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Bio-catalysts for oxygen reduction in proton exchange membrane fuel cells: Mechanistic insights, challenges and prospects

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HIGHLIGHTS

- Enzymes, porphyrins & biomass carbons are assessed as Pt/C substitutes for PEMFC cathodes.
- Bio-catalyst performance is governed by active-site chemistry & electron transfer.
- $4e^-$ oxygen reduction is promoted via molecular coordination & catalyst–support interactions.
- Catalyst poisoning, batch-to-batch variability & limited durability hinder bio-catalyst deployment.
- Ligand chemistry, catalyst immobilization, & electronic coupling dictate bio-catalysts' future for PEMFCs.

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ABSTRACT

Platinum (Pt) persists as an exceptionally prevalent catalyst material in the proton exchange membrane fuel cells (PEMFCs), especially for cathodic oxygen reduction kinetics. However, the high cost and limited supply of Pt are driving interest in bio-based catalyst systems. In this review, three families of such catalysts, which include enzymes, metalloporphyrins, and biomass-derived carbon, are evaluated for their performance under PEMFC-relevant conditions. Rather than evaluating these as potential alternatives, this review focuses on their four-electron route driving capabilities, impurity sensitivity, and stability. Contemporary reports indicate that laccase-based systems, Fe/Co porphyrinic carbons, and microbial-templated Pd and Pt catalysts can attain activity levels comparable to Pt/C when immobilization and electronic coupling are effectively regulated. The primary concerns lie in durability and poisoning, with sulfur species and CO being detrimental to various biological templates. Furthermore, challenges persist regarding scalability and reproducibility because, unlike the conventional heterogeneous catalysts used in PEMFCs, bio-catalysts are contingent upon parameters including ligand chemistry, protein structural arrangement, and microbial growth. This makes the production process and maintenance of batch uniformity laborious. Although recent advancements have mitigated performance limitations, mechanistic understanding, operational longevity, and manufacturing scalability remain critical considerations. Overcoming these challenges will establish biocatalysts as a viable alternative to traditional precious-metal catalysts in PEMFCs.

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List of Abbreviations

Abbreviations

AV Aloe vera.
BF Black fungus.
BNC Bacterial nanocellulose.
CB Carbon black.
CcO Cytochrome *c* oxidase.
Ci 1-Carboimidazole.
CL Catalyst layer.
CNF Carbon nanofiber.
CoNaSS Co/N-doped activated soybean straw.
DFTPP Difluoro tetraphenyl porphyrin.
E. coli *Escherichia coli*.
$E_{1/2}$ Half-wave potential.
ECSA Electrochemically active surface area.
ETN Electron transfer number.
FeDOH/C Phenol-substituted Fe-porphyrin dimer.
FeM/C Unsubstituted monomer.
FeMOH/C Phenol-substituted iron monomer variant.
FeO Iron oxide (transition metal oxide).
FePc Iron phthalocyanine.
Fe_2O_3 Iron(III) oxide.
GDE Gas-diffusion electrode.
GDL Gas diffusion layers.
H^+ Proton.
H_2O_2 Hydrogen peroxide.
$HClO_4$ Perchloric acid.
HOR Hydrogen oxidation reaction.
LSV Linear sweep voltammetry.
M-OMPCs Metallated ordered mesoporous porphyrinic carbons.
MEA Membrane electrode assembly.
MWCNT Multiwalled carbon nanotube.
N-F/PGPC N-/F-infused pomegranate peel carbon.
NCA Nitrogen-infused carbon.
NfnPPy Nafion-adorned polypyrrole.
NP Nanoparticle.
NSC N-/S-co-infused bio-carbon flocs.
OH^* Hydroxyl radical intermediate species.
OOH Adsorbed hydroperoxyl species.
OOH^* Hydroperoxyl radical intermediate species.
OP Onset potential.
OrP-AC Activated carbon from orange peel.
ORR Oxygen reduction reaction.
PDA Polydopamine.
PEMFC Proton exchange membrane fuel cell.
POXC *Pleurotus ostreatus* carbon.
Pt/C Platinum supported on carbon.
Pt/N-AVC N-doped AV carbon with Pt NPs.
Pt/NT1000 Pt on tamarind seed carbon @ 1000, °C.
Py Pyridine.
RDE Rotating disk electrode.
rGO Reduced graphene oxide.
rGO-TADPyCu Cu centers on triazole-dipyridine rGO.
RHE Reversible hydrogen electrode.
Rh(TCPP) Rhodium tetrakis(4-carboxyphenyl)porphyrin.
$R_{local}^{O_2}$ Local oxygen transport resistance.
TADPy Triazole-dipyridine.
TMPPCo Cobalt(II) tetrakis(4-methoxyphenyl)porphyrin.
tmpa Tris(2-pyridylmethyl)amine.
TRL Technology Readiness Level.
US DOE United States Department of Energy.
ZIF-8 Zeolitic imidazolate framework-8.

