下反应，产生了1.66 C的电荷和3.7 μmol氢气，法拉第效率为86%。

3 高分子固定化氢化酶

高分子作为固定化酶的载体有着生物相容性好、稳定性高等优点[82]。高分子固定化氢化酶可以提高氢化酶的稳定性（表3），增强氢化酶对氧气的耐受性[89]。

Ruth等[90]报道了使用环戊二烯基钴功能化的聚丙烯胺水凝胶将来源于*Clostridium pasteurianum*的铁铁氢化酶固定在旋转圆盘电极上。环戊二烯基钴功能化的聚丙烯胺水凝胶可以促进氢化酶与电极之间的间接电子转移（MET）。在改良的Britton-Robinson缓冲液（pH=6），−0.9 V *vs.* SHE的条件下进行电化学产氢，反应19 h后生产17 mmol/mg。Tapia等[91]使用二茂钴功能化支链聚乙烯亚胺（Cc-BPEI）将来源于*Desulfovibrio gigas*的氢化酶固定在金电极上，并和光系统Ⅰ（PSⅠ）蛋白相结合构建起光电催化体系。Cc-BPEI大幅提升光电催化性能，黑暗条件下的酶电极电

表3 高分子固定化氢化酶稳定性

Table 3 Stability of hydrogenases immobilized by polymers

固定化载体	氢化酶来源	分类	储存/反应条件及剩余酶活	参考文献
紫精凝胶	*Desulfovibrio vulgaris*	[NiFe]	4 ℃，磷酸盐缓冲液pH=7.0，储存20天保持50%的酶活	[83]
聚合物多孔凝胶	*Clostridium pasteurianum*	[Fe]	室温，厌氧缓冲液pH=8.0，储存28天保持70%的活性	[84]
聚合物多孔凝胶	*Lamprobacter modestogalophilus*	[NiFe]	室温，厌氧缓冲液pH=8.0，储存28天保持50%的活性	[84]
海藻酸钙凝胶	*Desulphovibrio desulphuricans*	[NiFe]	4 ℃，Tris-HCl缓冲液pH=7.5，储存40天保持60%的活性	[85]
海藻酸钙凝胶	*DesuEfouibrio* sp.	[NiFe]	30 ℃，Tris-HCl缓冲液pH=7.6，反应50 h保持40%的活性	[86]
阴离子交换树脂	*Ralstonia eutropha*	[NiFe]	35 ℃，Tris-HCl缓冲液pH=8.0，反应32 h保持50%的活性	[87]
琼脂糖凝胶	*Chromatium vinosum*	[NiFe]	65 ℃，Tris-HCl缓冲液pH=8.0，孵育80 min保持50%的稳定性	[88]

流密度达到了−30 μA/cm^2。

氧气对氢化酶活性和稳定性的影响显著。Kalms等[92]利用蛋白质X射线晶体学和计算研究来源于*Ralstonia eutropha*的膜结合镍铁氢化酶的氧失活机理。在7 MPa的环境下将镍铁氢化酶浸泡在分子氧中，随后，对气压池进行减压，并将晶体转移到液氮中，得到氧气衍生化镍铁氢化酶晶体。通过蛋白质X射线晶体学解出氧气衍生化氢化酶晶体的结构，从而界定了分子氧到酶的活性位点之间的直接路径，揭示了氧气对氢化酶的抑制作用机理，提出提高固定化氢化酶的耐氧性策略。高分子凝胶富含活性基团，价格低廉[93]，是固定化酶的优良载体。使用凝胶固定化氢化酶可以有效避免氢化酶受到氧气的毒害[94]。Rengaraj等[95]使用红素氧还蛋白自组装形成氧化还原纳米线，将其滴涂在玻碳电极上形成氧化还原水凝胶，固定来源于*Aquifex aeolicus*的镍铁氢化酶。氧化还原水凝胶形成了厌氧保护层，从而使镍铁氢化酶免受氧气的损害。Oughli等[96]使用硫化钠保护来源于*Desulfovibrio desulfuricans*的铁铁氢化酶，硫化钠和铁铁氢化酶可逆反应生成空气中稳定的无活性的氢化酶（H_{inact}），在有氧的环境下可稳定数天。将无活性的氢化酶（H_{inact}）包埋入氧化还原水凝胶中，在有氢气存在的情况下重新激活氢化酶（H_{act}），以达到保护氢化酶的目的。高分子固定化氢化酶可以应用于生物电催化、光电催化等方面。

