Journal of Inorganic Biochemistry 196 (2019) 110694

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Direct electrochemical reduction of carbon dioxide by a molybdenum-containing formate dehydrogenase

Cristina M. Cordas*, Mariana Campaniço, Rita Baptista, Luísa B. Maia, Isabel Moura, José J.G. Moura*

LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (FCT NOVA), 2829-516 Caparica, Portugal

ARTICLE INFO

Keywords:
Formate dehydrogenase
Direct electrochemistry
CO_2 reduction
Electrocatalysis

ABSTRACT

Formate dehydrogenase enzymes catalyse the reversible two-electron oxidation of formate to carbon dioxide. The class of metal-dependent formate dehydrogenases comprises prokaryotic enzymes holding redox-active centres and a catalytic site, containing either molybdenum or tungsten ion, that mediates the formate/carbon dioxide interconversion. The carbon dioxide reduction is of a particular interest, since it may be a route for its atmospheric mitigation with the simultaneous production of added-value products, as formate-derived compounds. Recently, the periplasmic formate dehydrogenase from *Desulfovibrio desulfuricans*, a molybdenum-containing enzyme, was proven to be an efficient enzyme for the CO_2 reduction to formate. In this work, the immobilized formate dehydrogenase isolated from *Desulfovibrio desulfuricans* direct electrochemical behaviour was attained in the presence and absence of substrates and the formal potentials associated with the catalytic centre transitions were determined in non-turnover conditions. The enzyme catalytic activity towards carbon dioxide reduction was observed using direct electrochemical methods.

1. Introduction

Formate dehydrogenase enzymes (FDHs) are a group of heterogeneous proteins that catalyse the reversible formate ($HCOO^-$) oxidation to carbon dioxide (CO_2) (Eq. (1)). These enzymes are classified in two families, one gathering the enzymes that hold no redox-active centres and another that includes all the metal-dependent enzymes [1–3].

The metal-dependent FDHs are found only in prokaryotic organisms and all have in common the presence of one molybdenum or one tungsten ion in the active site coordinated by four sulfur atoms of two pyranopterin cofactors and additional sulfur and/or selenium atoms [4–6]. Both FDH classes have been the subject of intense research, since it was unveiled that the enzymatic reaction can be run reversibly, depending on the experimental conditions, allowing the consumption (reduction) of carbon dioxide with formation of formate (Eq. (1)) [1–3,7–11].

$$CO_2 + 2\bar{e} + H^+ \rightleftharpoons HCOO^- \quad (1)$$

This enzymatic reaction is of great interest since it may contribute to decrease the high atmospheric carbon dioxide levels, converting this green-house gas back into fuel or other added-value chemicals [12,13]. The reaction of formate formation from CO_2 is one of the most interesting, because of the highest added value when compared with the energy required to form the product [14]. The reaction electrochemical control has been pursuit aiming an integration on bioelectrochemical systems (BES) that would allow the CO_2 conversion with (sustainable) low energy consumption, employing mild conditions, to produce a single and specific product – formate (or formic acid) [15]. The feasibility of the utilization of FDHs, in general as catalyst in BES, has already been shown, namely using FDH isolated from *Candida boidinii* (an enzyme that is NADH-dependent and metal-independent, available commercially) in the electrosynthesis of formate from CO_2, although the imposition of a high overpotential was necessary (-1 V vs. Ag/AgCl) [16]. A decrease in the required overpotential was described with the immobilization of *Methylobacterium extorquens* (*Me*) FDH (a tungsten-containing enzyme), through the use of modified carbon-based electrodes with several polymers incorporating mediators [17]. Recently, the co-immobilization of a redox polymer (cobaltocene) together with a molybdenum-containing FDH (isolated from *Escherichia coli*) allowed the enhancement of the three-dimension matrix and, as so, also the mediated electron transfer rate [18]. The use of direct electrochemical methods to tune the FDH activity has been widely searched [19–22].

