

特约评述

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氢化酶固定化研究进展

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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 (MOFs) 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。MOFs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 (MOFs)

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Advance of the immobilization of hydrogenases

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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 immobilized on carbon materials through adsorption, usually involving electrostatic interactions and hydrophobic interactions, and are used in bioelectrocatalysis, biofuel cells, bioreactors et al. Metals and semiconductors, known for high conductivity and excellent reactive activity, are expensive and less stable. Through adsorption involving electrostatic interaction and

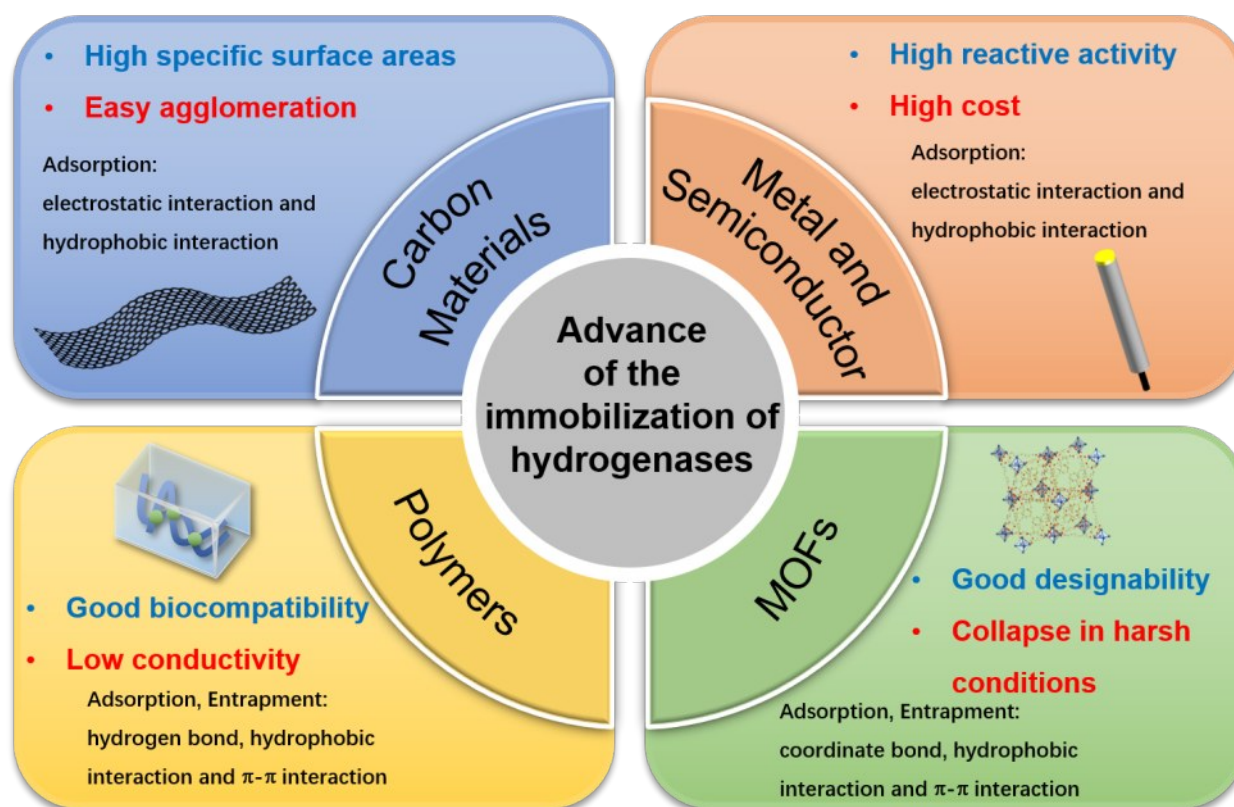
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hydrophobic interaction, immobilization of hydrogenases on metals and semiconductors are normally applied in bioelectrocatalysis, biofuel cells, photoelectrocatalysis et al. Polymers have good biocompatibility and mechanical strength but low conductivity. 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 $\pi - \pi$ interactions, and is often used in bioelectrocatalysis, photoelectrocatalysis et al. 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 $\pi - \pi$ 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蝶呤不对称加氢还原为亚甲基四氢甲蝶呤^[9], 这一催化过程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。^[14]。氢化酶固定化可以提高其稳定性和对氧气的耐受性^[15], 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^[16], 用于氢化酶固定化的载体可以提高氢化酶的稳定性^[17]。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。Wang等^[35]将聚(4-乙烯基吡啶) (P4VP) 共价固定在多壁碳纳米管 (MWNTs) 表面, 再用碘甲烷 (CH₃I) 烷基化, 生成MWNT-P4VPMc。MWNT-P4VPMc吸附固定化来源于 *Thiocapsa roseopersicina* 的氢化酶, 在室温 (25 °C) 下储存41天, 用磷酸盐缓冲液 (pH 7.0) 下测量酶活, 仍能保持最初活性的62.26%。Mazurenko等^[36]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。Qing等^[37]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

