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Review

Cathodes as electron donors for microbial metabolism: Which extracellular electron transfer mechanisms are involved?

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ABSTRACT

This review illuminates extracellular electron transfer mechanisms that may be involved in microbial bioelectrochemical systems with biocathodes. Microbially-catalyzed cathodes are evolving for new bioprocessing applications for waste(water) treatment, carbon dioxide fixation, chemical product formation, or bioremediation. Extracellular electron transfer processes in biological anodes, were the electrode serves as electron acceptor, have been widely studied. However, for biological cathodes the question remains: what are the biochemical mechanisms for the extracellular electron transfer from a cathode (electron donor) to a microorganism? This question was approached by not only analysing the literature on biocathodes, but also by investigating known extracellular microbial oxidation reactions in environmental processes. Here, it is predicted that in direct electron transfer reactions, *c*-type cytochromes often together with hydrogenases play a critical role and that, in mediated electron transfer reactions, natural redox mediators, such as PQQ, will be involved in the bioelectrochemical reaction. These mechanisms are very similar to processes at the bioanode, but the components operate at different redox potentials. The biocatalyzed cathode reactions, thereby, are not necessarily energy conserving for the microorganism.

1. Introduction

Bioelectrochemical systems (BESs) have become an exciting bioprocessing technology in the field of environmental engineering because of the promise of waste(water) treatment with sustainable bacterial metabolic reactions that use the anode as an electron acceptor. While originally mainly anodic biocatalytic reactions were targeted (with abiotic cathodic reactions), recent interest in biologically-catalyzed cathodic reactions opens new sustainable bioprocessing possibilities, such as: (1) sustainable treatment of waste(water); (2) carbon dioxide offsets; (3) production of chemicals; and (4) clean up of recalcitrant chemicals (bioremediation). In this review, the possible extracellular electron transfer (EET) mechanisms for the microbial electron uptake from a cathode are reviewed, but first a short classification of the underlying principles of microbial BESs are given: coupling electron-donating reactions to electron-accepting reactions.

1.1. Microbial fuel cells (MFCs)

Initially, workers used the spontaneous electron movement between electronegative bioanodes and electro positive abiotic cathodes in microbial fuel cells (MFCs) to generate electric power (Logan et al., 2006). However, unsustainable and/or expensive cathode systems were used, such as coatings of the noble metal platinum (Pt) to catalyze the oxygen reduction reaction or ferricyanide as a terminal electron acceptor, which is detrimental to further scale up in waste(water) treatment scenarios. To circumvent this problem, Clauwaert and colleagues (2007a,b) operated MFCs with biocathodes at which bacteria catalyze the electron transfer from the cathode to electro positive terminal electron acceptors, such as oxygen or nitrate. This resulted in a complete biological MFC with both bacteria at the anode and cathode, and therefore self-replenishing biological catalysts on just electrode materials, such as carbon or graphite (further examples are given in Table 1). Besides the circumvention of expensive metal catalysts, exciting recent work has shown that biocathodes in photosynthetic MFCs can also reduce carbon dioxide (CO_2) (Cao et al., 2009). In regards to the economical viability of BES technology, carbon recycling may become very important, especially for a political climate in which lowering the carbon footprint will have a monetary value.

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Table 1
Summary of reported biocathodes, sorted by the working potential of the biocathode.