---

* Corresponding authors.
E-mail addresses: cristina.cordas@fct.unl.pt (C.M. Cordas), jose.moura@fct.unl.pt (J.J.G. Moura).

https://doi.org/10.1016/j.jinorgbio.2019.110694
Received 12 March 2019; Received in revised form 3 April 2019; Accepted 12 April 2019

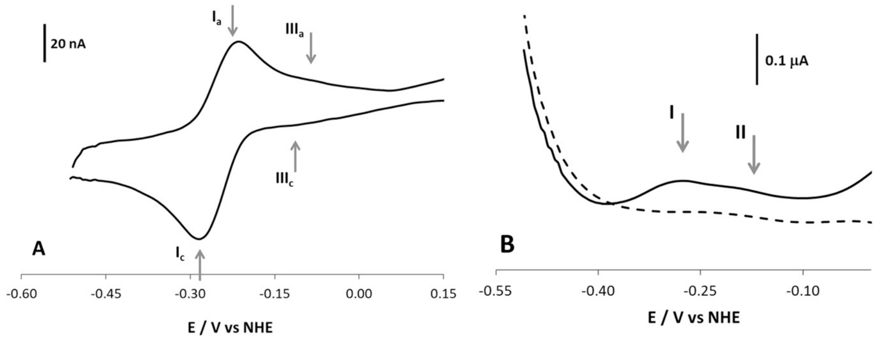

Fig. 1. *Dd*FDH electrochemical response. Representative results obtained in non-turnover conditions (300 mM Tris-HCl/100 mM NaCl, pH 7) observed by A) cyclic voltammetry (control subtracted), v = 10 mV·s^{-1}, E^{0}'(process I) = −250 ± 8 mV and E^{0}'(process III) ≈ −96 mV vs NHE; B) differential pulse voltammetry, step potential: 5 mV, potential amplitude 50 mV, step time 0.25 s, where two processes (processes I and II) can be observed, with formal potentials at approximately −262 mV and −170 mV vs NHE (full line: in the presence of *Dd*FDH; dashed line: control in the absence of *Dd*FDH).

Amongst the studies of the viability in using FDH enzymes to catalyse the CO_2 reduction, recently, it was found that the periplasmic FDH from *Desulfovibrio desulfuricans* (*Dd*FDH) is one of the most efficient carbon dioxide reducers so far described [7]. The enzyme has been demonstrated to possess high affinity for CO_2 ($K_m{}^{app}$ = 15.7 µM) and high rates for its reduction ($k_{cat}{}^{app}$ = 46.6 s^{-1}). *Dd*FDH is a heterotrimeric enzyme, harbouring one molybdenum-containing active site, two [4Fe-4S] centres and four haems *c*. The molybdenum centre, in its oxidised form, harbours the molybdenum ion coordinated by the *cis*-dithiolene group of two pyranopterin molecules, one selenium from a selenium-cysteine residue and one terminal sulfur atom [23,24]. The other metallic redox-active centres, besides the Mo active site, have an electron transfer role, allowing the existence of an intramolecular electronic pathway from the physiological electron partner to the active site [25]. During catalysis, the molybdenum ion cycles between the Mo (VI) and Mo(IV) oxidation states and the formate oxidation/carbon dioxide reduction were recently suggested to proceed through hydride abstraction/insertion (respectively), with the terminal sulfido group of the molybdenum centre acting as the direct hydride acceptor ($Mo^{(VI)}$ = S) / donor ($Mo^{(IV)}$-SH) (respectively) [7–11]. The two electrons (Eq. (1)) generated upon formate oxidation, or consumed during the carbon dioxide reduction are provided or accepted (respectively) by the physiological electron partner of each specific FDH enzyme. In vitro or in artificial systems, the electrons can be delivered/accepted by artificial electron donors/acceptors or by an electrode (as part of an electrochemical cell), via mediated or direct electron transfer [17,19,26]. Due to its unique kinetic parameters towards the carbon dioxide reduction [7], the *Dd*FDH is a good candidate to be incorporated in direct bioelectrochemical systems. Aiming to develop a future BES, in this work, we have characterised the *Dd*FDH electrochemical properties under non-turnover conditions, on pyrolytic graphite electrode, using direct electrochemical methods, without any mediators or surface modifiers. The electrochemical *Dd*FDH catalytic response towards the carbon dioxide reduction was also observed. The use of direct gaseous CO_2 injection into the electrochemical cell was attempted resulting in observable catalytic currents, showing to be a viable methodology.

