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Oxygen reduction reaction electrocatalysis in neutral media for bio-electrochemical systems

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Abstract

Oxygen reduction reaction (ORR) is an electrochemical process with uppermost importance to energy conversion and storage, corrosion and chemical technology. It plays major role in biological processes (respiratory biochemical chain of reactions) and is being incorporated in numerous bio-electrochemical devices and systems: microbial and enzymatic fuel cells, micro-biosynthesis processes and water desalination and purification technologies. Researchers from various background are joining to address the specifics of ORR in close-to-neutral environments, commensurate with the integration with bioprocesses. The importance of understanding ORR mechanism in this transitional and less-studied pH region is complex as it involves biotic (life) and abiotic (often inorganic materials or composite) catalysts. This review offers a summary of catalysts class-dependent ORR mechanisms and pathways with corresponding limiting steps relevant to the practical use in bio-electrocatalytic systems. We analyse the technology challenges often limited by the use of oxygen depolarisation as main driving force in practical applications.

Introduction

Oxygen in its gaseous form or dissolved (DO) in the electrolyte is probably the most frequently used reagent/oxidant in electrochemical systems due to its specific features¹. The oxygen reduction reaction (ORR) is one of the most studied reaction in the electrochemical panorama and often the limiting reaction of the system^{1,2}. Increased use of ubiquitously available oxygen in electrodes designed for ORR electrochemistry enables

many technical advances in energy harvesting, waste transformation and water purification, desalination, soil remediation, etc. integration biological catalysts. Integrating biocatalysts (microorganisms and biofilm, enzymes and cellular extracts) into electrochemical technology requires a pH match between the ORR, most effective at extreme high and low pH and the operational stability of the biotic component. The sluggish ORR kinetics in close to neutral media (circum-neutral), where under operating conditions the concentration of both protons (more precisely hydronium cations) and hydroxide anions are several orders of magnitude lower compared to extreme pHs³. Both H⁺ and OH⁻ are reactants for the ORR in acidic and alkaline media and their concentration dependence is a direct manifestation of the mass law controlling the reaction rate. As a result, in circum-neutral media the performance of bioelectrochemical systems (BESs) is dramatically lower compared to chemical fuel cells, however still promising for several applications. Inorganic electrocatalysts have been inherited from chemical fuel cells and widely used in BESs, sometimes being the most effective electrocatalysts during operation. In parallel, due to their natural adaptation to close to neutral electrolyte environment, biocatalysts such as enzymes, multi-enzyme macro-complexes or whole microorganisms have been explored as effective ORR electrocatalysts⁴⁻⁶. However, the addition of biological matter, acting as bio-electrocatalyst significantly increases the level of complexity of an already complex ORR process occurring over inorganic electrocatalysts.

This review focuses initially on the description of the ORR mechanism highlighting each reaction step and the critical/rate-limiting steps. The analysis of the ORR occurring over inorganic electrocatalysts highlighting electron transfer (ET) mechanisms and limitations is reported. The description of the enzyme-catalysed ORR focusing on the ET mechanism, limitations and applications in enzymatic electrochemical systems (EESs) is also described. Moreover, ORR mechanism occurring in the bacterial electrocatalysts, the ET mechanisms, rate-limiting steps involved in the process, bacterial formation and growth and its activity in microbial electrochemical systems (MESs) are reported. The different cathode architectures are identified and reported indicating their relative advantages and disadvantages, moreover the scaling up of the cathodes used in BESs is presented. Finally, the perspective and future directions related to the ORR operating in close to neutral pH (6<pH<9) are discussed.

Overview of ORR mechanism

The use of abiotic (inorganic or composite) electrocatalysts in BES is most immediate, as those have been most studied and developed in the general, mainstream electrochemical fuel cell technology usually practiced as alkaline and acidic for the low-temperature systems. In the case of inorganic electrocatalysts, ORR can follow different ET mechanisms that can be summarised into a 4e⁻, 2e⁻ or 2+2e⁻ transfer mechanism⁷. A direct 4e⁻ transfer mechanism is preferred and is the most efficient in transforming reagents to final product. A partial reduction of oxygen following a 2e⁻ transfer mechanism can also occur, but it is less efficient, and the reaction is better known as hydrogen peroxide

generation reaction (HPGR). A more complex $2+2e^-$ transfer mechanism is also plausible where the partial reduction of oxygen ($2e^-$) can occur and then the reaction product is further reduced ($2e^-$) on the same site or an adjacent active site⁷.

ORR processes in acid and alkaline media follow different ET mechanisms and pathways^{8,9}. For inorganic electrocatalysts, the identification and enumeration of the active sites on the surface, the morphological features such as porosity and surface area, the reaction(s) occurring on these electrocatalysts and their limiting kinetics steps are of utmost importance for understanding the ET mechanism and potentially improve the electrocatalytic activity during ORR. With biological electrocatalysts, ORR-related charge transfer process is being integrated in several hierarchies of scale in molecular, intracellular and extracellular arrangements¹⁰.

In general, complex and heterogeneous situation exists for inorganic electrocatalysts that can follow a direct $4e^-$ transfer mechanism over platinum group metal (PGM) electrocatalysts¹¹, a $2e^-$ transfer mechanism over pure carbonaceous-based electrocatalysts¹², a $2e^-$ transfer mechanism when metallic impurities or metallic oxides/carbides/nitrides are present in the carbon matrix^{13,14}. On a platinum group metal-free (PGM-free) electrocatalyst, $2e^-$, $2+2e^-$ (same active site or different active site) and $4e^-$ transfer mechanism can occur on metal coordinated with nitrogen (M-N-C) active sites (Figure 1.A-E)⁷. The above description can be considered true only if the electrocatalyst operates in a clean circum-neutral electrolyte environment. In general, electrolytes used in bioelectrochemical systems contain molecules or charged species that bind directly with the active sites and deactivate them. Therefore, the active sites are poisoned and no longer active towards ORR and as consequence, the ORR is occurring over the carbon matrix. In the latter conditions, secondary catalyst properties such as high porosity and high surface area, contribute significantly to the ORR kinetics.

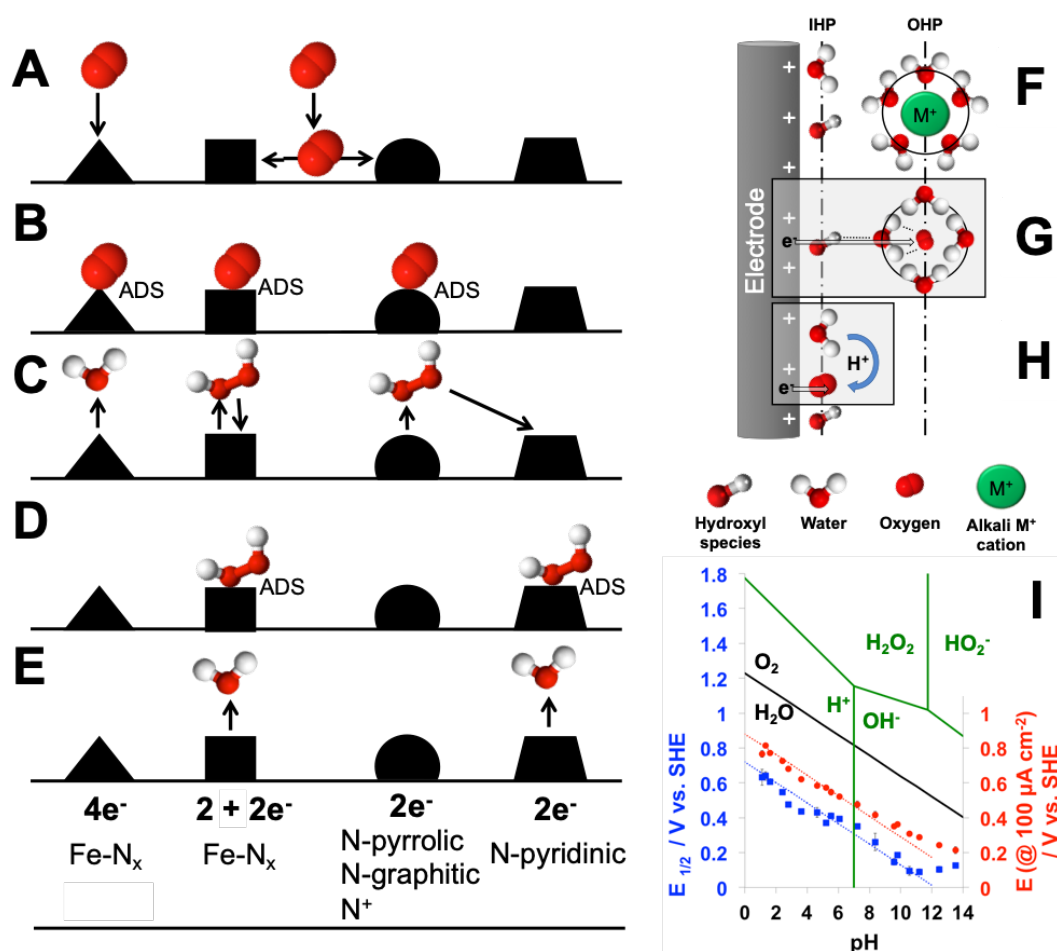


Fig. 1 Electron transfer mechanism in aqueous media for inorganic electrocatalysts