1. Introduction

Proton exchange membrane fuel cells (PEMFCs) represent a significant area of research in integrating hydrogen energy and sustainable electrochemical energy conversion. The constituent parts of a PEMFC stack are multiple individual cells arranged in a series. Each cell has a membrane electrolyte assembly (MEA), gas diffusion layers (GDL), and bipolar plates supporting it. The MEA comprises a polymer electrolyte (Nafion) sandwiched between anode and cathode catalysts [1]. The catalyst layers (CLs) have a longstanding history of investigation in PEMFCs, as they are essentially in charge of transforming the reactant fuel into functional electrical energy through hydrogen oxidation and the oxygen reduction reaction (ORR) [2]. On the anode side, the hydrogen oxidation process (HOR) takes place, generating protons (H^+) and electrons (e^-). ORR takes place on the cathode and is essentially a slower reaction that includes multiple reactive intermediates. In acidic media, the preferred four-electron pathway to H_2O is kinetically demanding due to the large activation barriers and O–O bond strength [3]. As a result, platinum supported on carbon (Pt/C) and other Pt group metal catalysts have dominated PEMFC cathodes for decades due to their high activity, durability, and tolerance to impurities [4,5]. Their behavior is consistent with Sabatier-type arguments for adsorption strength [6]; nonetheless, the higher expenses and the diminishing supply of these metals present significant challenges from a commercial perspective. Pt is scarce (about 37 ppb in the Earth's crust) and priced around 75 USD/g with market-driven fluctuations [7]. This poses significant difficulties for researchers aiming to meet the targets set by the United States Department of Energy (US DoE), which state that the cathode Pt loadings should be below 0.125 mg.cm^{-2} to achieve mass activities of 0.45 A.mg$_{Pt}^{-1}$ at 0.1 mg$_{Pt}$.cm^{-2} [8,9]. In anticipation, researchers have explored metal thrifting strategies that have reduced Pt usage, but durability and synthesis complexity continue to limit wider deployment [10]. These constraints have pushed interest toward bio-catalysts for the ORR in PEMFCs, motivated by the possibility of maintaining activity while reducing precious metal dependence.

Interest in bio-catalysts in fuel cells dates back to Jasinski's early work on phthalocyanine-based PEMFC cathodes in the 1960 s, which delivered power densities above 0.9 W.cm^{-2} under pure O_2 but dropped to 0.2–0.4 W.cm^{-2} in air [11]. Since then, a broader set of materials inspired by biological chemistry or derived from natural sources has emerged [12]. This classification primarily encompasses specific enzyme classes, including multi-copper oxidases like laccase for the ORR, hydrogenases for the HOR, as well as particulate methane monooxygenase alongside synthetic metalloporphyrins and carbon materials derived from biomass. These groups have demonstrated viability as cost-effective alternatives to traditional heterogeneous precious-metal catalysts. Metalloenzymes, in particular, rely on highly evolved transition-metal active centers protected by surrounding protein matrices, which can offer unique selectivity and localized pathways for electrocatalysis under targeted operational environments [13]. It is generally known that metalloporphyrins can undertake redox reactions, and they are very good catalysts even when there is no specific substrate in play [14]. Biomass and other natural sources of catalysts allow gas and ions to diffuse efficiently, which lowers internal resistance and raises power density [15]. This review examines these three bio-catalyst families for PEMFC cathodes, focusing on their catalytic activity, performance metrics, durability, and sensitivity to impurities.