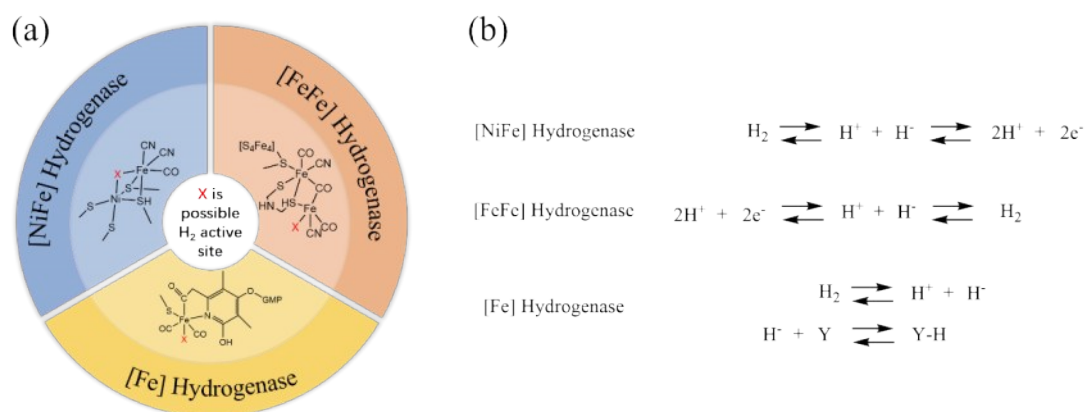


图1 镍铁氢化酶、铁铁氢化酶和唯铁氢化酶的活性位点和催化机理^[13]

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

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	<i>Escherichia coli</i> ([NiFe])	[NiFe]	生物电催化	[18]
	石墨	<i>Aquifex aeolicus</i>	[NiFe]	生物电催化	[19]
	石墨	<i>Clostridium acetobutylicum</i>	[FeFe]	生物电催化	[20]
	石墨	<i>Ralstonia eutropha</i>	[NiFe]	生物电催化	[21]
	石墨	<i>Desulfovibrio gigas</i>	[NiFe]	生物电催化	[22]
	石墨	<i>Ralstonia metallidurans</i>	[NiFe]	生物燃料电池	[23]
	石墨	<i>Allochrodatum vinosum</i>	[NiFe]	动力学研究	[24]
	碳黑	<i>Ralstonia eutropha</i>	[NiFe]	光谱电化学研究	[25]
	碳丝	<i>Thiocapsa roseopersicina</i>	[NiFe]	生物燃料电池	[26]
	碳丝	<i>Thiocapsa roseopersicina</i>	[NiFe]	生物反应器	[27]
	碳纸	<i>Pyrococcus furiosus</i>	[NiFe]	生物燃料电池	[28]
	碳毡	<i>Clostridium acetobutylicum</i>	[NiFe]	生物燃料电池	[29]
	单壁碳纳米管	<i>Clostridium acetobutylicum</i>	[FeFe]	生物电催化	[30]
	单壁碳纳米管	<i>Clostridium acetobutylicum</i>	[FeFe]	生物电催化	[31]
	单壁碳纳米管	<i>Allochrodatum vinosum</i>	[FeFe]	生物电催化	[32]
	多壁碳纳米管	<i>Aquifex aeolicus</i>	[NiFe]	电化学传感器	[33]
	多壁碳纳米管	<i>Ralstonia eutropha</i>	[NiFe]	生物燃料电池	[34]
		<i>Aquifex aeolicus</i>			

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^[38]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。Solène等^[39]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为0.89 mW/cm²，连续运行1 h后输出下降20%。Tsygankov等^[40]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/cm²的功率密度和6 mA/cm²的电流密度。该燃料电池在初始功率密度为1.78 mW/cm²测试49天，依然保持电催化活性。

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。Manuel等^[41]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值、盐浓度和电势对酶定向固定化的影响。pH值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^[42]将来源于*Pyrococcus furiosus*的氢化酶吸附在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）。