Microorganism/ mixed culture	Cathode material	Biocathode working potential (vs. SHE)	Redox mediator type (standard potential)	Terminal electron acceptor/reduced end product	Standard potential of the electron acceptor	Reference
Hydrogenophilic methanogenic culture	Carbon paper	<−0.650 V[a]	Not detected	H^+/H_2 CO_2/CH_4	−0.414 V −0.244 V	Villano et al. (2010)
Methanobacterium palustre	Graphite fiber brush	<−0.500 V[a]	Not detected	CO_2/CH_4	−0.244 V	Cheng et al. (2009)
Hydrogenophilic dechlorinating culture	Carbon paper	<−0.700 V[a]	Not detected	H^+/H_2	−0.414 V	Villano et al. (unpublished)
Hydrogenophilic mixed culture	Graphite felt	<−0.650 V[a]	Not detected	H^+/H_2	−0.414 V	Rozendal et al. (2008)
Hydrogenophilic mixed culture	Graphite felt	<−0.600 V[a]	Not detected	H^+/H_2	−0.414 V	Jeremiasse et al. (2010)
Desulfovibrio vulgaris Hildenborough	Glassy carbon	−0.500 V[b]	Methyl viologen (−0.446 V)	H^+/H_2	−0.414 V	Lojou et al. (2002)
Hydrogenophilic dechlorinating culture	Glassy carbon	−0.450 V[b]	Methyl viologen (−0.446 V)	H^+/H_2	−0.414 V	Aulenta et al. (2008)
Anaerobic sludge	Graphite felt	−0.550 V[b]	Methyl viologen (−0.446 V)	Acetate/ethanol	−0.433 V	Steinbusch et al. (2010)
Hydrogenophilic dechlorinating culture	Carbon paper	−0.550 V[b]	Not detected	TCE/*cis*-DCE (VC/ ethane)	+0.550 V	Aulenta et al. (2010)
Kingella kingae	Gassy carbon rod	<−0.460 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Staphylococcus carnusus	Gassy carbon rod	<−0.460 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Hydrogenophilic dechlorinating culture	Glassy carbon	−0.450 V[b]	Methyl viologen (−0.446 V)	TCE/*cis*-DCE (VC/ ethane)	+0.550 V	Aulenta et al. (2010)
Hydrogenophilic dechlorinating culture	Carbon paper	−0.450 V[b]	Self-excreted, unidentified	TCE/*cis*-DCE (VC/ ethane)	+0.550 V	Aulenta et al. (2010)
Shigella flexneri	Gassy carbon rod	<−0.450 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Escherichia coli	Gassy carbon rod	<−0.450 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Kingella denitrificans	Gassy carbon rod	<−0.450 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Enterobacter cloacae	Gassy carbon rod	<−0.430 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Micrococcus luteus	Gassy carbon rod	<−0.430 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Pseudonmonas aeruginosa	Gassy carbon rod	<−0.430 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Pseudomonas fluorescens	Gassy carbon rod	<−0.420 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Branhamella catarrhalis	Gassy carbon rod	<−0.420 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Bacillus subitilis	Gassy carbon rod	<−0.420 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Acinetobacter sp.	Gassy carbon rod	<−0.400 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Burkholderia cepacia	Gassy carbon rod	<−0.380 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Brevundimonas diminuta	Gassy carbon rod	<−0.370 V[a]	Not detected	O_2/H_2O	+0.820 V	Cournet et al. (2010)
Actinobacillus succinogenes	Graphite felt	n.a.[d]	Neutral red (−0.325 V)	Fumarate/ succinate	+0.031	Park and Zeikus (1999)
Geobacter metallireducens	Unpolished graphite rod	−0.300 V[b]	Not detected	NO_3^-/NO_2^-	+0.433 V	Gregory et al. (2004)
Geobacter sulfurreducens	Unpolished graphite rod	−0.300 V[b]	Not detected	Fumarate/ succinate	+0.031	Gregory et al. (2004)
Geobacter sulfurreducens	Graphite plate	−0.300 V[b] (−0.100 V[c])	Not detected	Fumarate/ succinate	+0.031	Dumas et al. (2008)
Geobacter sulfurreducens	Stainless steel	−0.300 V[b] (0.000 V[c])	Not detected	Fumarate/ succinate	+0.031	Dumas et al. (2008)
Geobacter sulfurreducens	Unpolished graphite rod	−0.300 V[b]	Not detected	U(VI)/U(IV)	+0.334	Gregory and Lovley (2005)
Geobacter lovleyi	Unpolished graphite rod	−0.300 V[b]	Not detected	PCE/*cis*-DCE	+0.560 V	Strycharz et al. (2008)
Dechloromonas agitata	Unpolished graphite rod	−0.250 V[b]	AQDS/$AQDSH_2$ (−0.184 V)	ClO_4^-/Cl^-	+0.809 V	Thrash et al. (2007)
Azospira suillum	Unpolished graphite rod	−0.250 V[b]	AQDS/$AQDSH_2$ (−0.184 V)	ClO_4^-/Cl^-	+0.809 V	Thrash et al. (2007)
Activated sludge	Granular graphite	−0.100 V[b]	Not detected	O_2/H_2O	+0.820 V	Cheng et al. (2010)
Anaerobic sludge	Granular graphite	+0.000 V[c]	Not detected	NO_3/N_2	+0.740 V	Clauwaert et al. (2007a)
Marine biofilm	Stainless steel	+0.000 V[b]	Not detected	O_2/H_2O	+0.820 V	Erable et al. (2010)
Acinetobacter johsonii	Stainless steel	+0.000 V[b]	Not detected	O_2/H_2O	+0.820 V	Erable et al. (2010)
Winogradkyella poriferorum	Stainless steel	+0.000 V[b]	Not detected	O_2/H_2O	+0.820 V	Erable et al. (2010)
Sphingobacterium sp.	Carbon fiber	+0.000 V[c]	Not detected	O_2/H_2O	+0.820 V	Rabaey et al. (2008)
Acinetobacter sp.	Carbon fiber	+0.000 V[c]	Not detected	O_2/H_2O	+0.820 V	Rabaey et al. (2008)
Acinetobacter calcoaceticus	Carbon paper	+0.100 V[b]	Self-excreted PQQ	O_2/H_2O	+0.820 V	Freguia et al. (2010b)
Shewanella putrefaciens	Carbon paper	+0.200 V[b]	Not detected	O_2/H_2O	+0.820 V	Freguia et al. (2010b)
Phototrophic mixed culture	Graphite felt	+0.242 V[b] (+light)	Not detected	CO_2/biomass (CH_2O)	−0.420 V	Cao et al. (2009)
Anaerobic sludge	Manganese treated graphite felt	+0.450 V[c]	Not detected	O_2/H_2O	+0.820 V	Clauwaert et al. (2007b)
Aerobic activated sludge	Graphite brush	+0.525 V[c]	Not detected	O_2/H_2O	+0.820 V	You et al. (2009)
Anaerobic digester effluent	Graphite plates	n.a.[d]	Not detected	Cr(VI)/Cr(III)	+1.33 V	Tandukar et al. (2009)

[a] Onset (starting) potential of the bioelectrocatalytic reaction in potentiostatically controlled potential scans.
[b] Potentiostatically applied working potential.
[c] Equilibrium potential of the biocathode in microbial fuel cell BES mode (regarded as an estimation of the equilibrium potential of the enzymatic complex that functions as the electron donor for the microbial electron transfer chain).
[d] Not available.

1.2. Microbial electrolysis cells (MECs)

Preliminary calculations have shown that the economic value of the generated electric power by MFCs from wastewater treatment is currently insufficient to warrant a large investment into BES technology, but that offsetting conventional costs of treatment with activated sludge would aid in returning the investment (Fornero et al., 2010). A recently published life-cycle assessment showed that producing a chemical product, such as hydrogen (or in their case hydrogen peroxide), at the cathode would provide considerably larger environmental benefits compared to generating electric power with MFCs (Foley et al., 2010). In addition, such product would also generate a larger monetary pay back that is necessary to warrant the investment, especially when the chemical product is useful at the wastewater treatment plant (Rosenbaum et al., 2010a). However, to generate a product, such as hydrogen, at the cathode, thermodynamic constraints must be overcome by providing an artificial potential increase between the anode and cathode (Logan et al., 2008; Rozendal et al., 2006). This is mostly performed with a 2-electrode BES for which the worker sets the potential difference between the electrodes by using a potentiostat or power supply, and this is referred to as a microbial electrolysis cell (MEC). MECs obtain all electrons to maintain a half reaction at the cathode from the anodic oxidation of organic material in waste(water). Therefore, the power supply is not the source of electrons, but rather it just overcomes cathodic reaction overpotentials by increasing the potential difference between the two electrodes. In other words, without the external power supply the desired reaction on the cathode cannot occur. Biocathodes in MECs have been used, for example, by Rozendal et al. (2008) to produce hydrogen and by Cheng et al. (2009) to produce methane from CO_2 (Table 1).