ORR mechanisms in acid media occurring on **Fe-N-C electrocatalysts** active sites. **(A)** O₂ present in solution. **(B)** O₂ adsorption on the different active sites. **(C)** O₂ reduction into H₂O or H₂O₂ followed by the diffusion of the product in the solution. **(D)** Re-adsorption of H₂O₂ on Fe-N_x sites or N-pyridinic sites. **(E)** H₂O₂ reduction to H₂O followed by the diffusion of the product in solution. Schematic illustration of ORR in correspondence to the double-layer structure, where the inner Helmholtz plane (IHP) and the outer Helmholtz plane (OHP) are identified: **(F)** Alkali metal ions fully solvated; **(G)** O₂ fully solvated involved in outer-sphere ET processes; **(H)** O₂ partially de-solvated involved in inner-sphere ET processes. **(I)** Potential @100 μ Acm⁻² and half-wave potential ($E_{1/2}$) trends of Fe-N-C electrocatalyst measured along the entire pH projected over the Pourbaix diagram of H₂O: potential/pH diagram identifying the equilibrium phases within the aqueous electrolyte. **A-E** adapted with permission from ref.⁷, Elsevier LTD. **F-H** adapted from ref.⁹, Hindawi Publishing Corporation under CC BY 3.0. **I** adapted with permission from ref.¹⁷, American Chemical Society.

In the case of enzymes, the ET mechanism follows a 4e⁻ transfer mechanism occurring in two 2e⁻ steps, where the first is the rate limiting¹⁵. For living biocatalysts, the bacterial respiration of oxygen occurs through enzymes of the hemi-copper oxidoreductase superfamily that act as redox proton pumps in the membrane-respiratory chain of bacterial cells that conserve part of the free energy released by the ORR to generate a proton motive force. They are associated with small hemoproteins (cytochromes) which play the leading

role as electron transporters. Generally, the amount of bioavailable oxygen impacts the cellular synthesis of the oxidoreductases catalysing the ORR. Multiple mechanisms are involved when is present a cohabitation of biotic and abiotic mechanisms already detailed in previous literature⁴.

The reagents and the products involved in acid and alkaline media electrolyte during ORR and their relative potentials are summarised in **Supplementary Figure 1**. It was shown that, **for inorganic electrocatalysts**, acidic mechanism occurs through the inner-sphere ET reaction with a direct ET. Instead, in alkaline environments, outer-sphere (OHP) ET reaction is expected with hydroxyl working **like a mediator** during the ET^{8,9} (Figure 1F-H). (Circum)neutral pH stands in the centre of the pH regions and therefore understanding the ET mechanism followed is crucial, controversial and not clearly defined. Recently, two independent studies showed that mechanism shift in Fe-N-C from acidic to alkaline occur at pHs around 10-12 showing the strict relation to the peroxide intermediate pK_a shift at 11.7^{16,17} (Figure 1.I). This is not necessarily true for other inorganic electrocatalysts where the mechanism shift can also occur at lower pHs. It is important to underline that the local pH on the electrode surface (highly basic) might differ from the (circum)neutral bulk pH in turn affecting the reaction mechanisms¹⁸.

Oxygen possesses unique features as it can be used without pre-treatment, is largely available in the atmosphere at no cost, space and weight, is easy to supply and it possess high redox potential. However, inadequate oxygen management might be deleterious to the overall biological or enzymatic reactions by being detrimental to the anaerobic/anoxic biofilm¹⁹ **and/or** interfering with some anodic enzymes²⁰. A well-defined physical/chemical gradient separation between anode and cathode or an oxygen scavenging system must be considered.

Oxygen is widely used as final electron acceptor in neutral media in technologies capable of delivering specific objectives such as water treatment/desalination, autonomous low-power source or sensing specific molecules such as microbial electrochemical systems (MESs) (e.g. microbial fuel cell (MFC), microbial desalination cell, Microbial Electrochemical Snorkel, microbial electrochemical sensors)^{21,22} and enzymatic electrochemical systems (EESs) (e.g. enzymatic fuel cell (EFC) or enzymatic biosensors)²³. In these applications, the anodic reaction is coupled with the ORR in order to operate without an external power source. Typical open circuit voltages (OCVs) and cathode open circuit potentials (OCPs) with their activation overpotentials are reported in **Supplementary Table 1**. All the ET mechanisms known till date involving anodic and cathodic reactions are schematised in the **SI (Supplementary Figure 2)**.

Inorganic electrocatalysts for ORR

Among non-biological (abiotic) electrocatalysts for ORR, the inorganic materials play critical role. They are the most developed and widely used branch in practical electrocatalysis technology and are often available as commercial products. Such

electrocatalyst are being widely used as cathode materials in BES systems. Inorganic electrocatalyst categories are very vast and diverse and are summarised in Figure 2.A²⁴. This category can be divided in groups considering the presence/absence of platinum group metal within the electrocatalyst. It is important to underline that, due to the presence of real close-to-neutral electrolytes (e.g. wastewater) containing a variety of pollutants and changed species, the electrocatalytic activity of a certain inorganic electrocatalyst does not necessarily reflect the trends heavily studied in acid and alkaline environments³ since the active sites can be poisoned and not working as well as in an unpolluted environment.

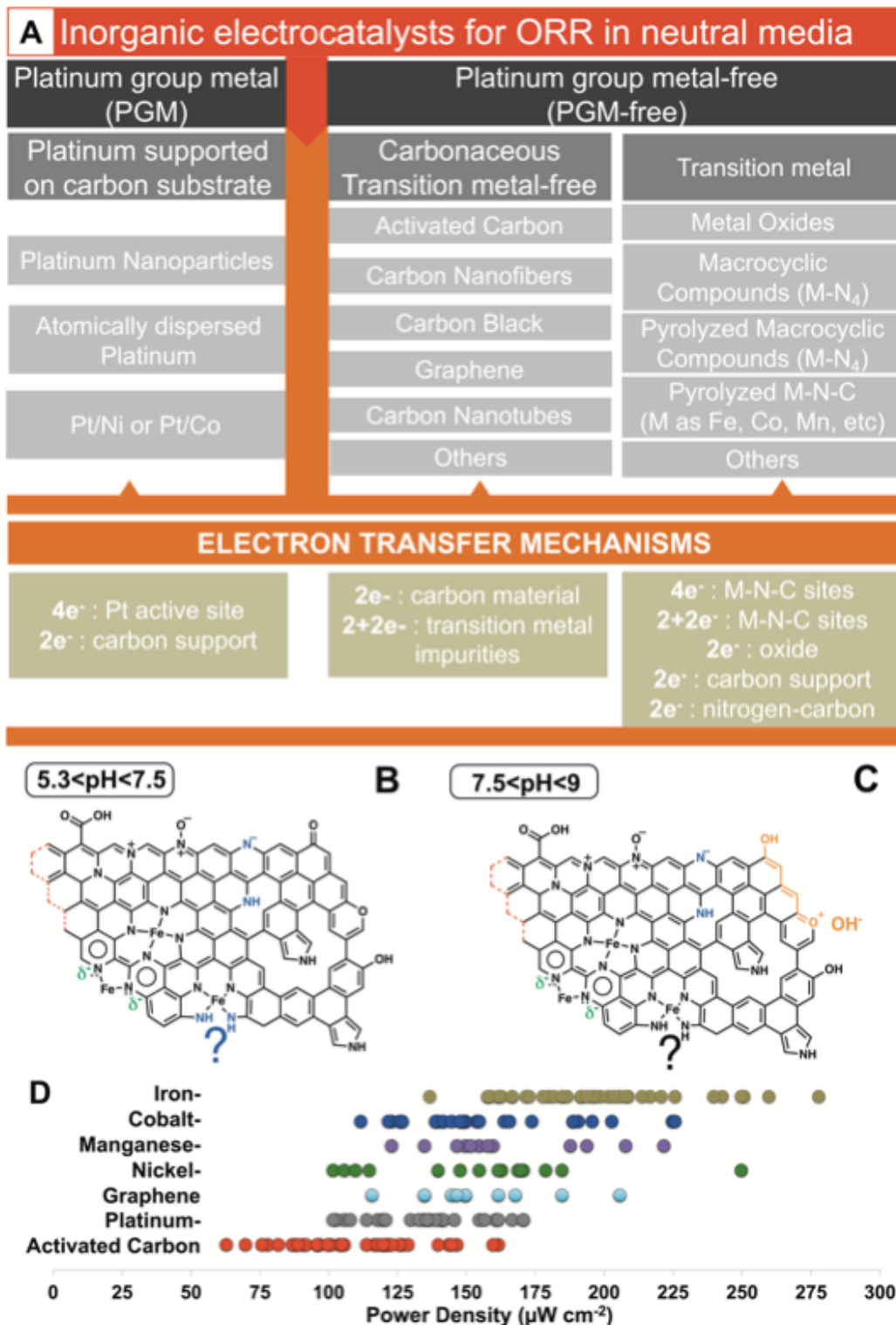


Fig.2 Type of inorganic electrocatalysts and their electrochemical performance

(A) Categories of inorganics electrocatalysts used in BESs operating in neutral media. (B) and (C) Protonation and deprotonation of active sites in Fe-N-C electrocatalyst in working electrolyte between 5.3 and 7.5 and 7.5 and 9, respectively (D) Trend of power density output of MFC using different inorganic electrocatalysts. The references of the value utilised are reported in the SI (Supplementary Table 2).