4 金属-有机框架（MOF）固定化氢化酶

金属-有机框架（metal-organic-framework，MOF）由金属离子和有机配体合成。MOF富含孔隙，具有比表面积大和结构可设计调控等优势，是固定化酶的良好载体[97]。MOF可以通过载体-酶之间配位、氢键、疏水、π-π相互作用等作用力固定化酶[98]。然而，目前为止MOF固定化天然氢化酶的文章还很少，更多的是固定化人工氢化酶，即使用MOF固定氢化酶活性位点以及使用MOF固定氢化酶模拟物。Pullen等[99]报道了使用Zr基MOF（UiO-66）固定具有铁铁氢化酶活性位点的络合物［Fe_2(dcbdt)$(CO)_6$］（dcbdt为1,4-二羧基苯-2,3-二硫醇酯），促进质子的还原。Wang等[100]报道了通过点击反应将铁铁氢化酶催化位点（Fe_2S_2）固定在［Ru(bpy)$_3$］$^{2+}$修饰的UiO-MOF上，提高了氢气产量。Castner等[101]使用有机氧化还原活性萘二酰亚胺基模拟电子传递链，使用络合物［Fe_2(dcbdt)$(CO)_6$］模拟铁铁氢化酶活性位点，将其固定在PCN-700中，加强了电子传递。Balestri等[102]将镍铁氢化酶模拟物（［$L^{N2S2}Ni^{II}Fe^{II}Cp(CO)$］$BF_4$）使用包埋的方法固定在PCN-777中，展现了优良的电化学催化性能。

MOF固定化氢化酶的难点在于与氢化酶生物相容性好的MOF较少。利用MOF具有可设计、调控的优势，根据不同来源氢化酶的理化性质和催化条件选择合适的MOF载体。部分MOF合成反应条件较为温和，例如ZIF-8在室温水溶液即可合成[103]。氢化酶固定化效果与MOF的孔径大小、带电性、亲疏水等性质有关，制备新型MOF有利于拓宽固定化氢化酶的应用场景。导电MOF有利于氢化酶的电催化效率。含Fe^{2+}的耐氧MOF[104]有利于为氢化酶提供无氧环境。与单金属MOF相比，双金属MOF可以提供更加丰富的活性位点，更好地对孔隙率进行调节[105]。双金属的协同作用可加速电子转移[106]，有望提高固定化氢化酶的活性。

5 总结与展望

体外氢化酶催化体系存在着氧敏感、传递电子速率慢等挑战。本文对碳材料、金属及半导体、高分子、MOF固定化氢化酶进行了总结（图3）。碳材料价格低廉、比表面积大，但容易团聚。碳材料主要通过吸附固定化氢化酶，碳和氢化酶之间的静电相互作用和疏水相互作用有利于提高固定化氢化酶的稳定性，在燃料电池、生物电催化、生物反应器等方面有着良好的应用。金属及半导体有着良好的导电能力和优异的催化性能，但是价格较高。金属及半导体与氢化酶之间存在静电相互作用和疏水相互作用，金属及半导体固定化氢化酶可以降低电解水的过电位，增强电子转移，常应用于生物燃料电池、光电催化等。高分子生物相容性好、稳定性高，但导电能力较弱。高分子通过吸附和包埋作用固定化氢化酶，利用其与氢化酶之间的氢键、疏水作用和π-π相互作用，增强氢化酶的稳定性和氧气耐受性。MOF具有结构可设计调控的优点，但是结构在极端条件下不稳定。MOF与氢化酶之间具有配位键、疏水作用和π-π相互作用，通过吸附和包埋作用固定化氢化酶。MOF固定化氢化酶在生物电化学催化、燃料电池等方面有着良好的应用前景。

复合材料有望结合不同材料的优点。制备碳材料与高分子复合的材料，具有碳材料的高导电性以及高分子的良好生物相容性。制备碳材料掺杂的MOF，可以增强导电性能。制备多金属MOF，利用多金属的协同作用加速电子转移，从而提高固定化氢化酶的活性，以适应多样化的应用条件。选取合适的固定化材料有利于克服氢化酶对氧气敏感、传递电子速率慢等问题。细胞层面的酶固定化具有酶的高负载率以及良好的生物相容性[107]，有望改善氢化酶对于氧气敏感的特点。新型纳米载体具有扩散限制小、表面积大和传质阻力低的优点[108]，有望提高氢化酶的电子传递速率。

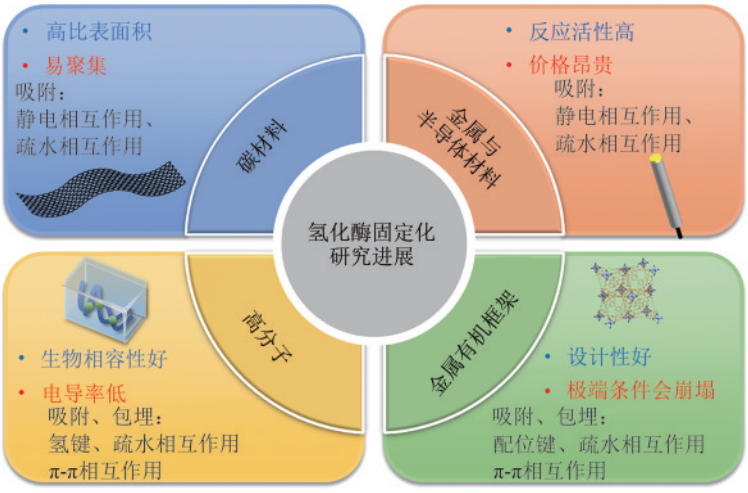


图3 不同材料固定氢化酶总结

Fig. 3 Summarization of different materials for hydrogenases immobilization

参 考 文 献

[1] WANG C, LAI Z L, HUANG G F, et al. Current state of [Fe]-hydrogenase and its biomimetic models[J]. Chemistry, 2022, 28(57): e202201499.