1.3. Microbial 3-electrode cells (M3Cs)

Several researchers have shown that an optimum anode potential should be maintained to guarantee efficient BES performance and anodic wastewater treatment (Aelterman et al., 2008; Torres et al., 2009; Wei et al., 2010). This can be performed with a 3-electrode BES for which the worker sets the working electrode potential (in this case for optimum anodic potentials) by using a potentiostat and a reference electrode, such as a Ag/AgCl reference system. Here, this BES is referred to as a microbial 3-electrode cell (M3C). *Vice versa*, the set potential at the working electrode can also maintain optimum cathodic potentials to, for example, support a bioelectrochemical electron-accepting reaction. Thus, an M3C can be regarded as a special case of an MEC for which an external power input helps to drive the reaction, while one electrode potential (working electrode) is controlled at favorable electrochemical conditions. Even more importantly, through optimization of the reaction rate, the M3C can boost the current density, which is also pertinent to ensure an economical scale up. Foley et al. (2010) have suggested a requirement of 1000 A/m^3 for a successful pilot or full-scale project, and such a high current is feasible with an M3C because after setting the working electrode potential (vs. the reference electrode), the maximum bioelectrochemical reaction rates at the working electrode are achieved by an automated increase in the potential difference between anode and cathode (the worker does not set the potential difference). Although this may require potentials ~2 V, this can still be economical if a product is formed with an added value that is higher than the value of electric energy consumed by the power supply. However, scale up of M3Cs may be difficult due to problems with maintaining a set working electrode potential for large surface areas. M3Cs without membranes (potentiostat-poised half cells) have been used to proof the concept of biocathodes even before applications in MFCs were considered (He and Angenent, 2006). Recently, M3Cs have been used to sustain biocathodes as powerful new devices for bioremediation applications, such as the reduction of chlorinated compounds (Aulenta et al., 2010), uranium (Gregory and Lovley, 2005), or chromium [(Tandukar et al., 2009) – here operated as an MFC, but M3C application is feasible] (more examples are given in Table 1).

2. Biocathodic electron transfer mechanisms

Although an increasing number of biocathode studies were published in recent years (e.g., 2 publications in 2004; 12 in 2007; and 34 in 2009; Source: Web of Knowledge, keyword "biocathode*", April 26, 2010) (Table 1), not much is known about the biochemical mechanisms of microbial electron uptake from a cathode. The main goal for this review is to suggest and discuss an array of possible bioelectrochemical electron-accepting reaction – none of which has been fully experimentally proven. This discussion is meant to motivate and inspire further research for the clarification of the respective biocathode reaction mechanisms. Since recent biocathode investigations (Cheng et al., 2010; Rozendal et al., 2008) have shown that "trained" electrochemically-active bioanodes may be turned into biocathodes upon changing the BES environmental and operating conditions, the investigative discussion of this review is initiated by recalling some known anodic EET mechanisms and evaluating their potential role in cathodic EET mechanisms.

2.1. Direct electron transfer

2.1.1. c-Type cytochromes play a pivotal role in direct anodic EET over a wide range of potentials

Thus far, the best studied anodic EET is the extracellular respiration of dissimilatory metal-reducing bacteria of the genera *Shewanella* and *Geobacter*. Both microorganisms are frequently found in the microbial community of MFCs (Butler and Nerenberg, 2010; Chae et al., 2009; Freguia et al., 2010a; Jung and Regan, 2007; Kim et al., 2006). They are capable of transferring metabolic electrons through a chain of *c*-type cytochromes across the cell envelope to extracellular electron acceptors. Comprehensive state of the art reviews on these electron transport chains have been recently published (Rosenbaum and Angenent, 2010; Weber et al., 2006). Terminal reductases – OmcA and MtrC for *Shewanella oneidensis* and, for example, OmcE and OmcS for *Geobacter sulfurreducens* – can either directly transfer electrons to solid extracellular electron acceptors, or donate electrons to soluble extracellular redox compounds [e.g., humic compounds (Cadena et al., 2007; Voordeckers et al., 2010) or riboflavins (Marsili et al., 2008)].

In bioelectrochemical studies of *G. sulfurreducens* grown with a positive electrode potential (anode: +240 mV; all redox potentials in this review are given vs. the standard hydrogen electrode, SHE), the mixed mid-peak potential of the electrochemical reaction is ~−150 mV (Marsili et al., 2010; Srikanth et al., 2008). This potential compares well to the mid-peak potentials of purified *c*-type cytochromes from *G. sulfurreducens* [OmcB: −190 mV (Magnuson et al., 2001); PpcA: −167 mV (Lloyd et al., 2003); PpcA expressed in *Escherichia coli*: −137 mV (Pessanha et al., 2006)]. However, recent studies with *G. sulfurreducens* suggested that this bacterium can adjust its redox activity to the potential of the electron acceptor (Wei et al., 2010; Yi et al., 2009). This finding is not surprising, considering that its genome contains 111 predicted *c*-type cytochromes most of which are still largely uncharacterized (Methe et al., 2003). Thus, it seems likely that the spectrum of *c*-type cytochromes (each with distinct redox properties) is selected to meet the availability and potential of the terminal electron acceptor.

As an example, electrochemical analysis by Marsili et al. (2010) found that additional redox peak systems appear with *G. sulfurreducens* cells when grown at an applied voltage of −160 mV instead of +240 mV over an extended period of time. Yi et al. (2009) evolved a *G. sulfurreducens* strain with superior performance by growing the culture at −400 mV for 5 months. The isolated strain KN400 showed higher current production at a lower biofilm thickness and a lower concentration of total *c*-type cytochromes, but a higher abundance of microbial nanowires than the original strain *G. sulfurreducens* DL1 [comment: nanowires are electronically conductive protein appendages and represent another mechanism of EET by *G. sulfurreducens* (Reguera et al., 2005; Yi et al., 2009)]. Electrochemical analyses were not performed, but the presented heme-stained protein gel indicates a different composition of *c*-type cytochromes for KN400 compared to DL1. Thus, it is possible that strain KN400 utilizes different cytochromes with lower mid-peak potentials.