2. Experimental

2.1. *DdFDH* purification and activity assessment

The *Dd*FDH was purified as described before [23,24]. The as-purified enzyme purity was accessed by electrophoresis and UV–visible spectroscopy and its formate oxidase activity, found to be 201 µmol/min/mg, was determined as described previously [23,24], following spectrophotometrically the reduction of benzyl viologen at 555 nm, in the presence of 10 mM formate.

2.2. *Electrochemical methods*

Assays were performed using an AUTOLAB 30 potentiostat/galvanostat, on a three electrodes configuration, in a single compartment electrochemical cell, inside a Faraday cage. The working, secondary and reference electrodes were a pyrolytic graphite disk (2 mm diameter), a platinum wire and a saturated calomel electrode, with a potential of +241 mV versus the Normal Hydrogen Electrode (NHE), respectively. The working electrode was polished with two different grades of alumina (1 and 0.3 µm), subjected to a Millipore water ultrasound bath for 2 min, and washed thoroughly with Millipore water. After the electrode was dried, 5 µL of the *Dd*FDH protein solution (8 µM) were pipetted on top of the electrode. The protein solution was then entrapped using a cellulose membrane (spectra/Pro) with a 3.5 kDa cut-off fitted with O-rings, as previously described [27], allowing the formation of a uniform protein thin layer on the electrode surface. Before starting the electrochemical assays, the electrolyte was degassed for a minimum 30 min using a continuous flow of argon. All the assays were performed in an anaerobic environment, at room temperature, with a positive pressure of argon maintained in the electrochemical cell headspace. The electrolyte used for the independent assays at pH 7 was 300 mM Tris-HCl/100 mM NaCl. The obtained reduction potential values were corrected for the NHE reference scale. The CO_2 solubility in water (1 atm, 25 °C, Henry's law constant) was considered to be 34 mM [28].

3. Results and discussion

3.1. *DdFDH direct electrochemical characterization*

The immobilized *Dd*FDH direct electrochemical behaviour was observed by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Cyclic voltammograms show a well-defined redox couple (process I, see Fig. 1A), with formal potential, E^{0}' = −250 ± 8 mV vs NHE, calculated by the midpoint of the anodic and cathodic potential peaks. Considering the experimental conditions, a good correlation was achieved for the I_p vs v plots (up to 50 mV·s^{-1}) for this process I, consistent with a quasi-reversible system in a thin layer regime (thickness, l ≈ 7 µm, considering a 2 electron process). In such experimental conditions, in which no diffusion of the electroactive species is considered, from the peak width at half height average values for process I (at least 10 replicates), the number of electrons involved was estimated to be 1.6, considered approximately as a two electron transfer (although not ideal behaviour). Using Laviron's mathematical approach [29], the heterogeneous electron transfer rate constant, k_{sh}, for process I, was then calculated to be 0.1 s^{-1} (10 mV·s^{-1}, using α = 0.5). Taking in consideration the electrochemical behaviour of process I, including the values of the potentials obtained for catalytic currents (see below in the text), this was assigned to the redox transition Mo(VI/IV) of the catalytic redox centre. The potential values observed seem in agreement with previous studies performed by EPR redox titrations (in different experimental conditions, namely temperature), where potential values of −330 and −160 mV were attributed to the redox couples Mo(V/IV) and Mo(VI/V), respectively [23] (in which the later couple is transient).