[2] AL-SHAMERI A, SIEBERT D L, SUTIONO S, et al. Hydrogenase-based oxidative biocatalysis without oxygen[J]. Nature Communications, 2023, 14(1): 2693.

[3] JI H S, WAN L, GAO Y X, et al. Hydrogenase as the basis for green hydrogen production and utilization[J]. Journal of Energy Chemistry, 2023, 85: 348-362.

[4] WANG X, FU J Q, LIU Z, et al. Review of researches on important components of hydrogen supply systems and rapid hydrogen refueling processes[J]. International Journal of Hydrogen Energy, 2023, 48(5): 1904-1929.

[5] 肖艳, 刘亚君, 冯银刚, 等. 热纤梭菌在生物质能源开发中的合成生物学研究进展[J]. 合成生物学, 2023, 4(6): 1055-1081. XIAO Y, LIU Y J, FENG Y G, et al. Progress in synthetic

biology research of *Clostridium thermocellum* for biomass energy applications[J]. Synthetic Biology Journal, 2023, 4(6): 1055-1081.

[6] MARTINO M, RUOCCO C, MELONI E, et al. Main hydrogen production processes: an overview[J]. Catalysts, 2021, 11 (5): 547.

[7] OGO S, KISHIMA T, YATABE T, et al. [NiFe], [FeFe], and [Fe] hydrogenase models from isomers[J]. Science Advances, 2020, 6(24): eaaz8181.

[8] OGATA H, LUBITZ W. Bioenergetics theory and components | Hydrogenases structure and function[M/OL]//Encyclopedia of Biological Chemistry Ⅲ. Oxford: Elsevier, 2021, 2: 66-73. (2021-08-02)[2024-02-01]. https://doi.org/10.1016/B978-0-12-809633-8.21396-4.

[9] HUANG G F, WAGNER T, WODRICH M D, et al. The atomic-resolution crystal structure of activated [Fe]-hydrogenase[J]. Nature Catalysis, 2019, 2(6): 537-543.

[10] LUBITZ W, OGATA H, RÜDIGER O, et al. Hydrogenases[J]. Chemical Reviews, 2014, 114(8): 4081-4148.

[11] ABOU HAMDAN A, LIEBGOTT P P, FOURMOND V, et al. Relation between anaerobic inactivation and oxygen tolerance in a large series of NiFe hydrogenase mutants[J]. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109(49): 19916-19921.

[12] RODRÍGUEZ-MACIÁ P, REIJERSE E J, VAN GASTEL M, et al. Sulfide protects [FeFe] hydrogenases from O_2[J]. Journal of the American Chemical Society, 2018, 140(30): 9346-9350.

[13] YUSHKOVA E D, NAZAROVA E A, MATYUHINA A V, et al. Application of immobilized enzymes in food industry[J]. Journal of Agricultural and Food Chemistry, 2019, 67(42): 11553-11567.

[14] JIANG Q Y, LI T P, YANG J, et al. Synthetic engineering of a new biocatalyst encapsulating [NiFe]-hydrogenases for enhanced hydrogen production[J]. Journal of Materials Chemistry B, 2023, 11(12): 2684-2692.

[15] PAVLIDIS I V, VORHABEN T, TSOUFIS T, et al. Development of effective nanobiocatalytic systems through the immobilization of hydrolases on functionalized carbon-based nanomaterials[J]. Bioresource Technology, 2012, 115: 164-171.

[16] MOROZOV S V, KARYAKINA E E, ZADVORNYI O A, et al. Bioelectrocatalysis by hydrogenase *Th. roseopersicina* immobilized on carbon materials[J]. Russian Journal of Electrochemistry, 2002, 38(1): 97-102.

[17] QUINSON J, HIDALGO R, ASH P A, et al. Comparison of carbon materials as electrodes for enzyme electrocatalysis: hydrogenase as a case study[J]. Faraday Discussions, 2014, 172: 473-496.

[18] LOJOU É, GIUDICI-ORTICONI M T, BIANCO P. Hydrogenases from the hyperthermophilic bacterium *Aquifex aeolicus*: electrocatalysis of the hydrogen production/consumption reactions at carbon electrodes[J]. Journal of Electroanalytical Chemistry, 2005, 577(1): 79-86.