2.1.2. c-Type cytochromes are also involved in direct cathodic EET

The fact that *c*-type cytochromes can donate electrons to an electrode at various electrode potentials was already discussed, but the important question of this review is whether they can take up electrons from the cathode. They can in the environment: *c*-type cytochrome-mediated electron uptake from solid electron donors is a very common process in nature, especially in acidic environments, such as mine drainages, where chemolithotrophic iron(II) and sulphur oxidation are the dominant microbial activities. Commonly, the oxidation process takes place at oxic/anoxic interfaces with Fe(II), S^0, or S^{2-} as electron donor and oxygen as electron acceptor. For example, it has been shown that the outer membrane *c*-type cytochrome Cyc2 from *Acidithiobacillus ferrooxidans* can accept electrons directly from Fe(II) minerals (e.g., pyrite) (Yarzabal et al., 2002). This protein then passes the electrons onto an electron transport chain with oxygen reduction as the final reaction step (Castelle et al., 2008). Thus far, the redox potential of purified Cyc2 (+0.560 V) is the highest potential observed for a *c*-type cytochrome (Yarzabal et al., 2002). Recently, Jeans et al. (2008) found cytochrome (Cyt_{572}), which was abundantly found in iron(II) oxidation environments, to posses the characteristic heme binding motive of *c*-type cytochromes. Considering that these naturally occurring electron uptake processes involve *c*-type cytochromes, it is, therefore, likely that *c*-type cytochromes are important for biocathodes (Fig. 1A).

Thus, here, it was discussed that a different set of *c*-type cytochromes is employed in the environment for iron oxidation (e.g., with *A. ferrooxidans*) than for iron reduction (e.g., with *G. sulfurreducens*). However, in BESs for which defined potentials can be applied to electrodes it may also be possible that identical *c*-type cytochromes are used by the microorganism on the anode and cathode. This strongly depends on the redox potential of the involved cytochromes and their eventual connection with an electron transfer chain or quinon pool. The electrochemical analysis of native and purified *Geobacter c*-type cytochromes shows that the electron transfer reaction of the outer membrane cytochromes is reversible (i.e., a reduction wave or peak appears in cyclic voltammetry if the potential scan is reversed). This finding, however, does not necessarily imply that the reduced cytochrome is further capable of passing electrons to a redox partner and ultimately to a terminal electron acceptor. Indeed, for the electrode to function as electron donor, the outer membrane cytochromes have to pass the electrons to more electro-positive electron acceptors within the periplasm and inner membrane. If the final reaction of the biocathode is the reduction of highly electro-positive electron acceptors, such as oxygen (Freguia et al., 2010b), nitrate (Lefebvre et al., 2008), or chlorinated organic compounds (Aulenta et al., 2010; Strycharz et al., 2008), then the potential range at which the microorganisms can take up electrons is broad and can leave enough potential difference for energy-conserving reactions between the electrode (electron donor) and the electron acceptor ($E^{\circ\prime}$[oxygen] = +820 mV, $E^{\circ\prime}$[nitrate] = +430 mV, $E^{\circ\prime}$[TCE] = +510 mV) (Fig. 1A). However, it is also possible that reactions mediated by outer membrane *c*-type cytochromes occur without the conservation of energy (i.e., ATP generation). For instance, the reduction of oxygen does not necessarily require enzymes – biological metal cofactors, such as heme molecules, can be sufficient to catalyze the oxygen reduction. As an example, Freguia et al. (2010b) found that a *Shewanella putrefaciens* strain can catalyze the oxygen reduction passively via *c*-type cytochromes, which are located on its cell surface, without the active involvement of the bacterium.

2.1.3. A powerful combination: c-type cytochromes and hydrogenases

The range of metabolic processes catalyzed by biocathodes is wide and ever increasing (Table 1). An interesting consideration is that a majority of these processes typically involve microorganisms capable of metabolizing H_2. Thus, they likely possess hydrogenases, which are enzymes catalyzing the reversible production and oxidation of H_2. Hydrogenases have been found in a wide variety of prokaryotes from aerobes to strictly anaerobes, and also in some eukaryotes, such as algae (Madigan and Martinko, 2006). Over the last years, the ability of hydrogenase-containing microorganisms to engage in EET processes with solid materials has been largely debated in the literature, especially in the field of anaerobic metal biocorrosion (i.e., the accelerated oxidation and deterioration of metals under a biological influence). Biocorrosion is frequently linked to the activity of sulfate-reducing bacteria (SRB) that are thought to act upon metal surfaces via (at least) two distinct mechanisms. The first mechanism is the chemical attack of the metal surface by biogenic hydrogen sulfide. The second mechanism involves the scavenging of hydrogen or possibly electrons from the metal surfaces by means of hydrogenases and/or other enzymes. Among the SRB, *Desulfovibrio* sp. (with their capacity to effectively consume hydrogen) have often been attributed a pivotal role in this second biocorrosion mechanism, even though the underlying mechanisms have not been completely elucidated.

Kloeke and colleagues (1995) isolated and partially characterized the outer and cytoplasmatic membranes of *Desulfovibrio vulgaris* (Hildenborough). Based on their findings, the authors proposed a new model for the biocorrosion of mild steel: a high molecular weight cytochrome (Hmc) localized in the outer membrane of the microorganism was suggested to be the "entrance point" of the electrons and the redox partner of a [Fe]-hydrogenase localized in the periplasm of the microorganism. Evidence for this model originated from adding the outer membrane fraction (containing Hmc and [Fe]-hydrogenase) to a phosphate solution (pH = 7) with mild steel as the source of electrons. H_2 generation was greatly enhanced compared to control tests lacking these enzymes. Different types of cytochromes were found in the outer membrane of other *Desulfovibrio* sp. and their role as redox partners of hydrogenase was confirmed (Barton et al., 2007; Guiral-Brugna et al., 2001; Pereira et al., 1996; Verhagen et al., 1994). Interestingly, the recently published genome sequence of *D. vulgaris* (Hildenborough) revealed the presence of a pool of *c*-type cytochromes, which create a vast network of interconnected hemes (Heidelberg et al., 2004). This network likely provides the electrical wiring for connecting multiple periplasmic enzymes, including hydrogenases, and may also serve as a temporary capacitor for storage of low-potential electrons. That this has a broader impact was shown by Dinh and colleagues (2004), who observed that a *Desulfobacterium*-like isolate and a *Methanobacterium*-like archaeon were capable of accepting electrons from metallic iron in a more direct manner than via H_2 consumption. To explain these findings, the authors hypothesized the occurrence of an efficient

