Electrochemical insights into bacterial respiration
upon polarized substrates: a proposal for tricking
bacteria and compelling them to exhibit desired
activities

Mohsen Lashgari^{1, 2}, Hadi Diarmand-Khalilabad¹*

¹Department of Chemistry, Institute for Advanced Studies in Basic Sciences
(IASBS), Zanzan 45137-66731, Iran

²Center for Research in Climate Change and Global Warming: Hydrogen fuel cell
and solar division, Zanzan 45137-66731, Iran

* Corresponding author. Tel.: +98 24 33153205; fax: +98 24 33153232. *E-mail*
address: Lashgari@iasbs.ac.ir

Abstract:

Based on the similarities between bacterial respiration and electrodic processes, we here present some propositions about microbial activities in electrochemical systems, which justify why microorganisms could interact with polarized surfaces and how these interactions could potentially be utilized for bioengineering as well as life science issues. In this paper, using a potentiodynamic polarization approach, the polarity effect on the bioelectrocatalytic response of a facultative anaerobe, viz. *Pseudomonas aeruginosa* was scrutinized. The investigations revealed that under the anodic regime, both respiratory options (aerobic and anaerobic) are available and the positively charged electrode could provide a suitable niche for bacterial growth. Microscopic observations illustrated that under such a circumstance, it is possible to electrochemically stimulate the bacterial cells on the polarized surfaces. Under cathodic polarization, the electrode serves as electron-source, and by decreasing the system impedance, the microorganism is able to catalyze the oxygen reduction reaction. Finally, a general insight was achieved and the phenomena were discussed in detail from physico-electrochemical viewpoint.

Keywords: Electrodic respiration; Facultative anaerobes; Biotic/abiotic systems; Electromicrobiology

1. Introduction

Although the energy of bio-globe (earth and its living creatures) is primarily supplied by solar radiation (nuclear reactions), gravitational forces, and geothermal activities [1], the energy of most terrestrial organisms is self-produced [2-3], via oxidation of food/fuel inside living cells or human-made power-generating reactors (e.g. fuel cells, heat engines, etc.):

In biological contexts, this energy-releasing oxidation process is routinely referred to as respiration [3-4]; using living organisms (e.g. bacteria), electrons are taken from the substrate (food/fuel; e.g. a carbon-containing substance) and delivered to their final recipients, i.e. oxygen diatomic molecules or any other non-oxygen species [5-7].

Electron transport and *transiently formation of radical species* are two interesting phenomena which commonly occur during both bacterial respiration/metabolism and electrodic processes [8-9]. For instance, the reduction of oxygen molecules on a cathode surface in aqueous media, is accompanied by the formation of superoxide ($\cdot O_2^-$), hydrogen ($\cdot H$), hydroxyl ($\cdot OH$) and hydroperoxyl ($HOO \cdot$) radicals [10-12]. Analogously, the same radicals are produced during the aerobic respiration/metabolism, by taking electrons (as well as protons) from the food material (substrate) and delivering them to the oxidant [13, 8]. In the case of electrodic processes, it is worth noting that some hydrogen atoms (radicals) are transiently generated on the cathode surface through the proton reduction reaction, viz. $H^+ + e^- \rightarrow H$ [14]. These atoms are reactive and could attack the oxygen species at the interface region, and eventually convert them to water or hydrogen peroxide [15-16]. The same reactions take place during

the bacterial respiration/metabolism: H atoms are transiently produced and biologically stored as NADPH [8, 13]; when necessary, by converting this biomolecule to NADP⁺, H-atoms are re-released as H^+/e^- and consumed in the subsequent reduction reactions to produce the final metabolites [8].