Despite notable advancements in PEMFC biocatalysts, the existing research remains predominantly scattered. As described by Panchal and Heena Shevde [16] in their review on biomimetic non-Pt group

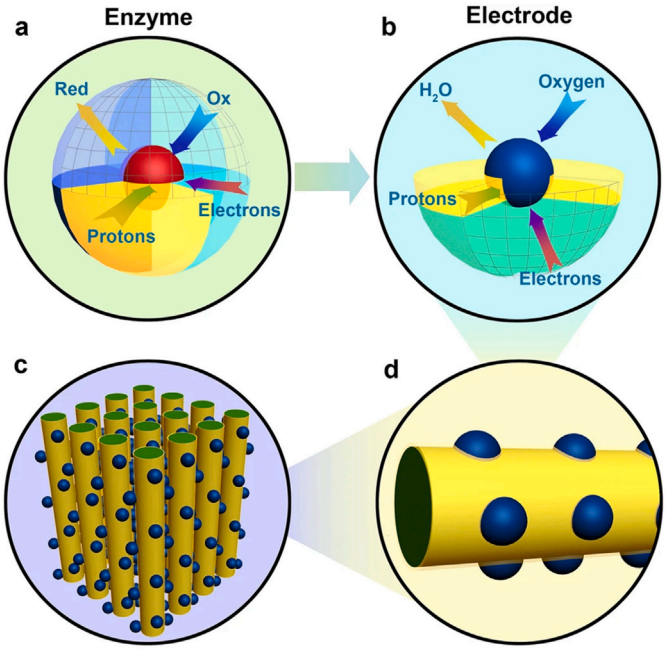


Fig. 1. a. A typical enzyme structure showing the active center inside the charge and mass transport channels; **b.** Nafion® adorned polypyrrole (NfnPPy) serves as a substrate for the encapsulated Pt NPs. (**c,d.**) the nanowire arrays provide a hierarchical structure in Pt-NfnPPy. From [19] Scientific Reports, 5, 16100 (2015), licensed under CC by 4.0.

metal catalysts for ORR in PEMFCs, comparing different families of bio-based catalysts is quite challenging, since most research reports ORR activity across varied experimental settings and performance metrics. Moreover, mechanistic comprehension has progressed unevenly, with significant exploration of enzymatic and metalloporphyrin active sites, as well as electron-transfer routes in biomass-derived catalysts. The existing research often focuses on outlining catalytic performance or specific raw material types, rather than integrating catalytic chemistry, compatibility with membrane electrodes, engineering scalability, and quantitative comparison [17]. A comprehensive review that brings together molecular processes, practical application, and technical readiness remains currently neglected. This report addresses these gaps by comprehensively and comparatively analyzing enzyme-based catalysts, metalloporphyrins, and carbon catalysts derived from biomass. This evaluation encompasses a holistic view of catalytic processes, performance comparisons, engineering challenges, and practical applications of PEMFC bio-catalysts. The fabrication challenges and the factors that affect reproducibility and scale are also discussed. By consolidating recent literature, the aim is to assess whether bio-catalysis can serve as a credible route toward reducing precious metal use in PEMFCs and to identify the main technical barriers that need to be addressed for practical implementation.

2. Enzymes as bio-based catalysts in PEMFCs

Enzymes form one of the main bio-based catalyst families considered for the ORR in PEMFCs. Sharma et al. [18] noted that enzymatic systems can catalyze reactions under relatively mild conditions, which lower energy input requirements and avoid the high-temperature or strongly acidic environments associated with conventional heterogeneous catalysts. Their high substrate specificity allows efficient turnover with limited by-products, and several enzymes show tolerance to contaminants such as carbon monoxide and hydrogen sulfide. Because they are derived from biological sources, enzymes do not suffer from the resource scarcity and cost issues associated with precious metal catalysts.