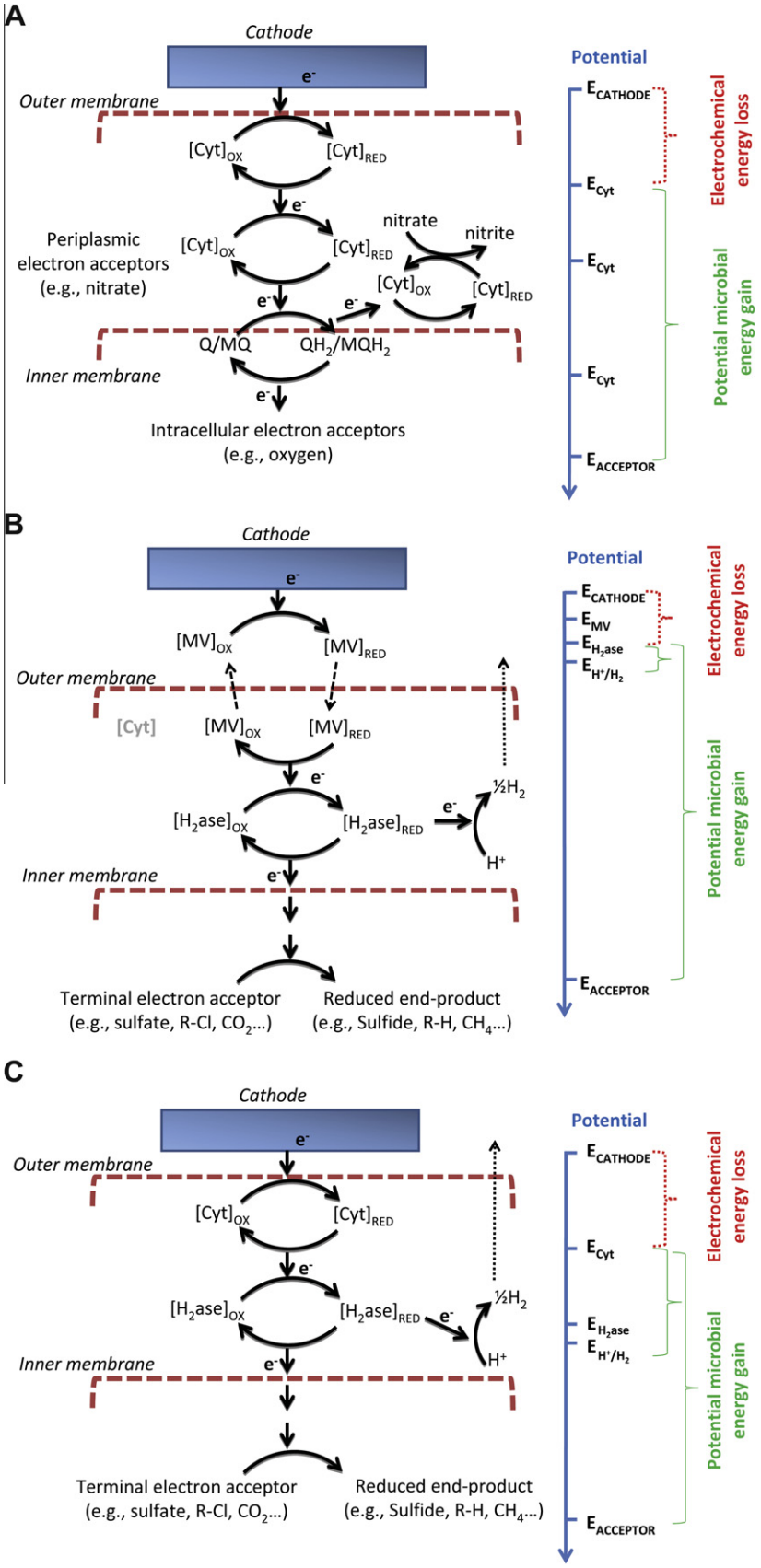

Fig. 1. Proposed cathodic extracellular electron transfer mechanisms and associated energy gains for biocathodic microorganisms: (A) direct electron transfer involving *c*-type cytochrome electron transfer chains. (B) Mediated electron transfer to a periplasmic hydrogenase. Examples are given for terminal electron acceptors; the reaction site for the terminal reduction depends on the type of electron acceptor. (C) Direct electron transfer involving cytochrome-hydrogenase partnerships.

electron uptake via a cell-surface-associated (yet unknown) redox active component. Cytochromes could very well be those redox components. These studies suggest a key role of *c*-type cytochromes in electronic interaction of microorganisms with their surrounding environment, which includes biocathodes.

2.1.4. The utilization of hydrogenase-containing microorganisms in biocathodes

Despite the many literature reports on the ability of hydrogenase-containing microorganisms to accept electrons from metal surfaces in the field of biocorrosion, only very few attempts have been made to characterize their performance and potential application in BES. While the electrocatalytic activity of purified hydrogenases adsorbed onto electrodes has received considerable attention (Cracknell et al., 2008; Lamle et al., 2003), very few studies have explored the possibility to utilize whole bacterial cells at the cathode. The first attempts to catalyze H_2 production via bacterial electrocatalysis was presented by Tatsumi et al. (1999) and Lojou et al. (2002). They demonstrated that in the presence of dissolved methyl viologen (MV), which is a low-potential redox mediator ($E^{o\prime}$ = −446 mV), *D. vulgaris* (Hildenborough) catalyzed H_2 production with a graphite electrode serving as electron donor. Since MV is considered to be unable to cross the cytoplasmatic membrane (Jones and Garland, 1977), they suggested that it could directly donate electrons to a periplasmic hydrogenase (Fig. 1B). Thus far, no information is available on the ability of *Desulfovibrio* sp. cells to accept electrons from a cathode via direct EET (without mediator). However, it seems likely that *Desulfovibrio* is capable of direct EET, since studies with purified *Desulfovibrio* enzymes have shown direct electron transfer from a cathode through cytochrome *c*3 to a [FeNi]-hydrogenase to produce hydrogen (or reversed) (Moreno et al., 1993; Niviere et al., 1988).