[19] BAFFERT C, DEMUEZ M, COURNAC L, et al. Hydrogen-activating enzymes: activity does not correlate with oxygen sensitivity[J]. Angewandte Chemie International Edition, 2008, 47(11): 2052-2054.

[20] GOLDET G, WAIT A F, CRACKNELL J A, et al. Hydrogen production under aerobic conditions by membrane-bound hydrogenases from *Ralstonia* species[J]. Journal of the American Chemical Society, 2008, 130(33): 11106-11113.

[21] RÜDIGER O, ABAD J M, HATCHIKIAN E C, et al. Oriented immobilization of *Desulfovibrio gigas* hydrogenase onto carbon electrodes by covalent bonds for nonmediated oxidation of H_2[J]. Journal of the American Chemical Society, 2005, 127 (46): 16008-16009.

[22] VINCENT K A, CRACKNELL J A, CLARK J R, et al. Electricity from low-level H_2 in still air: an ultimate test for an oxygen tolerant hydrogenase[J]. Chemical Communications, 2006(48): 5033-5035.

[23] LAMLE S E, VINCENT K A, HALLIWELL L M, et al. Hydrogenase on an electrode: a remarkable heterogeneous catalyst[J]. Dalton Transactions, 2003(21): 4152-4157.

[24] HEALY A J, ASH P A, LENZ O, et al. Attenuated total reflectance infrared spectroelectrochemistry at a carbon particle electrode; unmediated redox control of a [NiFe]-hydrogenase solution[J]. Physical Chemistry Chemical Physics, 2013, 15(19): 7055-7059.

[25] KARYAKIN A A, MOROZOV S V, KARYAKINA E E, et al. Hydrogen fuel electrode based on bioelectrocatalysis by the enzyme hydrogenase[J]. Electrochemistry Communications, 2002, 4(5): 417-420.

[26] SHASTIK E S, VOKHMYANINA D V, ZORIN N A, et al. Demonstration of hydrogenase electrode operation in a bioreactor[J]. Enzyme and Microbial Technology, 2011, 49(5): 453-458.

[27] JOHNSTON W, COONEY M J, LIAW B Y, et al. Design and characterization of redox enzyme electrodes: new perspectives on established techniques with application to an extremeophilic hydrogenase[J]. Enzyme and Microbial Technology, 2005, 36 (4): 540-549.

[28] HAMBOURGER M, GERVALDO M, SVEDRUZIC D, et al. [FeFe]-hydrogenase-catalyzed H_2 production in a photoelectrochemical biofuel cell[J]. Journal of the American Chemical Society, 2008, 130(6): 2015-2022.

[29] SVEDRUŽIĆ D, BLACKBURN J L, TENENT R C, et al. High-performance hydrogen production and oxidation electrodes with hydrogenase supported on metallic single-wall carbon nanotube networks[J]. Journal of the American

Chemical Society, 2011, 133(12): 4299-4306.

[30] MCDONALD T J, SVEDRUZIC D, KIM Y H, et al. Wiring-up hydrogenase with single-walled carbon nanotubes[J]. Nano Letters, 2007, 7(11): 3528-3534.

[31] HOEBEN F J M, HELLER I, ALBRACHT S P J, et al. Polymyxin-coated Au and carbon nanotube electrodes for stable [NiFe]-hydrogenase film voltammetry[J]. Langmuir, 2008, 24(11): 5925-5931.

[32] KYRPEL T, SASKA V, DE POULPIQUET A, et al. Hydrogenase-based electrode for hydrogen sensing in a fermentation bioreactor[J]. Biosensors & Bioelectronics, 2023, 225: 115106.

[33] MONSALVE K, MAZURENKO I, GUTIERREZ-SANCHEZ C, et al. Impact of carbon nanotube surface chemistry on hydrogen oxidation by membrane-bound oxygen-tolerant hydrogenases[J]. ChemElectroChem, 2016, 3(12): 2179-2188.

[34] LIU J, WU W J, FANG F, et al. Immobilization of hydrogenase on carbon nanotube polyelectrolytes as heterogeneous catalysts for electrocatalytic interconversion of protons and hydrogen[J]. Journal of Nanoparticle Research, 2016, 18(8): 220.

[35] MAZURENKO I, CLÉMENT R, BYRNE-KODJABACHIAN D, et al. Pore size effect of MgO-templated carbon on enzymatic H_2 oxidation by the hyperthermophilic hydrogenase from *Aquifex aeolicus*[J]. Journal of Electroanalytical Chemistry, 2018, 812: 221-226.

[36] SUN Q, ZORIN N A, CHEN D, et al. Langmuir-Blodgett films of pyridyldithio-modified multiwalled carbon nanotubes as a support to immobilize hydrogenase[J]. Langmuir, 2010, 26 (12): 10259-10265.

[37] WANG Y M, SONG Y H, MA C L, et al. Electrochemical characterization of a truncated hydrogenase from *Pyrococcus furiosus*[J]. Electrochimica Acta, 2021, 387: 138502.