From the CV assays, a second redox couple can be identified (labelled as process III), in particular after the control subtraction (Fig. 1A), for which it was estimated a formal potential value, $E^{0'}$ of approximately −99 mV vs NHE. This redox couple is not so well-defined in the used experimental conditions and, as so, no other parameters for this process were calculated at this time. Using DPV two processes were also identified, a first process that should correspond to process I observed by CV with $E^{0'} = -262$ mV vs NHE and a second process (process II) at −171 mV vs NHE (Fig. 1B). The second process (process II) observed by DPV is far more negative than the second previously observed by CV (labelled process III), although is very close to the potential value of the couple Mo(VI/V) found before (−160 mV) [23]. Moreover, an estimation of the number of electrons associated with process I observed by DPV (through the peak width at half height, making an approximation to a reversible system [30,31]) was performed and resulted in $n \approx 1.1$, which seems coherent with a one electron transfer process corresponding to the Mo(V/IV) couple. From the DPV measurements it was possible to differentiate two redox states of the molybdenum centre. It is not unusual that pulsed techniques (such as DPV) allow to discriminate reduction potentials that are observed by CV as overlapped signals (usually at low scan rates), in particular reactions involving transient/unstable species (such as Mo(V)), and/or species with close redox features, including enzymes with multiple centres [32–34]. Some studies on oxido-bisdithiolene inorganic models, similar to the molybdenum and tungsten centres of the FDH enzymes, have shown that is possible to discriminate the Mo(VI/V) and Mo(V/IV) reduction potentials. These models have formal potentials values around −0.2 V vs NHE at 25 °C [35]. Also, the models point to the more negative potential values for the W containing centres [35,36], which is in agreement with the more negative catalytic potentials found for W containing enzymes, as discussed later.

3.2. *Dd*FDH electrocatalytic activity

The electrocatalytic activity of *Dd*FDH towards the CO_2 reduction was observed directly (without the presence of mediators) by cyclic voltammetry, through the addition of carbon dioxide by three different methods (Fig. 2), namely: a) addition of an equilibrated solution of sodium hydrogen carbonate, as previously described [7], b) addition of a saturated CO_2 solution, previously prepared by continuous bubbling of 100% gaseous CO_2 for 30 min and c) direct injection of gaseous CO_2 into the electrolyte solution (in the electrochemical cell) and immediately starting the assays (in order that equilibrium between CO_2/CO_3^- in solution was not attained). All three methods have originated the increase of the cathodic current, with the maximum current observed around the potential of −250 mV, where the first cycle is the one presenting the highest current, consistently diminishing in the subsequent cycles (see Fig. 2A). This behaviour is in agreement with previous reports of Mo and W-containing enzymes [19,20]. After multiple cycling (typically around 10 cycles), the catalytic current is no longer observed and the voltammogram features are similar to the ones obtained in non-turnover conditions, in the absence of any added substrate. The observed catalysis is electrochemically controlled, since no mediators are included in the electrolyte, and the reducing equivalents are provided directly by the electrode. To guarantee that the observed catalytic currents are due to the *Dd*FDH-catalyzed CO_2 reduction, different control assays were performed, namely, in the presence of CO_2 but absence of the enzyme (Fig. 2A) and in presence of *Dd*FDH but with the addition of air instead of CO_2 (Fig. 2C; an air-equilibrated solution contains only 13 µM of carbon dioxide) where no significant current increase was detected. It is obvious that the results to be obtained using the third method are more difficult to control since gaseous CO_2 is directly injected into the cell, but opens promising experimental possibilities to explore the process as part of a mitigation atmospheric system for the CO_2 emissions, by using gas-diffusion type electrodes, as suggested by other studies [37].

In Fig. 2B, the Mo(VI)/Mo(IV) couple redox transition (process I), observed in the absence of CO_2, presents a slight shift (around 20 mV) towards more positive potential values. The electrochemical behaviour of this couple, in these experimental conditions, was attained after the complete CO_2 consumption, whose concentration in these assays was estimated to be, at the start of the experiment, 4.3 mM. The observed shift is related to the FDH catalytic activity and to the CO_2 presence and is probably due to the acidification of the medium, as verified by other authors [20].

Complementary, another set of assays were performed where both formate and CO_2 were simultaneously present in solution (Fig. 3), using the same experimental methodology described.