B-C adapted with permission from ref.¹⁷, American Chemical Society.

Platinum group metal electrocatalysts. PGM materials are by far the most popular inorganic (abiotic) electrocatalysts in electrochemical technology. PGM electrocatalysts singularly or alloyed, supported on a carbon matrix (Figure 2.A), exhibit high electrocatalytic activity towards ORR in extreme pHs⁹. The initial large employment can therefore be considered as a technology inherited from more established chemical fuel cells. PGM electrocatalyst materials advancements and limitations are described in recent reviews²⁵⁻²⁷. In general, PGM limiting step is the adsorption of oxygen on the active site. Importantly, the active sites in PGM electrocatalysts are prone to be poisoned easily and deactivated by several anions. Durability is a key issue when operating in BESs, in fact, after the deactivation of the PGM active sites, the carbon matrix itself operates as electrocatalyst^{3,21}. At last, PGM electrocatalysts are rare and expensive materials and therefore not suitable for bioelectrochemical applications.

These important limitations pushed the scientists to look for diverse alternatives moving their attention towards materials possessing intrinsic characteristics and simultaneously facilitating high ORR activity, reducing the costs, being durable in harsh/polluted environments¹¹.

Carbonaceous-based electrocatalysts. Carbon based materials are most often associated with BES systems as they support the premise of low cost and deployment at large scale unlimited by PGM materials availability. The PGM-free electrocatalysts category can also be divided into two subgroups in function of the presence or absence of transition metals within their structures named as metal-free carbonaceous-based or metal-based (PGM-free) electrocatalysts (Figure 2.A). Carbonaceous-based electrocatalyst can be used as ORR electrocatalysts or as carbon-matrix support to electrocatalysts. The most used carbonaceous-based electrocatalysts are reported in Figure 2.A^{11,21,28,29}. Generally, carbonaceous-based materials possess important features that are useful for utilisation in MESs/EESs^{28,29,30} by being resilient to corrosion, able to operate in harsh/polluted environments and electrically conductive. From the electrocatalytic point of view, carbonaceous-based electrocatalysts have low or no metal-containing active sites and their activity is much inferior compared to PGM operating in acid or alkaline electrolyte. However, they possess secondary electrocatalyst properties such as high porosity and high active surface area that are valuable characteristics for ORR by enhancing the interfacial surface and improving the mass transfer.

ORR on carbonaceous metal-free electrocatalysts led to a 2e⁻ transfer mechanism¹² supporting the HPGR. Importantly, despite carbonaceous-based electrocatalysts being considered metal-free, this is not necessarily a correct assumption/speculation/fact. In fact, impurities of transition metals (up to 1-2%wt)³¹ were detected and mainly attributable to the starting materials. For example, carbon nanofiber (CNF), carbon nanotubes (CNT) and graphene are synthesised through precursors containing transition metals (e.g., Fe, Co, Mn)

and impurities or nanoparticles are still detected at the end of the synthesis^{13,14}. Transition metals presence in any form and coordination could contribute to catalyse oxygen in a 4e⁻ mechanism or create active centres for disproportioning further peroxide in a 2+2e⁻ mechanism. The limiting step in carbonaceous **electrocatalysts** is implied to be the oxygen adsorption on the active site which in turn is not an electrochemical step^{32,33}.

Platinum group metal-free (PGM-free) electrocatalysts. An emerging group of nano-composite materials electrocatalysts that combine versatility of carbonaceous materials with selectivity and (to some extent) activity of the PGM ones are the PGM-free electrocatalysts. Termed often as PGM-free electrocatalysts, they most often contain transition metals and are often synthesised as atomically dispersed or single atom catalysts (SAC) for ORR showing high electrocatalytic activity towards ORR in neutral media demonstrating also high durability¹¹.

Four main types of PGM-free containing transition metal **electrocatalysts** can be identified as shown in Figure 2.A. PGM-free **electrocatalysts** derived by pyrolysis processes belong to the family of M-N-C (M=Mn, Fe, Co, Ni, Cu). Independently from the synthetic route, the M-N-C **electrocatalyst** surface is extremely diversified chemically and morphologically³⁴. It is logical to assume that a multitude of active sites on the plane and the edge of the graphene-like lattice is involved during ORR^{7,35,36,37,38}. The map of the edge and in plane active sites with 5.3<pH<9 is shown in Figure 2.B-C with each active site being protonated or deprotonated in function of its pKa¹⁷.

The active sites involved in ORR are the nitrogen-containing moieties (N-cyanide, N-imide, N-O, N-pyrrolic and N-pyridinic) and metal-containing particles, oxides, nitride and carbide and atomically dispersed metal coordinated with pyridinic nitrogen (M-N_x).^{33,39-41} An increasing content of nitrogen in the PGM-free **electrocatalyst** is beneficial for the electronic transfer, enhancing the **electrocatalyst** conductivity and in turn, improving the electrocatalytic activity⁴². Generally, the majority of nitrogen functionalities are related to a larger peroxide production except N-pyridinic which is suspected to have peroxide consumer functionalities³⁴. Metal-containing particles, oxides, nitrides and carbides can only perform a 2e⁻ reaction^{7,34}. Usually the majority of these particles are removed from the **electrocatalyst** after acid/basic washing which process typically occurs after pyrolysis. M-N_x (x=2,3,4) instead are the active sites main responsible for a direct 4e⁻ transfer mechanism⁴³. M-N-C **electrocatalyst** is composed of diverse active sites operating differently during ORR and generally, it can be claimed that a 2+2e⁻ transfer occurs³⁴ (Figure 1.A). In general, but not always, the limiting step in M-N-C **electrocatalysts** is actually electrochemical and is the intermediate transformation into the final product (peroxide reduction).

It was shown that the surface chemistry of PGM-free **electrocatalysts** is strictly related with electrocatalytic activity and, Fe-N_x and N-pyridinic active sites are beneficial for improving the overall ORR activity⁴⁴. MFC power peaks of selected literature

(Supplementary Table 2) in the past few years using PGM-free, carbonaceous-based and PGM **electrocatalysts** are reported in Figure 2.D. PGM-free **electrocatalysts** were responsible for the highest power output in MFCs. In neutral media, generally but not always, Fe-N-C **electrocatalysts** were shown to perform **better** compared to Co-N-C, Mn-N-C and Ni-N-C **electrocatalysts** (Figure 2.D)⁴⁵. **At the contrary than PGM**, PGM-free **electrocatalysts** are extremely resilient to poisoning due to the low affinity between the metallic centre and the anions such as NO₃⁻, Cl⁻, ClO₄⁻ and SO₄²⁻⁴⁶. Poisoning seems to occur in the presence of NO₂⁻ and CN⁻ that deactivate the metal centre **of the active site**⁴⁶⁻⁴⁸. **The durability** of PGM-free **electrocatalysts under** operating conditions has been validated **for** over one-year demonstrating the reliability towards ORR for MESS⁴⁹.

Fundamentals and applications of enzymatic ORR electrocatalysis

ORR can be catalysed also by a large number of enzymes belonging the family of copper proteins⁵⁰⁻⁵¹. In biological oxygen chemistry, copper and iron active sites play a key role in both homogeneous and heterogeneous catalysis. Among copper proteins, multicopper oxidases (MCOs) (e.g. laccase, bilirubin oxidase (BOx), ascorbate oxidase (AO), etc.) catalyse the 4e⁻ ORR to water coupled to a substrate oxidation⁵².

Homogeneous electron mechanism of multicopper oxidases. **Among the different types of electron transfer, the most studied and truly fascinating while being an extremely efficient ORR in circumneutral pH, is the homogeneous electron transfer mechanism.** The reaction is based on a four 1e⁻ oxidation of substrate that is coupled with a 4e⁻ reductive cleavage of O-O bond in molecular oxygen⁵³.