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	<i>Chlamydomonas reinhardtii</i>	[FeFe]	生物电催化	[43]
	金电极	<i>Desulfovibrio vulgaris</i>	[NiFe]	电化学研究	[44]
	金电极	<i>Desulfovibrio vulgaris</i>	[NiFe]	电化学分析	[45]
	金电极	<i>Ralstonia eutropha</i>	[NiFe]	电化学研究	[46]
	金电极	<i>Desulfovibrio vulgaris</i>	[NiFe]	生物电催化	[47]
	金电极	<i>Ralstonia eutropha</i>	[NiFe]	生物电催化	[48]
	硫醇修饰金电极	<i>Aquifex aeolicus</i>	[NiFe]	生物电催化	[49]
	紫精修饰金电极	<i>Desulfovibrio desulfuricans</i>	[FeFe]	生物燃料电池	[50]
	碳纳米管修饰金电极	<i>Desulfovibrio gigas</i>	[NiFe]	生物燃料电池	[51]
	碳纳米管修饰金电极	<i>Desulfovibrio fructosovorans</i>	[NiFe]	生物燃料电池	[52]
	纳米金电极	<i>Aquifex aeolicus</i>	[NiFe]	生物燃料电池	[53]
	纳米金电极	<i>Allochrochromatium vinosum</i>	[NiFe]	单酶分子电化学	[54]
	银纳米团簇	<i>Escherichia coli</i>	[NiFe]	光电催化	[55]
半导体	TiO ₂	<i>Thiocapsa roseopersicina</i>	[NiFe]	光电催化	[56]
	TiO ₂	<i>Desulfomicrobium baculatum</i>	[NiFeSe]	光电催化	[57]
	TiO ₂	<i>Desulfomicrobium baculatum</i>	[NiFeSe]	光电催化	[58]
	TiO ₂	<i>Clostridium acetobutylicum</i>	[FeFe]	生物电催化	[59]
	PVK IO-TiO ₂	<i>Desulfovibrio vulgaris</i>	[NiFeSe]	光电化学集成系统	[60]
	ITO	<i>Desulfovibrio vulgaris</i>	[NiFe]	光电催化	[61]
	ITO	<i>Desulfomicrobium baculatum</i>	[NiFeSe]	光电催化	[62]
	ITO	<i>Ralstonia eutropha</i>	[NiFe]	生物电子设备	[63]
	CN _x (氮化碳)	<i>Desulfomicrobium baculatum</i>	[NiFeSe]	光电催化	[64]
	CdS	<i>Clostridium acetobutylicum</i>	[FeFe]	光电催化	[65]
	CdS	<i>Clostridium acetobutylicum</i>	[FeFe]	电子转移动力学研究	[66]
	CdTe	<i>Clostridium acetobutylicum</i>	[FeFe]	光电催化	[67]
	CdTe	<i>Thiocapsa roseopersicina</i>	[NiFe]	光电催化	[68]
	In ₂ S ₃	<i>Desulfovibrio vulgaris</i>	[NiFeSe]	光电催化	[69]
	FTO-NiO-In ₂ S ₃	<i>Desulfovibrio vulgaris</i>	[NiFeSe]	光电催化	[70]

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，氢化酶与金属电极表面结合的越紧密。Alexandre 等^[71] 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（DET）和间接电子转移（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）。Cristina 等^[72] 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（Direct Electron Transfer，