When both substrates are present, the individual features of the molybdenum redox centre are not observed. Instead, typical cathodic and anodic catalytic waves are observed, starting to develop around −200 mV (both in the cathodic and anodic directions), corresponding to CO_2 reduction and formate oxidation, respectively, in a behaviour already described for other FDH enzymes [19,20]. Under these conditions, both observed catalytic currents increase with the applied

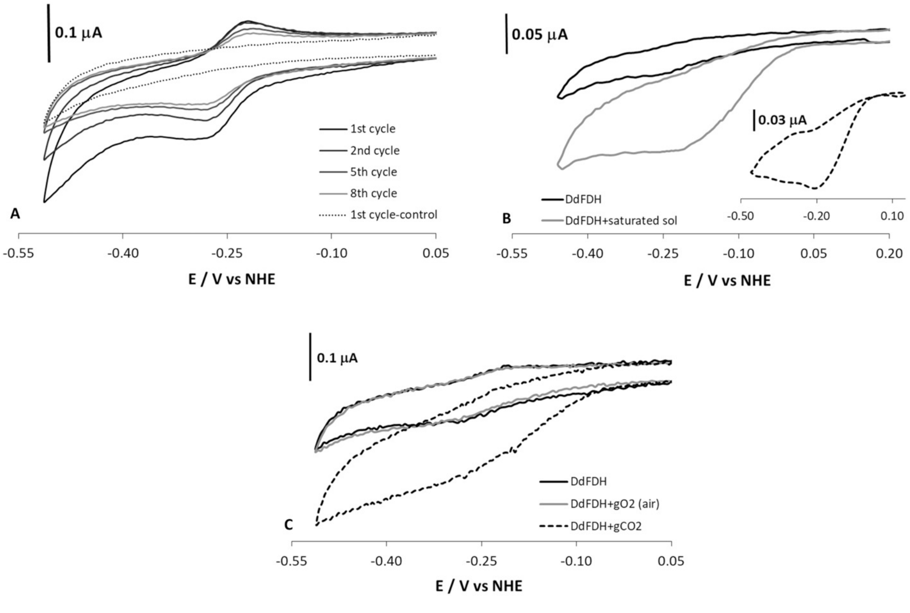

Fig. 2. Electrocatalytic response of *Dd*FDH. Cyclic voltammograms of *Dd*FDH electrocatalytic response towards the CO_2 reduction in 300 mM Tris-HCl/100 mM NaCl, pH 7: A) addition of an equilibrated solution of hydrogen carbonate, after equilibrium, $v = 10\ mVs^{-1}$, estimated CO_2 concentration 78 µM; B) addition of a CO_2 saturated solution, $v = 2.5\ mVs^{-1}$, estimated initial CO_2 concentration 4.3 mM; C) direct CO_2 injection in the electrochemical solution, $v = 10\ mVs^{-1}$, estimated initial CO_2 concentration 1.6 mM.

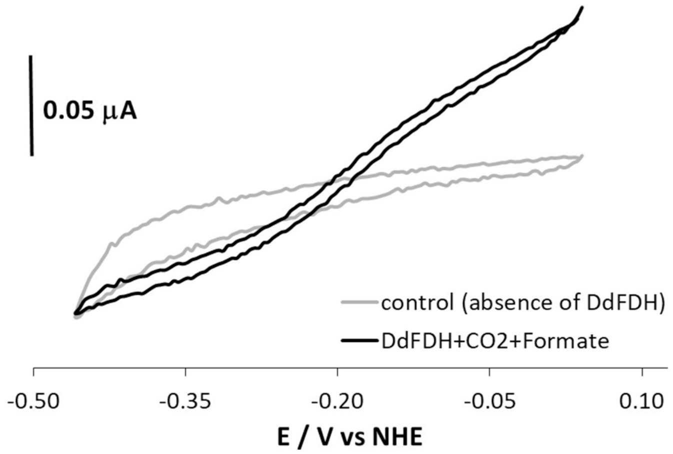

Fig. 3. Electrocatalytic response of *Dd*FDH in the presence of CO_2 and formate. Representative cyclic voltammograms of the *Dd*FDH electrocatalytic response towards the CO_2 reduction and formate oxidation, in 300 mM Tris-HCl/100 mM NaCl, pH 7.6 when both substrates are present in solution, showing catalytic currents towards the CO_2 reduction (cathodic currents) and formate oxidation (anodic currents); the initial concentrations were 5.2 mM for CO_2 (addition of a saturated CO_2 solution) and 1.4 mM of sodium formate; $v = 5\,mVs^{-1}$. The control was performed in the same electrolyte in the presence of both substrates (same concentrations and experimental conditions) but in the absence of the *Dd*FDH.

overpotential, but a steady state is not clearly met (where a limiting current is reached), reflecting the high activity of the enzyme, and indicating the need of optimization of the imposed experimental conditions (namely the scan rate and the need to use a rotating electrode). The inflection point at approximately −200 mV seems to agree with the reduction potentials observed for the Mo centre in non-turnover conditions, as well as previous results obtained by EPR spectroscopy [23].