Bioelectrocatalytic H_2 production in the presence of MV as a redox mediator and a carbon electrode polarized at −450 mV was also reported for dechlorinating bacteria (Aulenta et al., 2008, 2007). Similarly to SRB, dechlorinating bacteria are also very effective at consuming H_2 and are known to possess multiple periplasmic hydrogenases that probably accept electrons directly from reduced MV (Nijenhuis and Zinder, 2005). They suggested that H_2 production was a strategy to dispose of an excess of electrons, which could not be rapidly diverted to the physiological electron acceptor (i.e., TCE).

The first concept of mediatorless H_2 production with a microbial cathode was presented by Rozendal et al. (2008). Interestingly, their biocathode was initially operated as a bioanode, which was first fed with acetate and then with H_2 as electron donor (during bioanode-mode). The bioanode was characterized by an equilibrium potential of ∼−300 mV. This value is typical for systems dominated by *Geobacter* or *Shewanella* species and also consistent with the superior ability of these microorganisms to oxidize acetate or H_2, using an electrode as electron acceptor. When the electrode polarity was reversed, a cathodic current (linked to H_2 production) established without appreciable lag-phase for potentials lower than −650 mV. The finding that electrons were donated to the electrode at −300 mV, while they were accepted from the electrode at −650 mV, may either suggest the involvement of distinct redox active components (e.g., different outer membrane cytochromes) or substantial quasi-reversible catalytic behavior of a single redox system (e.g., the same outer membrane cytochrome) (Bard and Faulkner, 2001). Even though the direct involvement of a hydrogenase in H_2 production was indicated by means of carbon monoxide inhibition tests, it remains to be seen how the electrons were passed from the electrode surface to the hydrogenase. A tentative cathodic electron transfer pathway for the biocathode is proposed in Fig. 1C. This pathway could also work for *Shewanella*, which, in the absence of an external electron acceptor, has been reported to produce H_2 as a strategy to dispose of the excess of reducing equivalents (Meshulam-Simon et al., 2007). Disposing an excess of electrons by the production of hydrogen, however, is not an energy conserving reaction. No information is currently available on the ability of *G. sulfurreducens*, which contains hydrogenases, to produce sustainable amounts of H_2.

Recent studies have shown that a hydrogen-utilizing *Desulfitobacterium*-enriched dechlorinating culture catalyzes H_2 production with carbon electrodes serving as electron donors, either in the presence or in the absence of dissolved MV (Villano et al., submitted for publication). The bioelectrocatalytic H_2 production was affected by substantial overpotentials, only in the latter case (i.e., bioelectrocatalytic H_2 production required cathode potentials more negative than −700 mV), indicating that the passage of electrons from the solid electrode surface to the hydrogenases was the most sluggish and difficult step of the EET chain.

Very recently, hydrogen-utilizing methanogens were also reported to catalyze methane production from carbon dioxide reduction with carbon cathodes as electron donors, which were polarized at potentials more negative than −500 mV (Cheng et al., 2009; Villano et al., 2010). In one of these studies (Cheng et al., 2009), a *Methanobacterium* spp. was found to be the dominant microorganism at the cathode, recalling the previously described results of Dinh et al. (2004), who suggested that this methanogen possesses the ability to engage in microbial electron uptake from solid surfaces. The methanogens also contain membrane-associated hydrogenases with ferredoxin or methanophenazine as redox partners *in vivo* (Thauer et al., 2010), which could be directly or indirectly involved in biocathodic methane production. To prove this case, however, further experiments will have to be performed.

Most hydrogenases are reported to work very close to the thermodynamic potential for H_2 production (Armstrong and Fontecilla-Camps, 2008; Cracknell et al., 2008; Tatsumi et al., 1999). Thus, if electrons enter the metabolism directly at the hydrogenase level (at mediatorless H_2-producing biocathodes), no energy is likely to be gained by the microorganisms. Similar considerations are true for the MV-mediated biocathodic H_2 production, considering that the standard redox potential of MV (−446 mV) is very close to that of H_2 (−414 mV). Energy can only be gained if electrons enter the metabolism at the level of a sufficiently more electronegative component, such as an outer membrane cytochrome. A substantially different energetic scenario is expected when the electrons are ultimately transferred to a more electro-positive electron acceptor, such as sulfate, chlorinated compounds (R–Cl), carbon dioxide, or others as discussed in Section 2.1.2 (Fig. 1). Here, again, it depends on the potential difference between electron donor and acceptor and the metabolic "wiring" between the two, if and how much energy can be conserved.

Overall, it is becoming apparent that a wide range of hydrogenase-containing microorganisms are capable of using electrons from polarized electrodes (with and without soluble redox mediators) to catalyze the reduction of their physiological electron acceptors or to produce H_2 (i.e., if their physiological electron acceptors are not available). For the *Desulfovibrio* genus, *c*-type cytochromes seem to be required for electron transfer to and from hydrogenases. The electron transferring components (e.g., *c*-type cytochromes, other oxidoreductases, or mediators) to link the hydrogenase activity to cathodes in other genera remain unknown at this point.

2.1.5. Evidences of cathodic EET involving bacterial photosystems

Recently, the application of photosynthetic biocatalysts in BES has experienced great interest (Rosenbaum et al., 2010b). Cao et al. (2009) reported on direct cathodic EET in an anoxygenic photosynthetic mixed culture supplied with only light and carbon

dioxide. Although a light-dependent direct electron transfer in this study is possible, the use of a mixed microbial culture cannot exclude nonphotosynthetic microorganism to perform the necessary EET (e.g., hydrogen generation, see Section 2.1.4). While the authors suggest the involvement of the bacterial photosystem in the light-dependent electron uptake reaction, other metabolic reactions within the photosynthetic microorganism, which include redox proteins, such as cytochromes, could also be responsible for EET.