As shown in Figure 3.A, the reaction mechanism accounts for three steps, which are: the reduction of the type 1 Cu^{II} site (T1Cu) site through the electrons transferred from a substrate (or electrode considering the immobilised enzyme) **(step 1)**, the internal ET (IET) or tunnelling between the T1Cu and Trinuclear Cu Cluster (TNC) proceeding through the Cys-(His)₂ bridge over a distance of 13 Å **(step 2)** and O₂ reduction taking place at TNC⁵⁴ **(step 3)**. The ORR mechanism is displayed in detail in Figure 3.B (homogeneous mechanism). The enzyme in its fully reduced state reacts with O₂ generating a peroxy intermediate (PI)⁵⁵. Next, PI is further reduced to the native intermediate (NI) through the reductive O-O bond cleavage associated with the formation of T3μ₂OH bridge⁵⁶. NI can be rapidly reduced both through mediated ET (MET) and direct ET (DET) pathways to the initial fully reduced state, while other side reactions are really slow (oxygen atom decay with water rejection $k = 0.034 \text{ s}^{-1}$) and unlikely to occur. Therefore, the 4e⁻ enzymatic ORR from O₂ to H₂O occurs in two 2e⁻ steps. Importantly, the first step (peroxide formation) is the rate limiting, but the electron donor oxidation is considered the overall rate limiting step in the enzyme catalysed ORR⁵².

Heterogeneous electron mechanism of multicopper oxidases. The heterogeneous electron transfer occurring over MCOs provides the knowledge of how DET occurs with enzymes immobilised on electrode surface. Once the MCOs are immobilised on air-breathing or fully immersed electrodes, the kinetics of the enzyme ET might be affected by the immobilisation method⁵⁸⁻⁵⁹, as shown in Figure 3.C (heterogeneous mechanism).

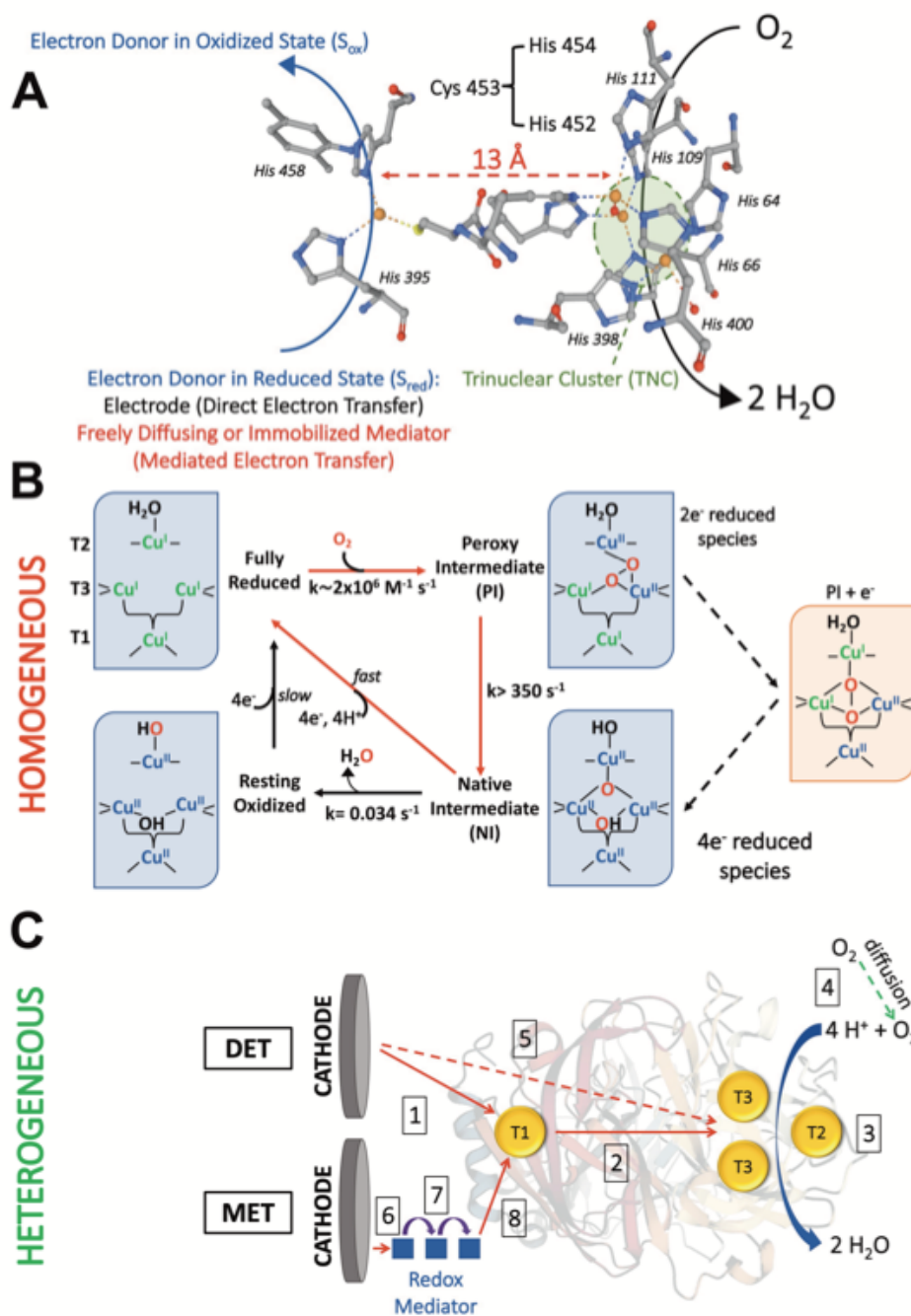


Fig.3. Electron transfer mechanism in multicopper oxidases

(A) The structure of the multicopper oxidases (MCOs) active site with arrows marking the flow of substrates, electrons (e^-) and O_2 . This drawing was taken directly from the Protein Data Bank (B) Homogeneous mechanism of O_2 reduction to water by the MCOs. Red arrows indicate the steps that take place in the catalytic cycle of the MCOs. Black arrows indicate steps that can be experimentally observed but are not part of the catalytic cycle. The dashed arrows at the right indicate the transfer of an electron from the T1Cu to the T2Cu to create $PI + e^-$, that occurs in going from PI to NI but is not experimentally observed in the wild type enzyme. (C) Heterogeneous ET mechanism of ORR occurring in direct ET (DET) and mediated ET (MET). A and B were adapted with permission from ref.⁵⁴, Wiley and Sons. C was adapted with permission from ref.⁵⁸, Elsevier LTD.

Considering the MCOs connected in DET motion, the proper enzyme orientation and the potential applied at the electrode are mainly limiting the step 1 (the reduction of the type 1 Cu^{II} site (T1Cu) site through the electrons transferred from a substrate)⁵⁴. The distance between the T1 and the electrode needs to be shortened as much as possible in order to ensure a fast ET. Moreover, the enzyme layer stability (e.g. enzyme leaching or structural reorganisation) might also significantly impact the step 1⁶⁰. Different from the homogeneous mechanism, the IET (step 2) between the T1 site and the TNC might be considered as a limiting step. For example, it was demonstrated that for BOx immobilised onto graphite electrode through diazonium coupling, the rate limiting step at high pH is the IET (step 2), while at pH 5 was the step 1 prevalent⁶¹. The ORR occurring at TNC (step 3) is usually a fast process with apparent bimolecular constants of about $10^6 \text{ M}^{-1} \text{ s}^{-1}$, thus it cannot be considered as a limiting step⁶². From the electrochemistry point of view, the O_2 diffusion towards the electrode surface (step 4) might also be included as a rate-limiting step. To tackle all conventional DET limiting steps, it was recently proposed the possibility to pump electrons directly through the TNC (step 5) to catalyse the ORR⁶³. By considering that the enzyme is immobilised according to MET configuration, three additional limiting steps have been identified. In particular, we must consider as limiting step the ET between the electrode and the redox mediator (step 6) and the ET between the redox mediator and the enzyme (step 8). When the redox mediator is a redox polymer (e.g., osmium redox polymers), we should also consider the ET within the redox mediator (step 7) as a limiting step⁶⁴.

Immobilisation methods and applications for enzymatic ORR. In order to enhance the durability and the stability of the enzymes as well as their electrochemical activity, immobilisation strategies are of critical importance for both DET and MET electrocatalysis. Despite the presence of many limiting steps, MCOs have been immobilised onto carbon-based nanomaterials by using several immobilisation methods (e.g., amide bonds formation through 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide (EDC-NHS) chemistry or Schiff-base intermediate, supramolecular chemistry, etc.) as shown in the Supplementary Figure 3⁶⁵. In addition, MCOs modified electrodes have been largely employed as enzyme-based fuel cells (EFCs)⁵², amperometric biosensors⁶⁶ and pH-switchable (bio)molecule release electrodes⁶⁷, as reported in Figure 4 (A-C)⁶⁸⁻⁷³. MCOs can be successfully applied in

different niche fields but there are significant barriers for their use on a large industrial scale due to high cost of the enzymes, limited durability, limited pH ranges (4-7), and low current densities because of low surface coverage.