Concerning the bacterial respiration and electrodic reactions, it is worth emphasizing that there is a close relationship between these biotic/abiotic phenomena [17-23]. As depicted in **Fig. 1**, under anodic polarization (positive polarity), the electrode serves as oxidant and provides a convenient place for anaerobic respiration of bacteria. Under this polarization regime, oxygen molecules could also be produced via a water oxidation process ($H_2O \rightarrow 1/2O_2 + 2H^+ + 2e^-$); thus, on the anode surface, a facultative bacterium would be able to alternatively respire through an aerobic pathway. This is however not the whole story. Because of the possibility of hydroxyl radical formation during anodic processes in aqueous media ($OH^- \rightarrow \cdot OH + e^-$) [24], the plasma membrane of bacterial cells (immobilized on the electrode surface) might also be damaged (oxidized) in some areas, through the reaction with hydroxyl radicals [25-27]. This phenomenon might result in the exchange of cellular contents and conduct the creation of conjugation channels (cell-to-cell contacts) between adjacent bacteria [28-30]; the generation of channels is ascribed to the genetic response and defense mechanism of bacterial communities to survive under harsh damaging conditions [25-26]. With altering the polarity of electrode to negative values (cathodic polarization), by contrast, the electrode acts as electron source (substrate) for bacterial cells [18-21]. Here, through the forced pumping of electrons from the electrode to oxidant (O_2), bacteria at the interface region might interfere with the electron-flow process and take their energy via an effortless

respiratory pathway. Under this circumstance, bacteria not only are able to respire aerobically on the electrode (cathode) surface but by participation in the generation of reactive oxygen species (ROS) [31-32], they could also facilitate the oxygen reduction reaction (ORR) at the interface region [for a detailed explanation, see **Section 3: equations 5 through 8** and discussion of **Fig. 3**]. This bioelectrocatalytic ability of bacteria is technologically interesting, because the slow rate of ORR is generally considered as the bottleneck of fuel cell technologies [33-34].

In this work, we propound some basic theorems which govern the bacterial life (ecology/physiology), justifying how microorganisms could interact with their environment and exhibit interesting behaviors upon different polarized substrates. Although the ideas proposed here are embryonic and each issue requires to be comprehensively investigated in a separate research, in the present study, we are just attempting to provide a general overview and preliminarily demonstrate that using a versatile electrochemical approach, it is practically possible to impose different respiratory situations, trick bacterial cells and compel them to exhibit desired activities. All these matters are highly interesting from biotechnological as well as medical viewpoints.

The bacterium under investigation is *Pseudomonas aeruginosa* –a facultative anaerobe, which is ubiquitously found in soil and water [35]. *P. aeruginosa* preferentially obtains its energy via aerobic respiration [36-37]. This bacterium is also well adapted to survive in limited oxygen media and grow even under anaerobic conditions [37-38]. From medical standpoint, *P. aeruginosa* is an opportunistic pathogen and considered as the main cause of cystic fibrosis, a pulmonary disease due to long-term colonization of the bacterium in the lung

tissues [35]. The ability of *P. aeruginosa* to respire by both aerobic and anaerobic pathways is a reason why we selected this ubiquitous bacterium as a model for our approach.

2. Material and Methods

2.1 Bacterial strain and culturing

The bacterial strain (ATCC 27853) was supplied by IROST as a lyophilized ampoule. To revive the bacterial cells, the content of ampoule was firstly transferred into MH broth (Merck Millipore) and then pre-cultured on MH agar through aerobic incubation for 72 h at 37 °C. For each bio-electrochemical test, the microorganism was re-cultured under the same condition overnight. To prepare the microbial electrolyte, the colony was removed from the agar surface, washed with phosphate buffer ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, pH=7) and transferred to the blank electrolyte solution ($\text{OD}_{600}=1.6$, obtained from turbidimetry measurements at wavelength 600 nm [39]; the cell density corresponds to 3.2×10^8 CFU/mL [40]).