2.1. Enzyme-mimetic nano-architectures

Synthetically mimicking the highly active coordination centers of specific metalloenzymes, primarily multi-copper oxidases and iron-sulfur proteins, holds significant promise for accelerating ORR kinetics without using fragile native protein backbones. As an example, Xia et al. [19] designed an enzyme mimic nanoscale catalyst in which Pt nanoparticles (NPs) were located at the edges of charge and mass transport channels (see Fig. 1). This arrangement was intended to facilitate reactant access and electron transfer in a manner analogous to enzymatic active sites.

Their polarization study showed an upward transition of 53 mV for bio-catalysts compared to commercial Pt/C, indicating improved ORR efficiency. Stress tests demonstrated that these bio-catalysts retained the majority of their electrochemically active surface area (ECSA), with only an 18.3% loss after 2000 cycles, whereas Pt/C catalysts underwent severe degradation, losing up to 80.4%. An enzyme-like electrode architecture that allowed concurrent charge transport surrounding Pt NPs stabilized by sulfonate group complexes ($R-SO_3$) likely made the observed effect possible. Sulfonate compounds are recognized for their strong interactions with metal surfaces and can stabilize Pt NPs by forming coordination bonds. This stabilization proves essential for guaranteeing the structural firmness of the developed catalyst while agglomeration remains minimal. Focusing specifically on multi-copper oxidases, Lee et al. [20] innovated by creating nano-scale flower-like particles through the structural integration of native laccase and copper ion interactions (see Fig. 2a), which significantly enhanced the ECSA, subsequently increasing ORR activity by 30% in an H_2/O_2 PEMFC setup. The cell possesses a biocathode (laccase) and an abiotic anode (Cu nanoflower/CNT) that work synergistically to reduce oxygen. The power density was maximized to 0.41 $mW.cm^{-2}$. After 15 days, the cell kept delivering a steady 0.35 $mA.cm^{-2}$, which is 85% of its original electrocatalytic prowess. This impressive performance arises from the dynamic correlations among the laccase and the bio-compatible conducting environment of the nanoflowers, enriched with $Cu_2{}^+$ ions. As illustrated

7.2. Cost

As an attempt to bring down the cost challenge of conventional PT catalysts, the bio-catalysts are yet to fall into the very economical scale, despite the present research momentum. This is said in concern with the significant expenses involved, because of the resource-dependent process in making enzyme-based catalysts. Enzymes, usually made by fermentation, are always in demand for precise regulation of temperature, pH, aeration, and nutrient scale for micro-organism growth [156], which positions itself as an overhead cost in the overall production. Metalloporphyrins and other bio-derived materials are also subdued when it comes to cost-reduction against the conventional catalysts, because of the cost of precursor chemicals, which is on the expensive side, particularly when it comes to functioning under rare or specialized molecules. However, cost-reduction synthesis strategies such as ball-milling mechanochemistry [157], sonochemistry [158], and self-assembly methods for porphyrin nano-structures [159], have been materialized as a resolution.

Maintaining active site consistency on a multi-gram scale during large-scale manufacture of these synthesis systems necessitates precise control over physical processing parameters. The ideal mechanical impact force for high-energy ball milling is produced by a ball-to-powder weight ratio of 20:1 and a consistent rotational speed of 500 rpm. Maintaining the core macrocycle structure while producing a dense network of edge defects and stable N_2-coordination routes requires precisely 8 hours of running time. Thermal processing of biomass-derived carbons necessitates well-defined technical constraints to forestall large-scale product inconsistency [160]. Rapid removal of volatile breakdown products requires a constant nitrogen infusion of 1.5 $L.m^{-1}$ when moving from tiny tube furnaces to continuous rotary kilns. An exact 2:1 mass ratio between the carbon precursor and solid KOH is required for the chemical processing step optimization. An ultimate calcination temperature of 800 °C is achieved by heating this combination uniformly at a rate of 5 °C per minute, resulting in the uniform development of thin pores. With a specific surface area of more than 1100 $m^2.g^{-1}$ and end product yields of more than 35%, this small thermal process effectively balances the density of catalytic sites against excessive carbon burn-up.