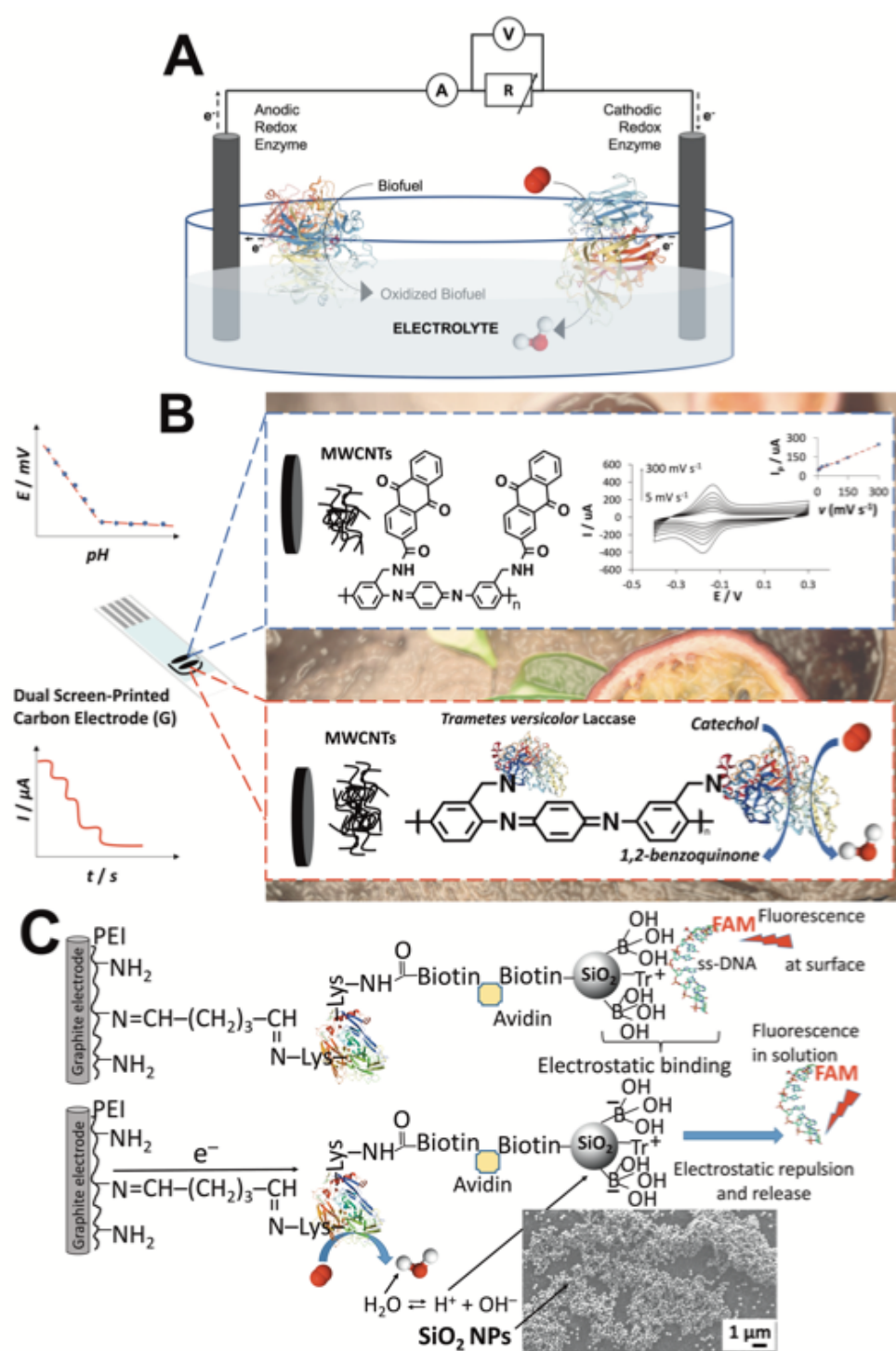


Fig.4. Enzymes based oxygen reduction reaction applications

(A) Representative scheme of a single compartment mediator- and cofactor-less direct ET based biofuel cell. (B) Scheme of an electrochemical biosensor array used for the evaluation of pH changes and total polyphenols content based on a conductive polymer used to immobilise anthraquinone-2-carboxylic acid (ANTQ), on the first electrode, and *Trametes versicolor* laccase on the second one. (C) Operational scheme of the modified electrode for the load and release of the FAM-DNA. Note that the bioelectrocatalytically produced pH change is localised at the interface, while the bulk pH is not changed. The inset shows a scanning electron microscopy (SEM) image of the electrode surface with the bound SiO₂-NPs. B was adapted with permission from ref.⁷⁰, Elsevier Ltd. C was adapted with permission from ref.⁷², American Chemical Society.

Bacterial electrocatalysis of ORR

Biofilms of aerobic electroactive bacteria that directly or indirectly use electrodes as metabolic electron donors (biocathodes) can also act as ORR electrocatalysts. This concept has been documented since the 1980s in the field of metals biocorrosion⁷⁴ and applied in MFCs since 2005⁷⁵. The formation of these bacterial biofilms on the electrodes is a spontaneous process towards which researchers are usually contemplative due to the lack of concrete tools or technological drivers to precisely control their implementation on the electrodes.

Several parameters can potentially impact the growth and the electrocatalytic activity of the electroactive biofilm (EAB) as depicted in Figure 5.A-D⁷⁶. The relationships that link the multi-scale properties of electrode materials and their effects on the formation, organisation and electrochemical activity of biofilms are reported in the Supplementary Figure 4. Multidisciplinary approaches have been used to design and optimise electrode-bacterial biofilm interfaces. Moreover, the electroautotrophic bacterial communities that catalyse ORR have been investigated with respect to both the rates and the mechanisms of extracellular ET (EET) between electrodes and bacterial biofilms.