2.2 Electrochemical analyses

All tests were carried out inside a sterile three-electrode glassy chamber in the presence and absence of bacteria, filled by 100 mL electrolyte with a suitable composition [41] (more details are available as Supplementary material). The pH of electrolyte was adjusted to 7, using NaOH (0.01 M) and HCl (0.1 M) solutions. In electrochemical experiments, we applied sterile glassy carbon (CHI 104; 0.071 cm^2), Pt foil (1.2 cm^2) and saturated calomel electrode (SCE) as working, auxiliary and reference electrodes, respectively. For the preparation of working

electrode (WE), the glassy carbon was successively polished with 1, 0.5 and 0.05 μm alumina slurries. Its surface was then cleaned using a water-ethanol solution (1:1 v/v) with sonication in an ultrasound bath (Bandelin DT 102 H). Potentiodynamic polarization data were recorded in two overvoltage ($\eta := E - OCP$) domains, low (0 to -50 mV) and high (0 to -950 mV), with the scan rate of 0.1 and 25 mV/s, respectively [42-43]. Concerning the choice of these overvoltage domains, we should declare that under the low overvoltage domain, since the situation is near the natural respiratory condition ($\eta = 0$), bacteria could easily tolerate this polarization condition. Under the high overvoltage domain, however, the bacteria are compelled to experience more violent situation. To mitigate this stressful condition –being encountered during the span of the high-overvoltage domain– by selection of the greater scan rate (25 mV/s), the period of test is deliberately shortened and thus the bacteria are less threatened due to short exposure to the harmful/high-overvoltage situation.

The polarization measurements were carried out using an Autolab PGSTAT-101 with Nova software. For electrochemical impedance studies (EIS), we employed a Zahner/Zennium Impedance workstation operating with Thales Z1.15 software. These experiments were performed at OCP superimposed by AC signal with the amplitude of 10 mV. The signal frequency was swept from 100 kHz to 0.1 Hz [44]; in some cases, the experiments were reexamined and a broader frequency range was applied. All prepolarization tests (anodic or cathodic pretreatment of the electrode) were independently conducted at the same time interval (4 h) with applying a small DC overvoltage ($|\eta| = 10 \text{ mV}$). The experiments were repeated at least three times and the mean values were reported.

2.3 Optical and scanning electron microscopy

The optical image of the planktonic bacteria (inside electrolyte) was captured in bright-field observation mode, using an inverted microscope (Olympus IX71) equipped with a DP30BW CCD camera. SEM images of the biofilm (bacterial cells attached to the electrode surface) were taken using a LEO 1430VP scanning electron microscope at 15 kV accelerating voltage. Prior to taking the SEM micrographs, bacterial cells were fixed on the electrode surface by one-hour suspension in an aqueous glutaraldehyde solution (4 °C; 2.5% v/v). Afterwards, the cells were dehydrated by successive 20 min immersions in a gradient series of 30, 50, 80 and 100% v/v ethanol/water solutions, and were finally dried in a vacuum desiccator.

3. Results and Discussion

Fig. 2 demonstrates the cathodic polarization behavior of the electrode in the absence of bacteria. The plots reveal that in the presence of oxygen, the cathodic current significantly increases. This observation is rationalized by the enhancement of electron consumption, through the following two- or four-electron oxygen reduction reactions [45]:

In the absence of O₂ molecules (purged by argon), **Fig. 2** also indicates that the cathodic current is not negligible, and increases at overvoltages greater than -10 mV. This background current is related to the proton reduction phenomenon –that is the characteristic of aqueous electrolytes, being normally observed under negative overvoltages [14]. Moreover, the observation of a linear tail in these

potentiodynamic plots indicates the existence of an ohmic relationship between current density and overvoltage, which could be ascribed to the water electrolysis phenomenon (H₂ gas evolution process on the cathode surface [14]) being taken place under high-overvoltage conditions.