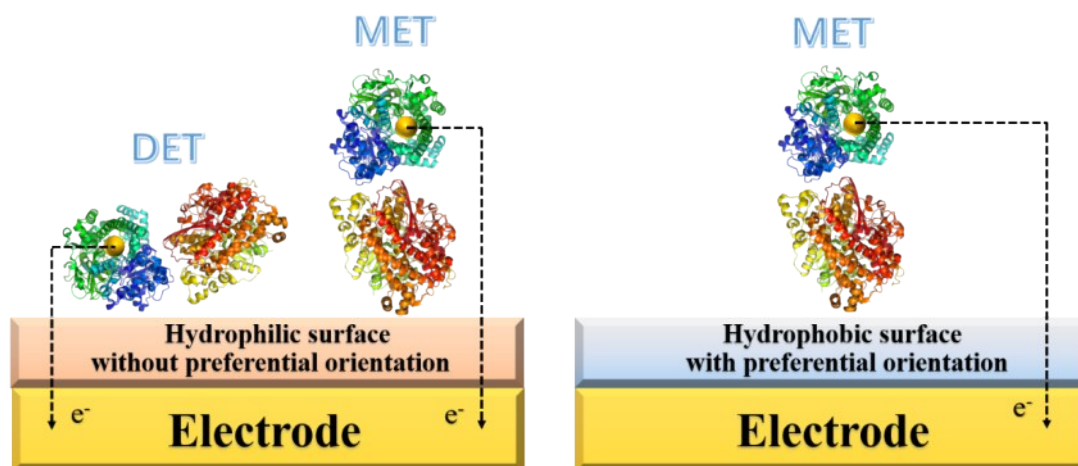


图2 电化学界面疏水性对氢化酶电子传递的影响^[71]

Fig. 2 The effect of hydrophobicity of the electrode interface on hydrogenases electron transfer^[71]

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（Mediated Electron Transfer, MET）。Tillmann等^[73]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。Henning等^[74]将来源于*Chlamydomonas 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-270 mV vs. NHE，降低了析氢过电位。Tomos等^[75]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。Murat等^[76]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。Xin等^[77]将铁氧还蛋白标记的铁氢化酶（*Chlamydomonas reinhardtii*）固定在黑色TiO₂纳米管上，实现直接电子传递。Davis等^[78]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/cm²，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/cm²。

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^[79]。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^[80]将来源于*CrHydA1*的氢化酶固定在Cu₂O-ZnO电极上，在电位为0 V vs. RHE的条件下100 mW/cm²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^[81]将来源于*Desulfomicrobium baculatum*的氢化酶固定在有TiO₂涂层的p-Si光电阴极上。在电位为0 V vs. RHE、10 mW/cm²光照1 h，产生了1±0.2 mC的电荷和25±2 nmol的氢气，法拉第效率达到95±6%。Gabriel等^[82]将来源于*Desulfovibrio vulgaris*的氢化酶固定在FTO-CuGaS₂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^[83]。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^[84]。

John等^[91]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 氢气/mg 氢化酶。Cristina等^[92]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流密度达到了 -30 $\mu\text{A}/\text{cm}^2$ 。

氧气对氢化酶活性和稳定性的影响显著。Jacqueline等^[93]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 70 bar 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^[94]，是固定化酶的优良载体。使用凝胶固定化氢化酶可以有效的避免氢化酶受到氧气的毒害^[95]。Saravanan等^[96]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^[97]使用硫化钠保护来源于 *Desulfovibrio desulfuricans* 的铁氢化酶，硫化钠和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（metal-organic-frameworks, MOFs）固定化氢化酶

金属-有机框架（MOFs）由金属离子和有机配体合成。MOFs 富含孔隙、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^[98]。MOFs 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^[99]。然而，目前为止 MOFs 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 MOFs 固定氢化酶活性位点以及使用 MOFs 固定氢化酶模拟物。Sonja等^[100]报道了使用 Zr 基 MOF（UiO-66）固定具有铁氢化酶活性位点的络合物 $[\text{Fe}_2(\text{dcbdt})(\text{CO})_6]$ （dcbdt

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	<i>Desulfovibrio vulgaris</i>	[NiFe]	4 °C, 磷酸盐缓冲液 pH=7.0, 储存 20 天保持 50% 的酶活	[85]
聚合物多孔凝胶	<i>Clostridium pasteurianum</i>	[Fe]	室温, 厌氧缓冲液 pH=8.0, 储存 28 天, 保持 70% 的活性	[86]
聚合物多孔凝胶	<i>Lamprobacter modestogalophilus</i>	[NiFe]	室温, 厌氧缓冲液 pH=8.0, 储存 28 天, 保持 50% 的活性	[86]
海藻酸钙凝胶	<i>Desulphovibrio desulphuricans</i>	[NiFe]	4 °C, Tris-HCl 缓冲液 pH=7.5, 储存 40 天保持 60% 的活性	[87]
海藻酸钙凝胶	<i>Desulfotomaculum</i> sp.	[NiFe]	30 °C, Tris-HCl 缓冲液 pH=7.6, 反应 50 h 保持 40% 的活性	[88]
阴离子交换树脂	<i>Ralstonia eutropha</i>	[NiFe]	35 °C, Tris-HCl 缓冲液 pH=8.0, 反应 32 h 保持 50% 的活性	[89]
琼脂糖凝胶	<i>Chromatium vinosum</i>	[NiFe]	65 °C, Tris-HCl 缓冲液 pH=8.0, 孵育 80 min 保持 50% 的稳定性	[90]