In the case of light-dependent EET by photosynthetic bacteria, the efficiency or value of this reaction for the microorganism depends on its membrane structure and the energetics of its photosynthetic component. Photosynthetic complexes of these bacteria are often located within intracytoplasmic membranes [e.g., in membrane vesicles = chromatophores (Madigan and Martinko, 2006), or sheet-like thylakoids (Miller, 1979)]. Thus, the majority of the photosynthetic complexes of a cell are typically not in contact with the outside of the cell. The light-dependent activation reaction of the anoxygenic bacterial photosynthesis complexes results in the excitation of reaction centres (e.g., P870 for nonsulphur purple bacteria, P840 for green sulphur bacteria, and P798 for *Heliobacterium*) (Madigan and Martinko, 2006). In its excited state, the reaction centre reduces other components of the photosynthetic complex, and therefore becomes oxidized itself. To complete the cycle, the reaction centre is typically re-reduced by a *c*-type cytochrome. How then can a cathode be involved in this cycle? Some bacterial photosynthetic reactions (e.g., of purple bacteria) only provide enough energy for the generation of ATP (through a proton motive force), but not for the formation of reducing equivalents, such as $NADH_2$, which are required for autotrophic growth with carbon dioxide. Thus, the photosynthetic electron flow is cyclic and does not involve the generation of electrons (i.e., reducing equivalents for metabolic reactions). Instead, during autotrophic growth, purple bacteria generate $NADH_2$ by the oxidation of reduced electron donors (e.g., S, H_2S, $S_2O_3^{2-}$, or Fe^{2+}) via *c*-type cytochromes, as was discussed above (Kappler et al., 2005; Madigan and Martinko, 2006). But even when the light reaction provides enough reducing power to produce $NADH_2$, as it is the case for green sulphur bacteria or Heliobacteria, the electrons are derived from the oxidation of inorganic or organic substrates through nonphotosynthetic reactions. Therefore, for electrochemically-active, anoxygenic photosynthetic bacteria, *c*-type cytochromes, which are used for the oxidation of inorganic electron donors and *not* the photosynthetic reaction centre, most likely are the entry point for cathodic electrons. Thus, even though the cathodic electron uptake may be energetically supported by a photosynthetic reaction, here, it is believed that not the photosynthetic reaction centre itself, but a *c*-type cytochrome will be responsible for the primary electron uptake.

2.1.6. Other oxidoreductases

Similar to *c*-type cytochromes, some organisms employ copper-containing oxidoreductases in their outer envelope to mediate EET. For example, the multi-copper proteins OmpB and OmpC from *G. sulfurreducens* are involved in extracellular respiration with iron(III) compounds or electrodes (Holmes et al., 2008). Rusticyanin, a small blue copper-containing protein, plays a central role in sulphur and iron oxidation by *A. ferrooxidans* and *Thiobacillus ferrooxidans* (Yamanaka and Fukumori, 1995; Yarzabal et al., 2004). Similarly, during iron(II) oxidation of *A. ferrooxidans*, the *c*-type cytochrome Cyc2 directly interacts with a copper-containing cytochrome *c* oxidase aa_3 (Castelle et al., 2008). Instead, for *T. ferrooxidans* the first step in the electron transfer chain for iron(II) oxidation is the uptake of electrons by a Fe_4S_4-cluster-containing oxidoreductase (Yamanaka and Fukumori, 1995). The electrons are then passed onto *c*-type cytochromes and copper-containing oxidases to finally reduce oxygen. Another example of environmentally abundant copper-containing redox enzymes are laccases, which catalyze the reduction of oxygen to water. Although laccases have mostly been described in eukaryotes (fungi, plants), evidence for their widespread presence in bacteria has been provided (Claus, 2003; Sharma et al., 2007). Owing to their high, but relatively unspecific oxidation capacity, laccases are useful biocatalysts for diverse biotechnological applications. It has been reported that laccases, which were adsorbed onto electrodes, catalyze the reduction of oxygen (e.g., in enzymatic biofuel cells), both via direct electron transfer (Gupta et al., 2004; Shleev et al., 2005) and with the mediation of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS) (Merle et al., 2008; Palmore and Kim, 1999). In living microorganisms, laccases are typically located in the outer membrane or are released in the surrounding environments. This suggests that laccase-containing microorganisms could potentially be able to engage in direct cathodic EET, although evidence is scarce. However, considering the very high potential of the copper reaction sites in the enzyme (up to +750 mV), electrons entering the cell at the laccase level, should not be available for metabolic energy generation. Given this wide spectrum of natural oxidoreductases for electron uptake, here, it is believed that other outer membrane redox proteins – besides the *c*-type cytochromes and hydrogenases – can be directly involved in electron uptake from a cathode.

2.2. Mediated electron transfer

2.2.1. Application of Artificial Electron Mediators

Extensive work with artificial redox mediators has been pursued to facilitate EET from a cathode to a microorganism, which by itself is not capable of doing so. Most commonly, methyl viologen (MV) (Aulenta et al., 2007; Lojou et al., 2002; Steinbusch et al., 2010), but also anthraquinone-2,6-disulfonate (AQDS) (Hatch and Finneran, 2008; Thrash et al., 2007) and neutral red (NR) (Park and Zeikus, 1999) have been used. Artificial redox mediators posses the great advantage of fairly well known redox and chemical properties. As already stated, MV can only penetrate the outer membrane of microorganisms, but cannot enter the cytoplasm, and therefore electrochemical interactions are only possible within the periplasm. This allows narrowing down the possible interaction pathways for MV-mediated EET. With its standard redox potential close to that of H_2, MV typically is employed as a redox partner of hydrogenases; both in H_2 production and oxidation studies. Nonetheless, a recent study has shown that reduced AQDS and natural quinones, despite their substantially higher redox potentials, enhanced the H_2 production (and altered the fermentative metabolism) by *Clostridium beijerinckii* (Hatch and Finneran, 2008). Previous studies also demonstrated that electron flow in cells and end product distribution could be manipulated by supplementing a culture of clostridia with viologen dyes as artificial electron shuttles (Rao et al., 1987). Unlike MV, electrically reduced NR ($E^{\circ\prime} = -325$ mV) has been reported to strongly bind to cell membranes and be reoxidized by intracellular NAD^+ or by the fumarate reductase complex (Park et al., 1999; Park and Zeikus, 1999). In summary, these data suggest that artificial mediators not only provide a useful tool for unraveling fundamental aspects of EET mechanisms, but also provide new and attractive strategies for fine tuning of microbial growth and metabolism in biocathodes.