The effect of microorganism on ORR was investigated and the results are shown in **Fig. 3**. This figure illustrates that in the presence of bacteria, a significant bioelectrocatalytic activity is observed and the rate of ORR becomes accelerated approximately 4 times as compared with that of blank (the electrolyte without bacteria). The bioelectrocatalytic response of *P. aeruginosa* is interpreted through the ability of the bacterium in the secretion of quorum sensing (QS) phenazine metabolites [47-48] –which are also referred to as exogenous or self-excreted redox mediators [20]. These virulent metabolites, which are naturally secreted in the response of the bacterium to environmental threats, are able to oxidize NADPH and serve as electron shuttles [46-47, 49-50]. By transferring these electrons to oxygen molecules and converting them to ROS [31-32], this bacterium could take its energy via an aerobic pathway and could potentially kill other competing cells [47,50]. From chemical standpoint, the ROS radicals, in comparison with oxygen molecules, are more reactive. Therefore, they can be more quickly converted to the final products, i.e. water or oxygenated water molecules through the following possible pathways [31-33, 50-52]:

These arguments justify why in the presence of bacteria, ORR becomes catalyzed.

It is also worth noting that the diagrams plotted in **figures 2** and **3** were recorded for the electrolytes containing CS. If this additive (CS) is omitted, the result will be amazing and the reaction will be accelerated; see **Fig. 4**. To justify this interesting observation, it should be declared that in the absence of CS, cathode is the main source of electrons for the bacterial respiration. In the presence of CS, the bacteria could also planktonically respire within the electrolyte solution through the oxidation of other alternative sources. With the elimination of CS, however, the bacteria seem to be forced to respire on the electrode surface; here, through the accumulation of bacteria (biofilm formation) at the interface region as well as the effective biological conversion of O₂ to ROS, ORR is therefore catalyzed.

As implicitly pointed out in the Introduction, by applying an anodic or cathodic overvoltage, it is possible to enhance the activity of microorganisms on the electrode surface. By using this strategy, we should be able to boost ORR via prepolarization of the electrode before its use as biocathode. Therefore, the electrocatalytic behavior of the biocathode was examined after the prepolarization tests (see **Fig. 5**). By comparing these results with those depicted in **Fig. 3**, our expectation was confirmed. Moreover, **Fig. 5** revealed a better performance for the anodic prepolarization. This finding is not surprising because under the anodic regime, the facultative anaerobe can use both respiratory pathways on the electrode surface. Under this circumstance, the electrons being transferred to the positively charged electrode can be supplied by two different sources: one through the anodic/anaerobic respiration of bacteria, and the other via the possibility of electrochemical oxidation of water molecules on the anode surface ($H_2O \rightarrow 1/2O_2 + 2H^+ + 2e^-$). The latter produces O₂ gas and provides an aerobic

respiratory condition for the microorganisms at the interface region. The evolution of oxygen molecules under the anodic regime was verified by O₂ measurements, carried out using a portable HQ40d dissolved-oxygen meter (HACH[®]): after 4 h prepolarization of the electrode, 2.61 ppm O₂ was generated ($\Delta C_{O_2} = 2.61$ mg/L). These explanations and evidence indicate how the positively charged surface could furnish a suitable niche for *P. aeruginosa* and why a better performance is achieved under the anodic prepolarization regime.

On the other hand, the lower bioelectrocatalytic activity observed in the case of cathodic prepolarization can be electrostatically rationalized through the negative surface charge of *P. aeruginosa* [53-55]. This negative charge of the bacterial cells is related to their outer membrane lipopolysaccharide (LPS) [56], which is naturally deprotonated in aqueous media and appears as anionic LPS [56-57]. Therefore, owing to the existence of a repulsive force between *P. aeruginosa* and the negatively charged electrode, the bacterial cells cannot easily attach to the electrode surface. After the formation of biofilm, however, *P. aeruginosa* is able to diminish its negative charge by making some modifications in the composition of its outer membrane [54]. By contrast, through altering the polarity of electrode to positive value, the negatively charged planktonic bacteria could effectively attach to the electrode surface. This alternatively justifies why the anodic prepolarization regime exhibits a better performance.