[38] GENTIL S, CHE MANSOR S M, JAMET H, et al. Oriented immobilization of [NiFeSe] hydrogenases on covalently and noncovalently functionalized carbon nanotubes for H_2/air enzymatic fuel cells[J]. ACS Catalysis, 2018, 8(5): 3957-3964.

[39] TSYGANKOV A A, ZORIN N A, STARODUBOV A S, et al. A simple method for oriented immobilization of HydSL hydrogenase of *Thiocapsa bogorovii* on carbon electrodes[J]. International Journal of Hydrogen Energy, 2023, 48(100): 39989-39999.

[40] RUIZ-RODRÍGUEZ M A, COOPER C D, ROCCHIA W, et al. Modeling of the electrostatic interaction and catalytic activity of [NiFe] hydrogenases on a planar electrode[J]. The Journal of Physical Chemistry B, 2022, 126(43): 8777-8790.

[41] WANG Y M, KANG Z P, ZHANG L L, et al. Elucidating the interactions between a [NiFe]-hydrogenase and carbon electrodes for enhanced bioelectrocatalysis[J]. ACS Catalysis, 2022, 12(2): 1415-1427.

[42] KRASSEN H, STRIPP S T, BÖHM N, et al. Tailor-made modification of a gold surface for the chemical binding of a high-activity [FeFe] hydrogenase[J]. European Journal of Inorganic Chemistry, 2011, 2011(7): 1138-1146.

[43] MILLO D, PANDELIA M E, UTESCH T, et al. Spectroelectrochemical study of the [NiFe] hydrogenase from *Desulfovibrio vulgaris* Miyazaki F in solution and immobilized on biocompatible gold surfaces[J]. The Journal of Physical Chemistry B, 2009, 113(46): 15344-15351.

[44] MILLO D, HILDEBRANDT P, PANDELIA M E, et al. *SEIRA* spectroscopy of the electrochemical activation of an immobilized [NiFe] hydrogenase under turnover and non-turnover conditions[J]. Angewandte Chemie International Edition, 2011, 50(11): 2632-2634.

[45] HEIDARY N, UTESCH T, ZERBALL M, et al. Orientation-controlled electrocatalytic efficiency of an adsorbed oxygen-tolerant hydrogenase[J]. PLoS One, 2015, 10(11): e0143101.

[46] SHIRAIWA S, SO K, SUGIMOTO Y, et al. Reactivation of standard [NiFe]-hydrogenase and bioelectrochemical catalysis of proton reduction and hydrogen oxidation in a mediated-electron-transfer system[J]. Bioelectrochemistry, 2018, 123: 156-161.

[47] RADU V, FRIELINGSDORF S, EVANS S D, et al. Enhanced oxygen-tolerance of the full heterotrimeric membrane-bound [NiFe]-hydrogenase of *Ralstonia eutropha*[J]. Journal of the American Chemical Society, 2014, 136(24): 8512-8515.

[48] LUO X J, BRUGNA M, TRON-INFOSSI P, et al. Immobilization of the hyperthermophilic hydrogenase from *Aquifex aeolicus* bacterium onto gold and carbon nanotube electrodes for efficient H_2 oxidation[J]. Journal of Biological Inorganic Chemistry, 2009, 14(8): 1275-1288.

[49] OUGHLI A A, VÉLEZ M, BIRRELL J A, et al. Viologen-modified electrodes for protection of hydrogenases from high potential inactivation while performing H_2 oxidation at low overpotential[J]. Dalton Transactions, 2018, 47(31): 10685-10691.

[50] ALONSO-LOMILLO M A, RÜDIGER O, MAROTO-VALIENTE A, et al. Hydrogenase-coated carbon nanotubes for efficient H_2 oxidation[J]. Nano Letters, 2007, 7(6): 1603-1608.

[51] LOJOU É, LUO X, BRUGNA M, et al. Biocatalysts for fuel cells: efficient hydrogenase orientation for H_2 oxidation at electrodes modified with carbon nanotubes[J]. Journal of Biological Inorganic Chemistry, 2008, 13(7): 1157-1167.

[52] MONSALVE K, ROGER M, GUTIERREZ-SANCHEZ C, et al. Hydrogen bioelectrooxidation on gold nanoparticle-based electrodes modified by *Aquifex aeolicus* hydrogenase: application to hydrogen/oxygen enzymatic biofuel cells[J]. Bioelectrochemistry, 2015, 106(Pt A): 47-55.

[53] HOEBEN F J M, MEIJER F S, DEKKER C, et al. Toward

single-enzyme molecule electrochemistry: [NiFe]-hydrogenase protein film voltammetry at nanoelectrodes[J]. ACS Nano, 2008, 2(12): 2497-2504.