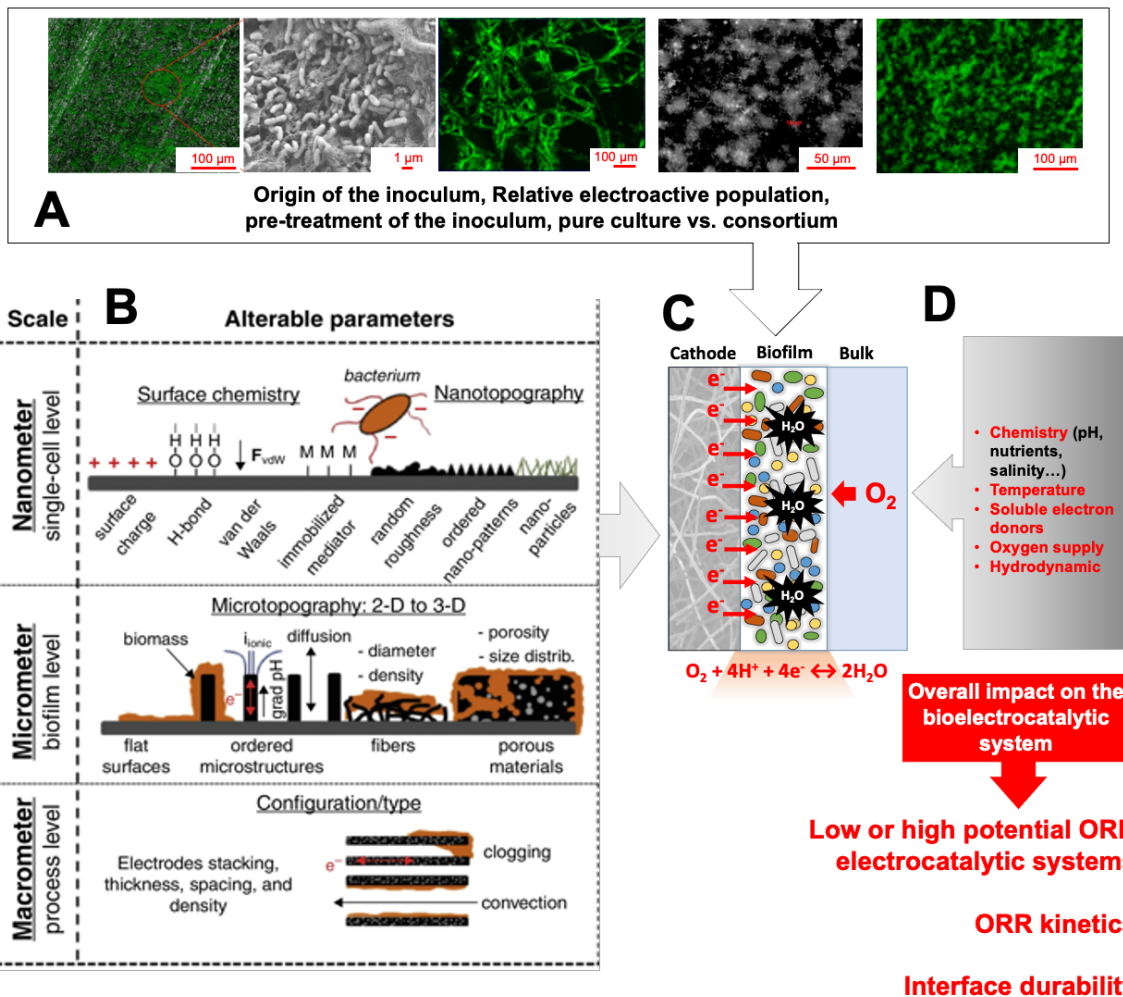


Fig. 5. Parameters affecting ORR biocathode

(A) SEM and Confocal Laser Scanning Microscopy (CLSM) images of aerobic electroactive biofilms illustrating the structural self-organisations in biofilms. (B) Multi-scale classification of the surface quality parameters and the structural and geometrical organisation of the electrodes. (C) Scheme illustrating an aerobic electroactive bacterial biofilm formed at the interface between the electrode and an aqueous electrolyte. (D) Physico-chemical parameters of the aqueous electrolyte solution, known to influence the development, mechanisms of EET and electrocatalytic activity of aerobic bacterial biofilms catalysing ORR. B is adapted with permission from ref.⁷⁶, Elsevier Ltd.

EET in aerobic bacterial biofilms. Extracellular electron transfer is one of the major charge transfer mechanisms enabling the utilisation of microbial bio-cathodes in BES. In bacterial ORR cathodes, the fundamental processes ensuring the EET from the electrode material to the final electron acceptor in solution, i.e. dissolved oxygen (DO), are still quite unknown and subject to much investigation and continued discussion. Nevertheless, several direct or indirect mechanisms, more or less speculative, have been proposed by analogy with the mechanisms already described in the EAB during oxidation⁷⁷, originating the concept of bidirectional EET mechanisms⁷⁸. Analogies with the oxidation mechanisms

of insoluble natural electron donors as described in the iron and manganese cycles **are also been speculated⁷⁹**. Ultimately, ORR mechanisms highlighted in the field of marine corrosion⁴ **have been suggested including** direct ORR **electrocatalysis** by bacterial cells, extracellular enzymes (superoxide dismutases, catalases, peroxidases) or autoxidation of compounds released by microbial cells (prosthetic groups, porphyrins, **haem** compounds and other biomolecules)⁸⁰ and also indirect ORR **electrocatalysis** mediated by intermediates produced by microbial cells⁴.

Bacterial electrocatalytic biofilms on cathode. The electroactive biofilm forming on the electrode surface is critical in practical deployment of BES and the central phenomena of such systems operational stability and field use. Pure and complex bacteria inoculum and different electrode materials were predominantly used for bacterial ORR biocathodes⁴. Three different methods are commonly shared for the conception of ORR bacterial cathodes. **The first one is** based on the natural ability of aerobic biofilms to grow spontaneously on material surfaces under OCP conditions⁸¹. **The second one relies on** the polarisation of the electrode at a potential lower than the OCP and particularly at varied⁸² or controlled constant potential⁸³ **and the third one counts on the inversion of** the polarity of already established bacterial anodes⁸⁴. These reversible bacterial electrodes benefit from a large biomass that develops preferentially during the cathodic phase. Periodic permutation allows the neutralisation of acidification during the cathodic phase and alkalisation during the anodic phase, leading to the perpetuation of the **electrocatalytic** efficiency in the long-term⁸⁵.

Biocathode Applications. The development of cathodic biofilm in MFCs is an emerging trend in BES design and it has received much attention in the past years. Given the number, versatility and co-occurrence of possible EET mechanisms that have been reported or speculated, the bacterial **electrocatalysis** of ORR is certainly at the nexus of many different pathways that could be activated or inhibited depending on the inoculum, electrode materials, conditions of aerobic biofilm formation and operation, and environmental parameters impacting the system microbiology. Different values of ORR half-wave potential have been reported using voltammetric techniques⁸⁶⁻⁸⁹. They can be categorised into two distinct groups. **The first group,** bacterial ORR electrocatalysis **occurs** at rather low potentials, ranging from -0.1 to 0.1 V/SHE (System I⁹⁰). **In the second group, ORR operates** at higher potentials, in the range of +0.4 to +0.5 V/SHE (System II⁹⁰). The half-wave potentials of the System I or II are not correlated with the inoculum source or the electrode material⁹⁰. Due to its higher redox potential, System II is of great interest for designing efficient ORR bacterial cathodes for MFC applications. The initiation of System II appears to be closely related to DO bioavailability, shear rate and electrode potential during the establishment of the biocathode. Most of the work reports current densities of

generally less than 1 A m^{-2} , except one study⁹¹ which reveals a current density of about 3-4 A m^{-2} due to an improved gas-liquid oxygen transfer.

Limiting factors in existing bacterial ORR cathodes. The maximum performance of oxygen microbial biocathodes is not yet up to the level of the best abiotic cathodes, but they offer other unrivalled advantages in terms of stability and resilience of their electrocatalytic activity when exposed in complex and polluted aqueous electrolytic solution (wastewater, leachate, etc.). The low availability of DO ($\sim 0.24 \text{ mM}$), the small amount of aerobic biofilm colonising the cathode, and the multiplicity of redox microbial systems catalysing the ORR are among the principal reasons explaining the poor performance of oxygen microbial cathodes^{4,92}.

Several methods for optimising DO and providing extra convective oxygen transfer in the aqueous electrolyte⁹¹ by stirring, forced aeration and air pumping have proven to be successful but remaining expensive alternatives. To overcome the problems, new intensification paths as floating systems⁹⁴ of ORR bacterial cathodes (Figure 6.A) and passive air breathing bacterial cathodes⁹⁴ (without abiotic **electrocatalyst**) have been recently successfully validated (Figure 6.B). The integration of planktonic microorganisms that produce DO in the electrolyte solution is also being studied⁹⁵.

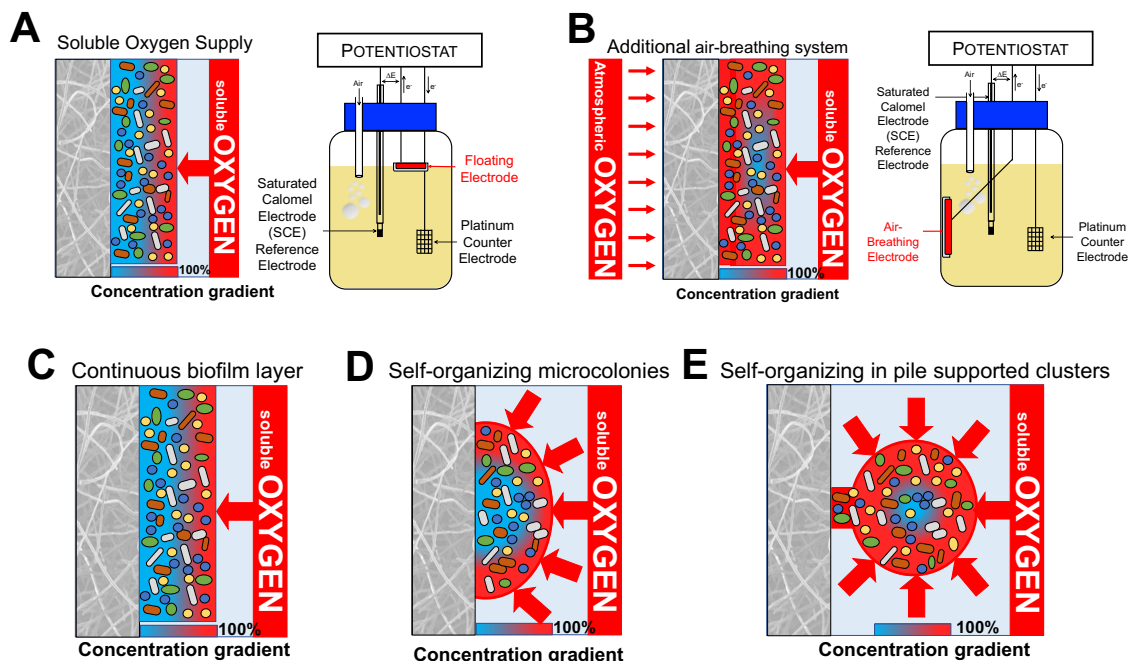


Fig. 6. Oxygen effect on biofilm formation and operations

Diffusion transport of soluble oxygen into the ORR aerobic electroactive biofilm through: (A) floating electrode; (B) an air-breathing electrode. Electroanalytical bioreactor system with: (C) floating electrode; (D) gas diffusion electrode (GDE). (E) Spherical self-organisation of the spatial microstructures of electroactive aerobic biofilms on the cathodes to improve oxygen mass transport.