The economic viability of biocatalysts is contingent upon the production scale, process integration, and composition of the catalyst, according to recent research. Based on a techno-economic analysis of commercial laccase production, the minimal selling price of laccase is USD 0.05 kU^{-1}. The main factors influencing production cost, instead of the fermentation stage, were found to be enzyme recovery efficiency and batch productivity [161]. These results show that rather than focusing on microbial cultivation, it would be more economically beneficial to enhance downstream processing. Notable findings have also been documented for ORR catalysts that do not contain platinum. The manufacturing costs of catalysts were calculated at USD 3.41 kW^{-1} and USD 0.79 kW^{-1}, respectively, in a recent techno-economic analysis that compared Pt/C and Fe-N-C cathode catalysts [162]. Nevertheless, the anticipated costs of the PEMFC stack for the Pt/C system were 13.8 kW^{-1}, whereas for the Fe-N-C systems, it was 41.6 kW^{-1}. This shows that inexpensive catalysts don't always mean cost-effective systems since factors like integration, process complexity, catalyst size, and durability all have a major impact on commercial viability.

At present, there is a lack of comparative market and industry cost data for the majority of the enzyme-, metalloporphyrin-, and biomass-carbon catalysts that have been studied for use as PEMFC anodes. The majority of the research that has been reported primarily deals with electrochemical performance in controlled laboratory settings, whereas the techno-economic assessments that have been conducted are rather limited and tend to be route-specific [163]. Thus, standardized industry databases do not yet allow for direct cost comparisons between various bio-catalyst types. Therefore, to evaluate the commercial viability of biocatalysts beyond their performance in the lab, it is necessary to provide standardized frameworks for techno-economic and life cycle assessments.

8. Conclusions and outlook

Biocatalysts have broadened the spectrum of catalyst materials for PEMFCs by offering sustainable alternatives to platinum-based systems. Enzymes offer superior ORR selectivity, metalloporphyrins offer precisely defined catalytic centers, and bio-based carbons integrate wide-ranging raw materials with versatile reaction site chemistry. Investigations have indicated that, under ideal laboratory settings, all three catalyst families can achieve the ORR activity necessary for practical PEMFC applications.

Nonetheless, catalyst enhancement continues to prevail over catalyst processing in recent studies. The majority of research emphasizes the OP or $E_{1/2}$ potential, or ETN, while minimal attention is given to long-term durability, membrane electrode compatibility, toxicity under actual working conditions, product reproducibility, and scalability. Consequently, catalysts exhibiting comparable electrochemical performance may vary considerably in their practical applications.

There are also a great deal of research gaps that remain unaddressed. The relationship between the chemistry of biomaterial precursors and the evolution of active sites has not yet been completely established. Additionally, there is a lack of standardized measurement protocols across the various families of biocatalysts, and the influence of catalyst layer structure on ORR performance is rarely investigated in a systematic manner. Moreover, techno-economic evaluations and technical readiness are hardly analyzed alongside electrochemical performance.

Consequently, further research should concentrate on the integration of design with membrane electrode technology, standardized experimental protocols, scalable production, and pragmatic performance assessment. Addressing these factors will facilitate the transition of promising biocatalysts from laboratory-scale research to PEMFC applications.

CRediT authorship contribution statement

D.K. Madheswaran: Conceptualization, Methodology, Data curation, Writing – original draft. **S. Vengatesan:** Investigation, Validation, Formal analysis. **J. Selvaraj:** Investigation, Data curation, Visualization. **E.G. Varuvel:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **H. Togun:** Formal analysis, Visualization, Validation. **A. Pugazhendhi:** Supervision, Project administration, Writing – review & editing. **J. Matijošius:** Methodology, Validation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon request.

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