4. Conclusions

In this work we presented the direct electrochemical behaviour of the Mo containing formate dehydrogenase, purified from *D. desulfuricans*, where the Mo centre redox features were observed. The results allowed to calculate the reduction potentials associated with the couples Mo(VI/IV) (by CV) and Mo(VI/V) and Mo(V/IV) (by DPV). The catalytic currents associated with the carbon dioxide reduction were attained using direct cyclic voltammetry (without mediators) and different methodologies to add CO_2 into the solution. When both CO_2 and formate are present, the Mo centre redox features are not observed, but the development of characteristic sigmoidal catalytic currents towards the CO_2 reduction and formate oxidation are observed. We believe that these qualitative results constitute a first step as a proof of concept that *Dd*FDH can be used as a basis for a BES device for the CO_2 reduction, using gas-diffusion type electrodes, allowing the direct injection of atmospheric carbon dioxide and its reduction in situ.

Abbreviations

BES	bioelectrochemical systems
CV	cyclic voltammetry
CO_2	carbon dioxide
Dd	*Desulfovibrio desulfuricans*
*Dd*FDH	formate dehydrogenase isolated from *Desulfovibrio desulfuricans*
DPV	differential pulse voltammetry
$HCOO^-$	formate
FDH	formate dehydrogenase
NADH	reduced nicotinamide adenine dinucleotide

Acknowledgments

This work was supported by the Associate Laboratory for Green Chemistry-LAQV, with national funds from FCT/MCTES (UID/QUI/50006/2019). LBM thanks to FCT/MCTES for the CEEC-Individual 2017 Program Contract.