Limitations by bacterial colonisation of electrode surfaces. Bacterial biocathode encounter several limitations that are being viewed from both nature of the microorganisms and their metabolism and the chemistry and morphology of the electrode, playing the role of biofilm substrate. Microscopic analysis of bacterial ORR cathodes reveals heterogeneous colonisation structures on the cathode⁸⁷. Indeed, aerobic biofilms self-organise in response to low DO, which is enhanced with biofilms increasing thickness (Figure 6.E-G). Thus, under limiting oxygen bioavailability, biofilm patterns either take the form of thin layers containing only a few superimposed layers of bacteria, independent, isolated adherent colonies, or networks of biofilm clusters interconnected with the electrode through parts serving as electron gates⁹⁶.

However, increasing the amount of biofilm and the number of electroactive bacterial cells on the cathode surface remains a major challenge. Electrode designs or engineering systems need to maximise DO transfer leading to massive and homogeneous colonisation rates. The modification of electrode surfaces⁹⁷ with increased hydrophilicity allows a more homogeneous electroactive biofilm distribution. The reversible electrode method leads to dense biofilm thicknesses that significantly restrict the oxygen penetration to the inner layers. The bidirectional EET mechanisms seem to be less effective than those occurring in conventional ORR bacterial cathodes⁸⁹.

Cathode architecture and upscaling

Electrocatalysts integration into the cathode electrodes and electrodes architecture optimisation are two key points. The electrocatalyst integration into the cathode architecture led to several issues related to transport phenomena and effects of chemical and physical nature ranging from angstrom/nanometric scale, up to micrometric scale, summarised in Figure 7.A where SEM images of Fe-N-C (Figure 7.B)⁹⁸, BOx cartoon representation (Figure 7.C), manganese oxides/C (Figure 7.D)⁹⁹ and single bacterium (Figure 7.E)¹⁰⁰ are also inserted into a spatial scale for understanding their involvement at different physical/chemical level.

Cathode fully immersed into an aqueous solution and cathode open to air are the two main cathode configurations used in MESs or EESs²¹. The first configuration (cathode fully immersed), in order to improve the interface electrocatalyst-electrolyte, the electrode surface has to be completely hydrophilic. Importantly, the reaction occurs in a two-phase interface, liquid (protons and DO) and solid (catalytic sites). Particularly, DO is needed for bacterial ORR.

As the ORR is often limiting for MESs and EESs, a larger oxygen concentration is needed and therefore the gaseous oxygen transport is more efficient compared to the DO (~8.6 mM vs. ~0.24 mM). Instead, the second configuration (open-to-air cathode-type) operates with passive oxygen transfer based on natural diffusion phenomenon avoiding oxygen transfer through energy-consuming devices¹⁰¹.

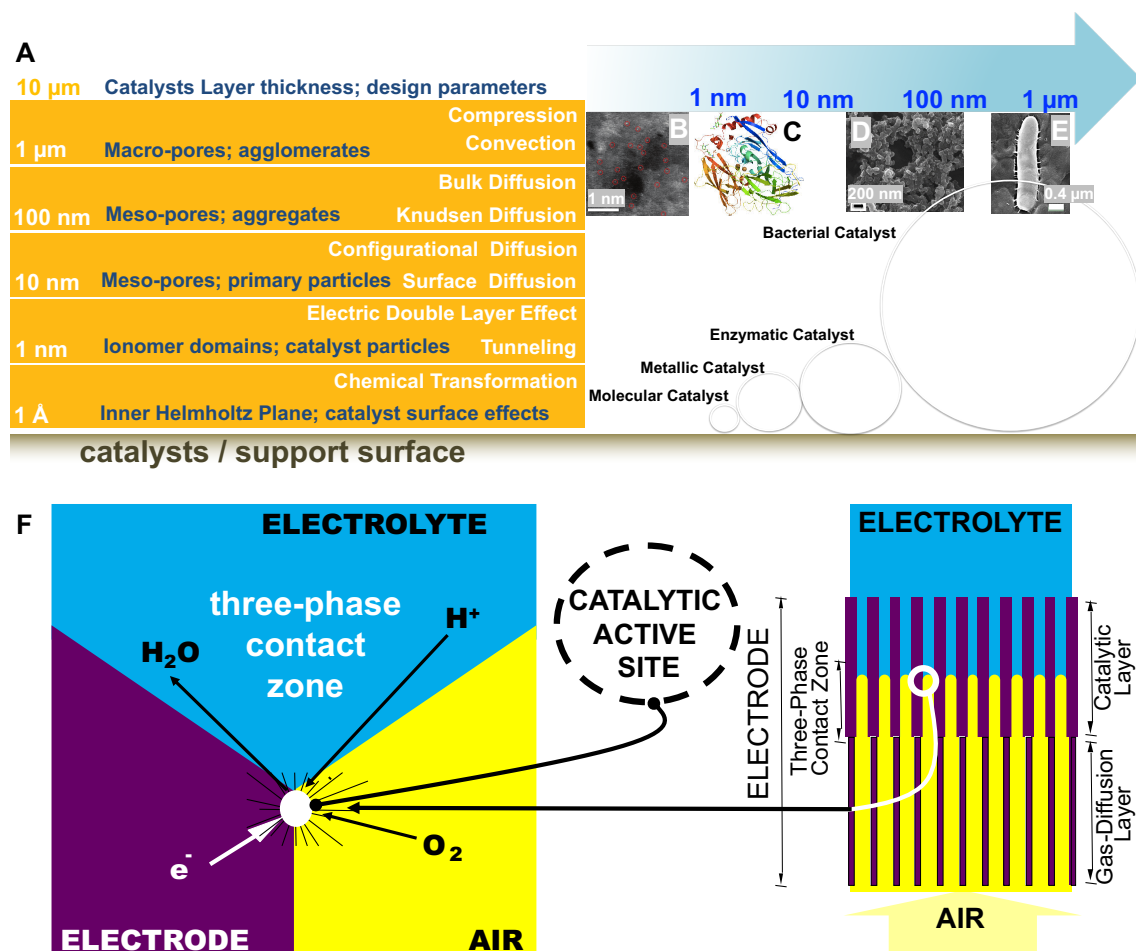


Fig. 7. Oxygen transport phenomena within the cathode architecture

(A) Transport phenomena and interaction occurring at different scale. (B) High-angle annular dark field scanning transmission electron microscopy of Fe-N-C electrocatalyst with subangstrom resolution. (C) BO_x from *Myrothecium Verrucaria*: cartoon representation of the X-ray structure. (D) SEM image of MnO_x/C (E) A magnified image of *Shewanella Oneidensis* MR-1 single bacterium. (F) Schematic of the three-phase interface (TPI).

B was adapted with permission from ref.⁹⁸, Wiley. C was adapted from Wikipedia under CC BY-SA 3.0. D was adapted with permission from ref.⁹⁹, Elsevier Ltd. E was adapted from ref.¹⁰⁰, MPDI under CC BY 4.0.

Air-breathing gas-diffusion electrode architecture. This particular cathode structure is designed particularly for enhancing the three-phase interface (TPI) as represented in Figure 7.F. In the ideal point, the TPI zone is ensured and the ORR occurs optimally. Three different phases (liquid, gaseous and solid) are involved playing a crucial role within the ORR. The liquid phase is responsible for the proton transport to the catalytic centre; the gaseous phase is responsible for the gaseous oxygen transport to the active site and the solid and conductive phase is responsible for the electron transport²¹. Therefore, the cathodes are mainly built using a pellet-type structure composed by carbonaceous-based/electrocatalyst mixture pressed over a carbonaceous or metallic porous current

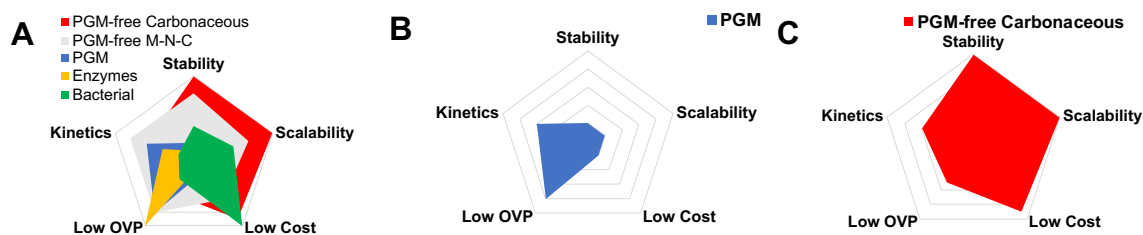
collector (CC)¹⁰². A rigorous air-breathing cathode design might optimise each single phase and merge these optimisations within the structure. Both enzymes and inorganic electrocatalysts find their optimal operation conditions once integrated in this cathode architecture. Another layer, named as diffusion layer/layers, might be added on the CC facing the air for increasing the hydrophobicity gradient and/or containing the liquid electrolyte.