2.2.2. Intrinsic and Natural Electron Mediators

Microbially produced redox mediators, such as phenazines from *Pseudomonas* spp. (Rabaey et al., 2005; Venkataraman et al., 2010) or flavins from *S. oneidensis* (Marsili et al., 2008; von Canstein et al., 2008), represent very important vehicles for electron transfer between bacteria and from bacteria to anodes. Benefits of these

mediators are: (1) they can also be utilized by other microorganism than their producer; and (2) they can react at multiple reaction sites within the periplasm, at the cytoplasmic membrane, and sometimes even within the cytoplasm. Depending on the equilibrium potential of the mediator and the working potential of the cathode, it is likely that intrinsic redox mediators, such as phenazines, also play an important role in microbial biocathodes. Indeed, Freguia et al. (2010b) found that the native pirroloquinoline quinone (PQQ) could act as a reversible redox mediator for EET during biological oxygen reduction at a biocathode with *Acinetobacter calcoaceticus*. In its natural function, PQQ is a co-factor of glucose dehydrogenase and serves as the electron acceptor during glucose oxidation. In enzymatic biofuel cells, the enzyme gained importance as an anodic biocatalyst with an electrode as electron acceptor (Ivnitski et al., 2007). Additional evidence for intrinsic mediators in biocathodes was recently obtained by Aulenta and colleagues (2009), who detected an unidentified redox mediator with a mid-peak potential of $\sim$−400 mV in the filter-sterilized supernatant of a TCE dechlorinating culture. This redox compound could be an excreted microbial product (e.g., phenazines, riboflavins), or it could be released upon death and lysis of microbial cells. Dechlorinating bacteria, for example, are known to contain cyanocobalamin (vitamin B_{12}) and other redox active cofactors, which may be released upon cell death and possibly could then act as redox shuttles. One further possibility for intrinsic electron mediation is given by the electronic properties of DNA. Individual purine and pyrimidine bases resulting from the degradation of extracellular DNA and from cell lysis could potentially play a role as mediators for biocathodic EET (although the reduction potential of <−2.0 V of many individual bases might be too negative to be applicable) (Boussicault and Robert, 2008).

Freguia et al. (2010b) also investigated if PQQ could be involved in an abiotic oxygen reduction process at the biocathode, but found only minor catalytic effects. However, in the same paper they did find abiotic autooxidation activity with AQDS and a heme compound (hemin): the cathode reduces these mediators, which subsequently undergo autooxidation with oxygen (Freguia et al., 2010b). This autooxidiation effect has also been described for flavin-type mediators (Tatsumi et al., 1998). Since riboflavins are produced by *S. oneidensis* in BES (Marsili et al., 2008), this abiotic electrocatalysis could play an important role in oxygen reduction biocathodes. Indeed, Freguia et al. (2010b) found electrocatalytic oxygen reduction activity with *S. putrefaciens* (this species is very similar to *S. oneidensis*) for the culture broth with microorganism and for the supernatant, indicating that soluble components without bacteria catalyze the reaction. However, the typical reversible redox signal of flavins was not detected in these experiments and the mechanism of this activity is, thus, unclear at this point. The authors suggest heme-containing proteins (on the outer membrane and resulting from cell lysis) catalyzed the reaction.

Thus far, intrinsic electron mediators at the biocathode that originate from microbial metabolism were discussed. For biocathode applications in soils and sediments (e.g., bioremediation) it is further possible that quinone-like humic substances or manganese species can act as natural redox mediators (He and Angenent, 2006; Rhoads et al., 2005; Roche et al., 2010). De Schamphelaire et al. investigated natural electron mediation under exploitation of the manganese cycle at a biocathode in sediment applications (De Schamphelaire et al., 2008, 2007). In soils, manganese(IV) oxide is reduced at the cathode to produce soluble manganese(II), which in turn is back oxidized by manganese-oxidizing bacteria. Overall, the biotic and abiotic function of soluble redox compounds must play an important role in EET processes of biocathodes, just as it is an important electron transfer pathway in all environmental microbial transformations.

3. Outlook

With increasing interest in the application of electrodes as electron donors for a large number of microbially-catalyzed reactions of industrial or environmental relevance, the biochemical mechanisms involved in microbial electron uptake from a cathode are required to be uncovered. In this review, possible strategies that microorganisms can employ to exploit electrodes as electron donors for their metabolisms were elucidated and proposed. It is now the task of the scientific community to elucidate the true mechanisms of cathodic EET. A good starting point for this research endeavor will be pure culture studies with natural iron-oxidizing or hydrogen-utilizing microorganisms. In this effort, selective knock-out mutants of possibly involved enzymes, the smart application of different artificial redox mediators, and powerful electrochemical analysis will help the researcher to resolve the underlying mechanisms.

4. Conclusions

A deep understanding of the biochemical energetics of the reaction mechanisms to optimize functional biocathodes is necessary. This is important because the energy gain for biocathode application must be maximized, and therefore the energy consumption by the microorganism should be minimized. It was discussed, here, that the microbial energy gain of biocathodic reactions is strongly affected by the type and efficiency of the EET mechanism that is utilized. In some instances, microbes will not conserve energy during the biocathodic reaction – then additional energy resources (e.g., light, organics) have to be provided to sustain their biocatalytic activity. Clearly, it is now time to perform mechanistic studies and to learn and understand how current biocathodes work besides screening for new applications and designing systems.

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