Because of the bacterial respiration/metabolism and the ability of *p. aeruginosa* to biocatalyze the electron transport phenomenon at the interface region, we expect to observe a decrease in the impedance of system. **Fig. 6** confirms this expectation. In the presence of bacteria, the impedance plots also reveal a suppressing effect on the mass transfer issue [58]; see the inset and notice

the squashed tail of spectra. This indicates that the formation of biofilm can enhance the electrode interaction with oxygen molecules, and hence can mitigate the mass transport limitation. At high frequencies (see the circle-marked zone: the inset of **Fig. 6**), the impedance plots in the presence of *P. aeruginosa*, are similar to those being routinely observed in abiotic systems [59]. At lower frequencies, however, the impedance response of the bacterial cells seems complicated. This behavior might be attributed to the complex nature of biological processes occurring in living organisms [60], which could not be exactly interpreted through the conventional models classically developed for abiotic systems [14, 59-61].

Since the best bioelectrocatalytic activity was achieved for the anodically prepolarized electrode, we also carried out a microscopic study on this electrode. Therefore, we took some microscopic images (optical and SEM) of the bacterial cells before and after the immobilization process; see **Fig. 7**. Concerning the immobilized microorganisms, **Fig. 7b** clearly approves the presence of a considerable number of bacterial cells on the electrode surface. This figure also reveals that although the most of bacterial cells have an approximately uniform morphology, in some spots, their diameter is greater than normal. In line with this fact, it is worth declaring that the size of bacterial cells is not always constant and can vary with nutritional conditions and growth rate [62-63]. Regarding this issue, we should also remind that during the prepolarization process and growth of microorganisms on the electrode surface, owing to the presence of a strong electric field at the interface region [64], nutrient intake as well as metabolic processes might be influenced. The possibility of electrodic stimulation of bacterial cells seems highly interesting. However, it needs extra research, which

could be collaboratively examined via some joint projects between electrochemical and microbiological scientists.

Concerning the massive cells witnessed in **Fig. 7b**, it should also be noted that even if identified these species are contaminants (foreign entities), the SEM image of bacteria taken at higher magnification (**Fig. 8**) may still support the possibility of the formation of cell-to-cell contacts and the variation of cell size. This issue similarly needs more multidisciplinary scrutiny, which is worth pursuing in the future and seems to be technologically as well as medically intriguing.

Finally, we should remark that with the present approach [the possibility of electrodic stimulation of bacterial cells], one could electrodically stimulate/kill the bacterial cells, accelerate/diminish the formation of biofilm, and ultimately compel the microbial communities to exhibit desired activities.

4. Conclusions

There are many similarities between electrodic reactions and bacterial respiration/metabolism. Using a polarized substrate, it is possible to electrodically provide different respiratory situations, trick microorganisms and force them to exhibit some intriguing behaviors. Under anodic polarization, a stimulative/oxidative regime governs the bacterial growth on the electrode surface. Moreover, under this circumstance, both respiratory options (anaerobic/aerobic) are locally available for the facultative anaerobes. During the cathodic pumping of electrons to dissolved oxygen molecules, the electrode serves as electron source. Through the aerobic respiration and interfering with the electron-flow process, *P. aeruginosa* can decrease the system impedance and catalyze the charge transport phenomenon (ORR).

ABBREVIATIONS AND NOMENCLATURE

AC	= alternative current
ATCC	= American type culture collection
CS	= the carbon containing substance (sodium bicarbonate; a kind of carbon source)
DC	= direct current
E	= applied potential
IROST	= Iranian research organization for science and technology
LPS	= lipopolysaccharide
MFC	= microbial fuel cell
MH	= Mueller-Hinton
NADPH	= the reduced form of nicotinamide adenine dinucleotide phosphate
OCP	= open circuit potential
OD ₆₀₀	= optical density measured at 600 nm
ORR	= oxygen reduction reaction
<i>P. aeruginosa</i>	= <i>Pseudomonas aeruginosa</i> (an opportunistic pathogen)
QS	= quorum sensing
ROS	= reactive oxygen species (including superoxide, hydroxyl and hydroperoxyl radicals, which could be generated electrochemically or biologically)
SCE	= saturated calomel electrode
SEM	= scanning electron microscopy
WE	= working electrode (glassy carbon)
ΔC_{O_2}	= the extent of oxygen molecules being released during the anodic prepolarization process

η	= overvoltage, a measure of polarization, which is defined as the difference between applied and open circuit voltages.
H	= hydrogen atom (radical)
H^+	= proton (hydrogen cation)
$HOO \cdot$	= hydroperoxyl radical
$\cdot OH$	= hydroxyl radical
$\cdot O_2^-$	= superoxide anion radical

Acknowledgements

The authors would like to acknowledge the anonymous Referees for their useful/constructive comments to enhance the quality of the work. We should also thank INSF (Grant no. 91001676) for partial financial support of this research.