Scaling up the cathodes are of paramount for the bioelectrochemical technology development and deployment. Images of successful pilot scale adopting large scale air-breathing cathodes are presented in Supplementary Figure 5.

Conclusions and perspective

Oxygen reduction electrocatalysis plays central role in technical electrochemistry and is critical for energy conversion and energy storage. BESs provide us with ways of energy harvesting, water treatment and desalination, sites decontamination and biosensing. All these processes benefit by or directly utilise ORR in circum-neutral media. Complex electrocatalysis and hierarchy of scale and co-presence of living systems and inorganic interfaces at realistic technology scale is both a challenge and an opportunity. The realisation of large-scale and commercially available BESs for any of the above-mentioned applications requires focusing on key problems related to cost, durability, power density, biosensing/detection/degradation, etc. ORR is often being considered the limiting reaction in METs and EESs, therefore a substantial improvement and the right compromise among the issues discussed is to be established. The advantages and disadvantages of inorganic, enzymatic and bacterial electrocatalysts are presented in Figure 8. Similar comparison was previously presented more than ten years ago¹⁰³, however important efforts have been carried out in improving the activity and stability of PGM-free electrocatalysts and more long-term studies have been conducted highlighting previously unknown durability issues. Moreover, we have introduced also carbonaceous-based electrocatalyst, not considered up to now.

It was the intention of this Review article to introduce via a collaborative work the fundamentals and technology of oxygen reduction in the most common and least advantageous circumneutral pH to the most diverse audiences of chemical, environmental and biological scientists and engineers.



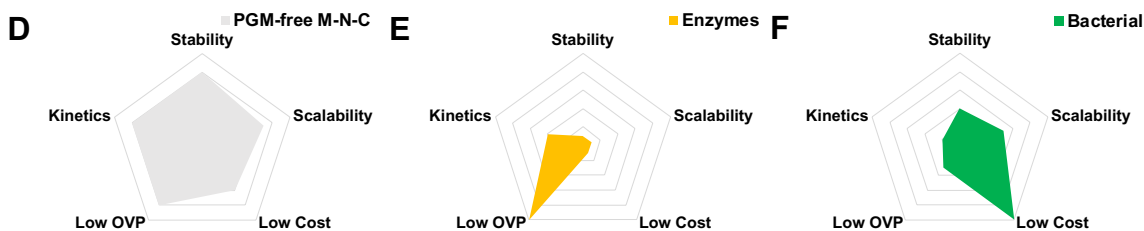


Figure 8. Key performance parameters of biotic and abiotic electrocatalysts

Overlapped comparisons of the key parameters among the different electrocatalysts used in ORR operating in neutral media (A). The parameters identified are stability, scalability, low cost, low OVP (overpotentials) and kinetics. Comparisons of the key parameters for single type of electrocatalysts used in neutral media ORR. (B) PGM, (C) PGM-free carbonaceous, (D) PGM-free metal-nitrogen-carbon (M-N-C) electrocatalysts, (E) enzymes and (F) bacteria.

Inorganic electrocatalysts are the most technologically mature ORR electrocatalysts and are successfully deployed in both acidic and alkaline media, dominantly as PGM cathode electrocatalysts. PGM electrocatalysts meet most activity requirements of acid and alkaline fuel cell technology and are on trajectory to attain to the continuously increasing durability demands. PGM electrocatalysts are not suitable for bioelectrochemical systems because are expensive and their durability is compromise rapidly with the presence of pollutants. Recent introduction of PGM-free electrocatalysts for the mainstream chemical fuel cell provoked renewed interest in studying the ET mechanisms. These are currently studied to chart new paths for both activity and durability improvements. Major effort in shifting towards PGM-free materials is warranted to ensure broad introduction to BESSs.

Carbonaceous materials are currently largely used as electrocatalysts in BESSs because they offer the right compromise of cost, activity and durability. PGM-free electrocatalysts are gaining more foothold as the demand for activity and selectivity raises. PGM electrocatalysts are still used for research purposes but due to their high cost are not suitable for practical applications. So far, the higher electrocatalytic activity is provided by M-N-C electrocatalysts showing durability and pollutant/impurities tolerance while operating in polluted environments. Further enhancement of both activity and durability requires additional effort. An increase of the atomically dispersed Fe-N_x active sites should be accompanied with an increase of the accessibility to the active sites tuning the morphology. Novel synthetic routes are being examined coupled with theoretical studies and microscopic/spectroscopic tools, which all help to understand and predict the electrochemical output. Crucially, PGM-free costs should be reduced substantially to be comparable to carbonaceous materials.

Enzymatic electrocatalysts for ORR have been deeply studied over the last decades with particular attention on the elucidation of the ET mechanism occurring in multicopper

oxidases-which is of critical importance for developing a new generation of enzymatic fuel cells-overcoming the current limitations. In general, the ORR at MCOs modified electrodes is characterised by a high thermodynamic parameter with open circuit voltage being close to the theoretical value. This is associated with the high selectivity of the enzymatic electrocatalysis and the low tendency for side-reactions thus no mixed potential generation. Moreover, MCOs modified electrode exhibited relatively short lifetime. Most of the future research in enzymatic electrocatalysts for ORR would need to focus on improving the interfacial ET between MCOs and the electrode surface, and storage/operational stability, since at the they are suitable only for short-term applications (e.g., disposable biosensors). Some of the main future research directions will focus on electrode bioengineering and immobilisation techniques. For example, to overcome interfacial ET issues, scientists are currently involved in the electrode bioengineering in order to enhance the ET rates. The electrode bioengineering would be optimised with-diazonium coupling or oxidation of amines and physical adsorption or site-directed enzyme immobilisation by the combination of site-directed mutagenesis, electro-grafting, and solid-phase synthesis methodology.

The progression of fundamental knowledge about ET mechanisms for bacterial ORR requires isolates of model aerobic bacteria catalysing ORR. Aerobic cathodic bacteria isolates are still rare⁸⁹ and their electroactivity remains quite unpredictable and difficult to manipulate¹⁰⁴. However, their studies in electroanalytical BES individually, associated in co-cultures, or in the form of synthetic biofilms, would provide essential information firstly on the ET mechanisms, their induction and their effectors; but also on the cooperation phenomena and cell-cathode and cell-cell interactions existing within the more complex mixed biofilm systems.

To overcome the low solubility of oxygen in aqueous media, new engineering innovations need to be envisioned and pursued. Finally, the increase in oxygen bioavailability should naturally induce a much higher rate of bacterial colonisation of the electrodes. In the upcoming years, encapsulating bacteria onto the surface in polymer or silica matrix could be used for pre-colonisation and electrode microbial colony standardisation and it could be envisioned as major technology shift in microbial BESs¹⁰⁵.

The cathode architecture plays a key role to advance the BES technology. The ORR electrocatalytic activity of an electrocatalyst using Rotating Ring Disk Electrode (RRDE) technique doesn't always translate into high cathode performance once integrated into the cathode structure. This observation propagates throughout every fuel cell technology including BESs. Oxygen is supplied directly from ambient air via passive delivery system preferably air-breathing GDE. This places emphasis on optimised cathode architecture with designed TPI to address all these requirements for BES practical applications¹⁰¹. Particular attention has to be devoted to the materials used as current collector, conductive matrix/electrocatalyst to find the optimum cost, performance and durability. The cathode architecture has to contain hydrophilic/hydrophobic features for an optimised TPI. Scaling up and industrial type manufacturing of air-breathing GDEs have been implemented in

BESs¹⁰⁶⁻¹⁰⁷. Fabrication of gas-diffusion cathode with large geometrical area is limited by the difficulty of homogeneous pressing/stamping with size increase. This either limits the design to multiple medium-sized cathodes or calls for a different roll-to-roll fabrication technique. Decrease in cost in any preparation step and consumables is also desired.

As the technology of bio-catalysed cathodes mature and becomes integrated into practical devices, more and more engineering and design issues are being brought in to be addressed, all building on the success of ORR kinetics in circumneutral media.

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Author contributions

C.S., P.B. and B.E. conducted the literature search and wrote the manuscript. P.A. and D.P. supervised the study and wrote the manuscript. All the authors have contributed to the response of the reviewers.

Competing interests

The authors declare no competing interests.