[54] ZHANG L Y, MORELLO G, CARR S B, et al. Aerobic photocatalytic H_2 production by a [NiFe] hydrogenase engineered to place a silver nanocluster in the electron relay[J]. Journal of the American Chemical Society, 2020, 142(29): 12699-12707.

[55] NIKANDROV V V, SHLYK M A, ZORIN N A, et al. Efficient photoinduced electron transfer from inorganic semiconductor TiO_2 to bacterial hydrogenase[J]. FEBS Letters, 1988, 234(1): 111-114.

[56] CAPUTO C A, WANG L D, BERANEK R, et al. Carbon nitride-TiO_2 hybrid modified with hydrogenase for visible light driven hydrogen production[J]. Chemical Science, 2015, 6(10): 5690-5694.

[57] REISNER E, POWELL D J, CAVAZZA C, et al. Visible light-driven H_2 production by hydrogenases attached to dye-sensitized TiO_2 nanoparticles[J]. Journal of the American Chemical Society, 2009, 131(51): 18457-18466.

[58] MORRA S, VALETTI F, SADEGHI S J, et al. Direct electrochemistry of an [FeFe]-hydrogenase on a TiO_2 electrode [J]. Chemical Communications, 2011, 47(38): 10566-10568.

[59] EDWARDES MOORE E, ANDREI V, ZACARIAS S, et al. Integration of a hydrogenase in a lead halide perovskite photoelectrode for tandem solar water splitting[J]. ACS Energy Letters, 2020, 5(1): 232-237.

[60] ASAKURA N. Photoinduced hydrogen evolution with Ni-Fe hydrogenase-viologen-porphyrin immobilized ITO electrode [J]. ECS Meeting Abstracts, 2020, MA2020-02(44): 2807.

[61] MERSCH D, LEE C Y, ZHANG J Z, et al. Wiring of photosystem Ⅱ to hydrogenase for photoelectrochemical water splitting[J]. Journal of the American Chemical Society, 2015, 137(26): 8541-8549.

[62] HARRIS T G A A, HEIDARY N, FRIELINGSDORF S, et al. Electrografted interfaces on metal oxide electrodes for enzyme immobilization and bioelectrocatalysis[J]. ChemElectroChem, 2021, 8(7): 1329-1336.

[63] CAPUTO C A, GROSS M A, LAU V W, et al. Photocatalytic hydrogen production using polymeric carbon nitride with a hydrogenase and a bioinspired synthetic Ni catalyst[J]. Angewandte Chemie, 2014, 53(43): 11538-11542.

[64] BROWN K A, WILKER M B, BOEHM M, et al. Characterization of photochemical processes for H_2 production by CdS nanorod-[FeFe] hydrogenase complexes[J]. Journal of the American Chemical Society, 2012, 134(12): 5627-5636.

[65] WILKER M B, SHINOPOULOS K E, BROWN K A, et al. Electron transfer kinetics in CdS nanorod-[FeFe]-hydrogenase complexes and implications for photochemical H_2 generation [J]. Journal of the American Chemical Society, 2014, 136(11): 4316-4324.

[66] BROWN K A, DAYAL S, AI X, et al. Controlled assembly of hydrogenase-CdTe nanocrystal hybrids for solar hydrogen production[J]. Journal of the American Chemical Society, 2010, 132(28): 9672-9680.

[67] GREENE B L, JOSEPH C A, MARONEY M J, et al. Direct evidence of active-site reduction and photodriven catalysis in sensitized hydrogenase assemblies[J]. Journal of the American Chemical Society, 2012, 134(27): 11108-11111.

[68] TAPIA C, ZACARIAS S, PEREIRA I A C, et al. *In situ* determination of photobioproduction of H_2 by In_2S_3-[NiFeSe] hydrogenase from *Desulfovibrio vulgaris* hildenborough using only visible light[J]. ACS Catalysis, 2016, 6(9): 5691-5698.

[69] LUNA-LÓPEZ G, DEL BARRIO M, FIZE J, et al. Photobio-electrocatalytic production of H_2 using fluorine-doped tin oxide (FTO) electrodes covered with a NiO-In_2S_3 p-n junction and NiFeSe hydrogenase[J]. Bioelectrochemistry, 2023, 150: 108361.

[70] CIACCAFAVA A, INFOSSI P, ILBERT M, et al. Electrochemistry, AFM, and PM-IRRA spectroscopy of immobilized hydrogenase: role of a hydrophobic helix in enzyme orientation for efficient H_2 oxidation[J]. Angewandte Chemie International Edition, 2012, 51(4): 953-956.

[71] GUTIÉRREZ-SÁNCHEZ C, OLEA D, MARQUES M, et al. Oriented immobilization of a membrane-bound hydrogenase onto an electrode for direct electron transfer[J]. Langmuir, 2011, 27(10): 6449-6457.

[72] UTESCH T, MILLO D, CASTRO M A, et al. Effect of the protonation degree of a self-assembled monolayer on the immobilization dynamics of a [NiFe] hydrogenase[J]. Langmuir, 2013, 29(2): 673-682.