为1,4-二羧基苯-2,3-二硫醇酯), 促进质子的还原。Wang等^[101]报道了通过点击反应将铁氢化酶催化位点(Fe_2S_2)固定在 $[\text{Ru}(\text{bpy})_3]^{2+}$ 修饰的UiO-MOF上, 提高了氢气产量。Ashleigh等^[102]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 $[\text{Fe}_2(\text{dcdbt})(\text{CO})_6]$ 模拟铁氢化酶活性位点, 将其固定在PCN-700中, 加强了电子传递。Davidet等^[103]将镍铁氢化酶模拟物($[\text{L}^{\text{N}2\text{S}2}\text{Ni}^{\text{II}}\text{Fe}^{\text{II}}\text{Cp}(\text{CO})]\text{BF}_4$)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。

MOFs固定化氢化酶的难点在于与氢化酶生物相容性好的MOFs较少。利用MOFs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MOFs载体。部分MOFs合成反应条件较为温和, 例如ZIF-8在室温水溶液即可合成^[104]。本课题组将来源于*Pyrococcus furiosus*的镍铁氢化酶(pfSHI)与 Zn^{2+} 及有机配体2-甲基咪唑在室温水溶液的条件下混合, 实现氢化酶的ZIF-8包埋固定化。使用比色法检测电子中介体苜蓿紫精(BV, 578 nm, 8.65 mM/cm)^[105]的变化, 从而检测氢化酶的酶活。以第一次使用的酶活为100%, 在90 °C、pH = 8.5的条件下重复使用7次仍能保持45%的酶活(图3)。然而, 大部分MOFs

需要有机溶剂溶解配体和高温合成。因此需先制备MOFs再除去有机溶剂, 最后进行氢化酶的吸附固定化。

氢化酶固定化效果与MOFs的孔径大小, 带电性, 亲疏水等性质有关, 制备新型MOFs有利于拓宽固定化氢化酶的应用场景。导电MOFs有利于氢化酶的电催化效率。含 Fe^{2+} 的耐氧MOFs^[106]有利于为氢化酶提供无氧环境。与单金属MOFs相比, 双金属MOFs可以提供更加丰富的活性位点, 更好地对孔隙率进行调节^[107]。双金属的协同作用可加速电子转移^[108], 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。本文总结了碳材料、金属及半导体、高分子和MOFs固定化氢化酶(图4)。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