References

- [1] M. Lashgari, Use of solar and alternative energy to reduce emissions, U.S.-Iran Symposium on Climate Change: Impacts and Mitigation, Irvine, California (2015).
- [2] T. Gold, The deep, hot biosphere, Proc. Natl. Acad. Sci. USA (PNAS) 89 (1992) 6045–6049.
- [3] L. Konieczny, I. Roterman-Konieczna, P. Spólnik, Systems biology: functional strategies of living organisms, Springer, Dordrecht (2014).
- [4] N.S. Gel'man, M. A. Lukoyanova, D.N. Ostrovskii, Respiration and phosphorylation of bacteria, Springer, New York (1967).

- [5] P. John, Aerobic and anaerobic bacterial respiration monitored by electrodes, *J. Gen. Microbiol.* 98 (1977) 231–238.
- [6] D.J. Richardson, Bacterial respiration: a flexible process for a changing environment, *Microbiology* 146 (2000) 551–571.
- [7] B.J. Little, J.S. Lee, Microbiologically influenced corrosion, John Wiley & Sons, Inc., New Jersey, 2007.
- [8] B. Alberts, A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts, P. Walter, J. Wilson, T. Hunt, *Molecular biology of the cell*, sixth ed., Garland Science (Taylor & Francis Groups), New York, 2015.
- [9] J.O'M. Bockris, A.K.N Reddy, M. Gaboa-Aldeco, *Modern electrochemistry*, Kluwer Academic/Plenum Publishers, New York, 2000.
- [10] L. Dai, Y. Xue, L. Qu, H.-J. Choi, J.-B. Baek, Metal-free catalysts for oxygen reduction reaction, *Chem. Rev.* 115 (2015) 4823–4892.
- [11] Q. Yue, K. Zhang, X. Chen, L. Wang, J. Zhao, J. Liu, J. Jia, Generation of OH radicals in oxygen reduction reaction at Pt–Co nanoparticles supported on graphene in alkaline solutions, *Chem. Commun.* 45 (2010) 3369–3371.
- [12] A. Morozan, B. Jousselme, S. Palacin, Low-platinum and platinum-free catalysts for the oxygen reduction reaction at fuel cell cathodes, *Energy Environ. Sci.* 4 (2011) 1238–1254.
- [13] R. Luc, C. Vergely, Forgotten radicals in biology, *Int. J. Biomed. Sci.* 4 (2008) 255–259.

- [14] M. Lashgari, D. Matloubi, Atomistic understanding of hydrogen loading phenomenon into palladium cathode: A simple nanocluster approach and electrochemical evidence, *J. Chem. Sci.* 127 (2015) 575–581; DOI: 10.1007/s12039-014-0764-z.
- [15] J. Greeley, I.E.L. Stephens, A.S. Bondarenko, T.P. Johansson, H.A. Hansen, T.F. Jaramillo, J. Rossmeisl, I. Chorkendorff, J.K. Nørskov, Alloys of platinum and early transition metals as oxygen reduction electrocatalysts, *Nat. Chem.* 1 (2009) 552–556; DOI: 10.1038/nchem.367.
- [16] Z. Peng, Y. Chen, P.G. Bruce, Y. Xu, Direct detection of the superoxide anion as a stable intermediate in the electroreduction of oxygen in a non-aqueous electrolyte containing phenol as a proton source, *Angew. Chem. Int. Ed.* 54 (2015) 8165–8168; DOI: 10.1002/anie.201502039.
- [17] K. Rabaey, J. Rodríguez, L.L. Blackall, J. Keller, P. Gross, D. Batstone, W. Verstraete, K.H. Nealson, Microbial ecology meets electrochemistry: electricity-driven and driving communities, *ISME J.* 1 (2007) 9–18.
- [18] D.R. Lovley, Electromicrobiology, *Annu. Rev. Microbiol.* 66 (2012) 391–409.
- [19] K.B. Gregory, D.R. Bond, D.R. Lovley, Graphite electrodes as electron donors for anaerobic respiration, *Environmental Microbiology* 6 (2004) 596–604.
- [20] S.A. Patil, C. Hägerhäll, L. Gorton, Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems, *Bioanal. Rev.* 4 (2012) 159–192.