[73] KRASSEN H, STRIPP S, VON ABENDROTH G, et al. Immobilization of the [FeFe]-hydrogenase CrHydAl on a gold electrode: design of a catalytic surface for the production of molecular hydrogen[J]. Journal of Biotechnology, 2009, 142 (1): 3-9.

[74] HARRIS T G A A, HEIDARY N, KOZUCH J, et al. *In situ* spectroelectrochemical studies into the formation and stability of robust diazonium-derived interfaces on gold electrodes for the immobilization of an oxygen-tolerant hydrogenase[J]. ACS Applied Materials & Interfaces, 2018, 10(27): 23380-23391.

[75] SEZER M, FRIELINGSDORF S, MILLO D, et al. Role of the HoxZ subunit in the electron transfer pathway of the membrane-bound [NiFe]-hydrogenase from *Ralstonia eutropha* immobilized on electrodes[J]. The Journal of Physical Chemistry B, 2011, 115(34): 10368-10374.

[76] LIU X, RISBAKK S, ALMEIDA CARVALHO P, et al. Immobilization of FeFe-hydrogenase on black TiO_2 nanotubes

as biocathodes for the hydrogen evolution reaction[J]. Electrochemistry Communications, 2022, 135: 107221.

[77] DAVIS V, HEIDARY N, GUIET A, et al. Immobilization of O_2-tolerant [NiFe] hydrogenase from *Cupriavidus necator* on tin-rich indium oxide alters the catalytic bias from H_2 oxidation to proton reduction[J]. ACS Catalysis, 2023, 13(9): 6312-6327.

[78] JANG Y J, LEE J S. Photoelectrochemical water splitting with p-type metal oxide semiconductor photocathodes[J]. ChemSusChem, 2019, 12(9): 1835-1845.

[79] TIAN L, NÉMETH B, BERGGREN G, et al. Hydrogen evolution by a photoelectrochemical cell based on a Cu_2O-ZnO-[FeFe] hydrogenase electrode[J]. Journal of Photochemistry and Photobiology A: Chemistry, 2018, 366: 27-33.

[80] LEE C Y, PARK H S, FONTECILLA-CAMPS J C, et al. Photoelectrochemical H_2 evolution with a hydrogenase immobilized on a TiO_2-protected silicon electrode[J]. Angewandte Chemie International Edition, 2016, 55(20): 5971-5974.

[81] LUNA-LÓPEZ G, SAINZ R, COITO A M, et al. Hybrid biological/inorganic photocathode for H_2 production based on a NiFeSe hydrogenase immobilized on electrodeposited CuGaS2 [J]. Catalysis Today, 2023, 423: 114281.

[82] LYU X Y, GONZALEZ R, HORTON A, et al. Immobilization of enzymes by polymeric materials[J]. Catalysts, 2021, 11(10): 1211.

[83] NOSAKA Y, KUWABARA A, KOBAYASHI T, et al. Immobilization of hydrogenase in nylon gel containing electron mediator and application to an artificial photosystem [J]. Biotechnology and Bioengineering, 1986, 28(3): 456-460.

[84] ELGREN T E, ZADVORNY O A, BRECHT E, et al. Immobilization of active hydrogenases by encapsulation in polymeric porous gels[J]. Nano Letters, 2005, 5(10): 2085-2087.

[85] ZIOMEK E, MARTIN W G, VELIKY I A, et al. Immobilization of *Desulphovibrio desulphuricans*: cell-associated hydrogenase in beaded matrices[J]. Enzyme and Microbial Technology, 1982, 4(6): 405-408.

[86] BARRETO M C, CABRAL J M S. Immobilization of *Desulfovibrio vulgaris* cells with hydrogenase activity[J]. Journal of Chemical Technology & Biotechnology, 1991, 50 (4): 563-570.

[87] HERR N, RATZKA J, LAUTERBACH L, et al. Stability enhancement of an O_2^- tolerant NAD^+-reducing [NiFe]-hydrogenase by a combination of immobilisation and chemical modification[J]. Journal of Molecular Catalysis B: Enzymatic, 2013, 97: 169-174.

[88] KLIBANOV A M, KAPLAN N O, KAMEN M D. Thermal stabilities of membrane-bound, solubilized, and artificially immobilized hydrogenase from *Chromatium vinosum*[J]. Archives of Biochemistry and Biophysics, 1980, 199(2): 545-549.

[89] LI H G, MÜNCHBERG U, OUGHLI A A, et al. Suppressing hydrogen peroxide generation to achieve oxygen-insensitivity of a [NiFe] hydrogenase in redox active films[J]. Nature Communications, 2020, 11(1): 920.

[90] RUTH J C, MILTON R D, GU W Y, et al. Enhanced electrosynthetic hydrogen evolution by hydrogenases embedded in a redox-active hydrogel[J]. Chemistry, 2020, 26 (32): 7323-7329.