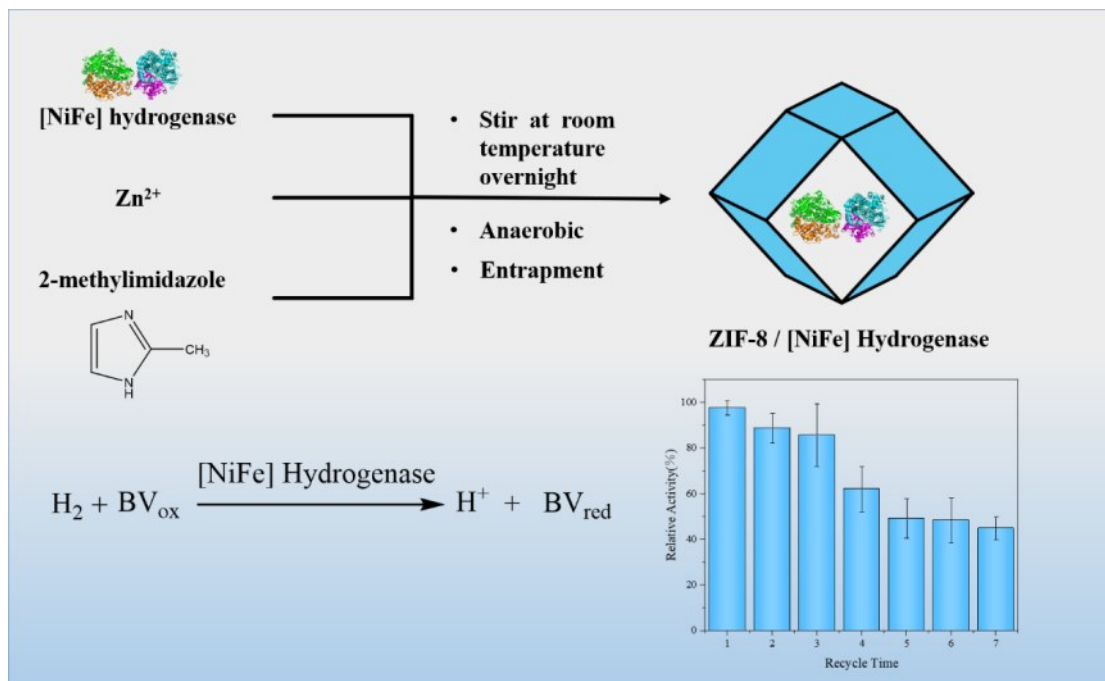


图3 ZIF-8固定化来源于*Pyrococcus furiosus*的 $[\text{NiFe}]$ 氢化酶

Fig. 3 Immobilization of $[\text{NiFe}]$ hydrogenase from *Pyrococcus furiosus* on ZIF-8

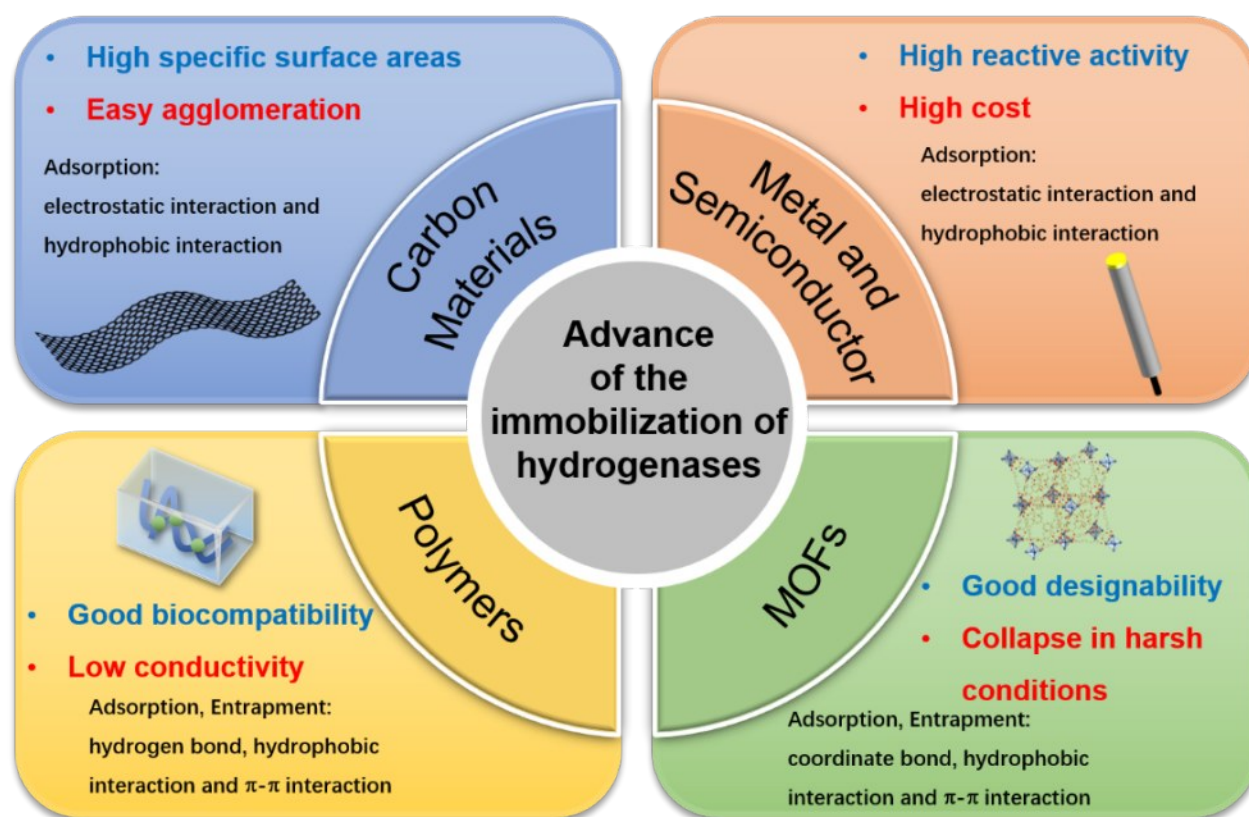


图4 不同材料固定氢化酶总结

Fig. 4 summarization of different materials for hydrogenases immobilization

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。MOFs具有结构设计调控的优点，但是结构在极端条件下不稳定。MOFs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。MOFs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MOFs，可以增强导电性能。制备多金属MOFs，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^[109]，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^[110]，有望提高氢化酶的电子传递速率。

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