References

[1] L. Maia, J. Moura, I. Moura, J. Biol. Inorg. Chem. 20 (2015) 287–309 (Springer Science & Business Media B.V.).
[2] L.B. Maia, I. Moura, J.J.G. Moura, Inorg. Chim. Acta 455 (2017) 350–363.
[3] D. Niks, R. Hille, Protein Science, vol. 28, John Wiley & Sons, Ltd, 2019, pp. 111–122.
[4] R. Hille, J. Hall, P. Basu, Chem. Rev. 114 (2014) 3963–4038 (American Chemical Society).
[5] L.B. Maia, I. Moura, J.J.G. Moura, Molybdenum and Tungsten Enzymes: Biochemistry, The Royal Society of Chemistry, 2017, pp. 1–80.
[6] L.B. Maia, J.J.G. Moura, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Elsevier, 2018.
[7] L.B. Maia, L. Fonseca, I. Moura, J.J.G. Moura, J. Am. Chem. Soc. vol. 138, (2016) 8834–8846 (American Chemical Society).
[8] D. Niks, J. Duvvuru, M. Escalona, R. Hille, J. Biol. Chem. 291 (2016) 1162–1174.
[9] X. Yu, D. Niks, A. Mulchandani, R. Hille, J. Biol. Chem. 292 (2017) 16872–16879.
[10] D. Niks, R. Hille, in: F. Armstrong (Ed.), Methods in Enzymology, vol. 613, Academic Press, 2018, pp. 277–295.
[11] G. Dong, U. Ryde, J. Biol. Inorg. Chem. 23 (2018) 1243–1254.
[12] R.F. Service, Science 349 (2015) 1158–1160.
[13] A.M. Appel, J.E. Bercaw, A.B. Bocarsly, H. Dobbek, D.L. DuBois, M. Dupuis, J.G. Ferry, E. Fujita, R. Hille, P.J.A. Kenis, C.A. Kerfeld, R.H. Morris, C.H.F. Peden, A.R. Portis, S.W. Ragsdale, T.B. Rauchfuss, J.N.H. Reek, L.C. Seefeldt, R.K. Thauer, G.L. Waldrop, Chemical Reviews, vol. 113, American Chemical Society, 2013, pp. 6621–6658.
[14] C. Finn, S. Schnittger, L.J. Yellowlees, J.B. Love, Chem Commun, vol. 48, The Royal Society of Chemistry, 2012, pp. 1392–1399.
[15] L. Zhang, J. Liu, J. Ong, S.F.Y. Li, Chemosphere 162 (2016) 228–234.
[16] S. Srikanth, M. Maesen, X. Dominguez-Benetton, K. Vanbroekhoven, D. Pant, Bioresour. Technol. 165 (2014) 350–354.
[17] K. Sakai, Y. Kitazumi, O. Shirai, K. Kano, Electrochem. Commun. 65 (2016) 31–34.
[18] M. Yuan, S. Sahin, R. Cai, S. Abdellaoui, D.P. Hickey, S.D. Minteer, R.D. Milton, Angewandte Chemie International Edition, vol. 57, Wiley-Blackwell, 2018, pp. 6582–6586.
[19] T. Reda, C.M. Plugge, N.J. Abram, J. Hirst, Proc. Natl. Acad. Sci. 105 (2008) 10654.
[20] A. Bassegoda, C. Madden, D.W. Wakerley, E. Reisner, J. Hirst, J. Am. Chem. Soc. vol. 136, (2014) 15473–15476 (American Chemical Society).
[21] M. Miller, W.E. Robinson, A.R. Oliveira, N. Heidary, N. Kornienko, J. Warnan, I.A.C. Pereira, E. Reisner, Angewandte Chemie, vol. 0, John Wiley & Sons, Ltd, 2019.
[22] K.P. Sokol, W.E. Robinson, A.R. Oliveira, J. Warnan, M.M. Nowaczyk, A. Ruff, I.A.C. Pereira, E. Reisner, J. Am. Chem. Soc. 140 (2018) 16418–16422 (American Chemical Society).
[23] C. Costa, M. Teixeira, J. LeGall, J.J.G. Moura, I. Moura, J. Biol. Inorg. Chem. 2 (1997) 198–208.
[24] M.G. Rivas, P.J. González, C.D. Brondino, J.J.G. Moura, I. Moura, J. Inorg. Biochem. 101 (2007) 1617–1622.
[25] M. Jormakka, S. Törnroth, B. Byrne, S. Iwata, Science 295 (2002) 1863.
[26] K. Schuchmann, V. Müller, Science 342 (2013) 1382–1385.
[27] T.C. Santos, A.R. de Oliveira, J.M. Dantas, C.A. Salgueiro, C.M. Cordas, Biochim. Biophys. Acta Bioenerg. 1847 (2015) 1113–1118.
[28] R. Sander, Atmos. Chem. Phys. 15 (2015) 4399–4981.
[29] E. Laviron, J. Electroanal. Chem. Interfacial Electrochem. 101 (1979) 19–28.
[30] A.M.O. Brett, C.M.A. Brett, Journal (1993) (Pages).
[31] A.M.O. Brett, C.M.A. Brett, Electrochemistry: Principles, Methods and Applications, 1st edition, Oxford University Press, Oxford, 1993.
[32] F. Folgosa, C.M. Cordas, J.A. Santos, A.S. Pereira, J.J.G. Moura, P. Tavares, I. Moura, Biochem. J. 438 (2011) 485–494.
[33] Z. Guo-hua, L. Ming-fang, L. Ming-li, Cent. Eur. J. Chem. 5 (2007) 1114–1123.
[34] C. Moreno, A. Campos, M. Teixeira, J. LeGall, M.I. Montenegro, I. Moura, C. Dijk, J.G.J. Moura, Eur. J. Biochem. 202 (1991) 385–393 (Wiley/Blackwell (10.1111)).
[35] C. Schulzke, Dalton Trans. (2005) 713–720 (The Royal Society of Chemistry).
[36] H. Sugimoto, K. Sugimoto, Inorg. Chem. Commun. 11 (2008) 77–80.
[37] K. Sakai, Y. Kitazumi, O. Shirai, K. Takagi, K. Kano, Electrochem. Commun. 73 (2016) 85–88.