[91] TAPIA C, MILTON R D, PANKRATOVA G, et al. Wiring of photosystem Ⅰ and hydrogenase on an electrode for photoelectrochemical H_2 production by using redox polymers for relatively positive onset potential[J]. ChemElectroChem, 2017, 4(1): 90-95.

[92] KALMS J, SCHMIDT A, FRIELINGSDORF S, et al. Tracking the route of molecular oxygen in O_2-tolerant membrane-bound [NiFe] hydrogenase[J]. Proceedings of the National Academy of Sciences of the United States of America, 2018, 115(10): E2229-E2237.

[93] SHAKERI F, ARIAEENEJAD S, GHOLLASI M, et al. Synthesis of two novel bio-based hydrogels using sodium alginate and chitosan and their proficiency in physical immobilization of enzymes[J]. Scientific Reports, 2022, 12(1): 2072.

[94] PLUMERÉ N, RÜDIGER O, OUGHLI A A, et al. A redox hydrogel protects hydrogenase from high-potential deactivation and oxygen damage[J]. Nature Chemistry, 2014, 6 (9): 822-827.

[95] RENGARAJ S, HADDAD R, LOJOU E, et al. Interprotein electron transfer between FeS-protein nanowires and oxygen-tolerant NiFe hydrogenase[J]. Angewandte Chemie International Edition, 2017, 56(27): 7774-7778.

[96] OUGHLI A A, HARDT S, RÜDIGER O, et al. Reactivation of sulfide-protected [FeFe] hydrogenase in a redox-active hydrogel[J]. Chemical Communications, 2020, 56(69): 9958-9961.

[97] MUKOYOSHI M, KITAGAWA H. Nanoparticle/metal-organic framework hybrid catalysts: elucidating the role of the MOF [J]. Chemical Communications, 2022, 58(77): 10757-10767.

[98] HU Y L, DAI L M, LIU D H, et al. Progress & prospect of metal-organic frameworks (MOFs) for enzyme immobilization (enzyme/MOFs) [J]. Renewable & Sustainable Energy Reviews, 2018, 91: 793-801.

[99] PULLEN S, ROY S, OTT S. [FeFe] Hydrogenase active site model chemistry in a UiO-66 metal-organic framework[J]. Chemical Communications, 2017, 53(37): 5227-5230.

[100] WANG W J, SONG X W, HONG Z X, et al. Incorporation of iron hydrogenase active sites into a stable photosensitizing

metal-organic framework for enhanced hydrogen production [J]. Applied Catalysis B: Environmental, 2019, 258: 117979.

[101] CASTNER A T, JOHNSON B A, COHEN S M, et al. Mimicking the electron transport chain and active site of [FeFe] hydrogenases in one metal-organic framework: factors that influence charge transport[J]. Journal of the American Chemical Society, 2021, 143(21): 7991-7999.

[102] BALESTRI D, ROUX Y, MATTAROZZI M, et al. Heterogenization of a [NiFe] hydrogenase mimic through simple and efficient encapsulation into a mesoporous MOF[J]. Inorganic Chemistry, 2017, 56(24): 14801-14808.

[103] WU X L, GE J, YANG C, et al. Facile synthesis of multiple enzyme-containing metal-organic frameworks in a biomolecule-friendly environment[J]. Chemical Communications, 2015, 51 (69): 13408-13411.

[104] FERNANDEZ-BARTOLOME E, SANTOS J, KHODABAKHSHI S, et al. A robust and unique iron(ii) mosaic-like MOF[J]. Chemical Communications, 2018, 54(44): 5526-5529.

[105] AHMED M. Recent advancement in bimetallic metal organic frameworks (M'MOFs): synthetic challenges and applications [J]. Inorganic Chemistry Frontiers, 2022, 9(12): 3003-3033.

[106] SHEN H, SHI H M, YANG Y, et al. Highly efficient synergistic biocatalysis driven by stably loaded enzymes within hierarchically porous iron/cobalt metal-organic framework *via* biomimetic mineralization[J]. Journal of Materials Chemistry B, 2022, 10(10): 1553-1560.

[107] DU C, ZHOU Y L, LIU L, et al. Bacterial surface-assembled chitinosome for dismantling chitin into *N*-acetyl glucosamine [J]. ACS Sustainable Chemistry & Engineering, 2023, 11(30): 11239-11247.

[108] ZHOU C, HE N S, LIN X F, et al. Site-directed display of zearalenone lactonase on spilt-intein functionalized nanocarrier for green and efficient detoxification of Zearalenone[J]. Food Chemistry, 2024, 446: 138804.

通讯作者：王世珍（1982—），女，副教授，硕士生导师。研究方向为合成生物学、生物催化与转化、酶工程等。
E-mail：szwang@xmu.edu.cn

第一作者：雷航彬（2000—），男，硕士研究生。研究方向为固定化酶。
E-mail：lhb20000410@qq.com