- [21] O. Choi, B.I. Sang, Extracellular electron transfer from cathode to microbes: application for biofuel production, *Biotechnol. Biofuels* 9 (2016) 1–14; DOI: 10.1186/s13068-016-0426-0
- [22] L. Shi, H. Dong, G. Reguera, H. Beyenal, A. Lu, J. Liu, H.-Q. Yu, J.K. Fredrickson, Extracellular electron transfer mechanisms between microorganisms and minerals, *Nat. Rev. Microbiol.* 14 (2016) 651–662; DOI: 10.1038/nrmicro.2016.93
- [23] S. Kato, Microbial extracellular electron transfer and its relevance to iron corrosion, *Microb. Biotechnol.* 9 (2016) 141–148; DOI: 10.1111/1751-7915.12340
- [24] G. Fóti, D. Gandini, C. Comninellis, A. Perret, W. Haenni, Oxidation of organics by intermediates of water discharge on IrO₂ and synthetic diamond anodes, *Electrochem. Solid-State Lett.* 2 (1999) 228–230.
- [25] E. Cabiscol, J. Tamarit, J. Ros, Oxidative stress in bacteria and protein damage by reactive oxygen species, *Internatl. Microbiol.* 3 (2000) 3–8.
- [26] I.F.F. Benzie, Evolution of antioxidant defence mechanisms, *Eur. J. Nutr.* 39 (2000) 53–61.
- [27] J.A. Imlay, Pathways of oxidative damage, *Annu. Rev. Microbiol.* 57 (2003) 395–418.
- [28] G.P. Dubey, S. Ben-Yehuda, Intercellular nanotubes mediate bacterial communication, *Cell* 144 (2011) 590–600.

- [29] S. Pande, S. Shitut, L. Freund, M. Westermann, F. Bertels, C. Colesie, I.B. Bischofs, C. Kost, Metabolic cross-feeding via intercellular nanotubes among bacteria, *Nat. Commun.* 6 (2015) 6238; DOI: 10.1038/ncomms7238.
- [30] S. Benomar, D. Ranava, M.L. Cárdenas, E. Trably, Y. Rafrafi, A. Ducret, J. Hamelin, E. Lojou, J.-P. Steyer, M-T. Giudici-Orticoni, Nutritional stress induces exchange of cell material and energetic coupling between bacterial species, *Nat. Commun.* 6 (2015) 6283; DOI: 10.1038/ncomms7283.
- [31] Y.J.H.J. Taverne, A.J.J.C. Bogers, D.J. Duncker, D. Merkus, Reactive oxygen species and the cardiovascular system, *Oxid. Med. Cell Longev.* 2013 (2013), Article ID: 862423, 15 pages; <http://dx.doi.org/10.1155/2013/862423>.
- [32] W. Dröge, Free radicals in the physiological control of cell function, *Physiol. Rev.* 82 (2002) 47–95; DOI: 10.1152/physrev.00018.2001.
- [33] B. Erable, D. Féron, A. Bergel, Microbial catalysis of the oxygen reduction reaction for microbial fuel cells: a review, *ChemSusChem* 5 (2012) 975–987.
- [34] Y. Liang, Y. Li, H. Wang, J. Zhou, J. Wang, T. Regier, H. Dai, Co_3O_4 nanocrystals on graphene as a synergistic catalyst for oxygen reduction reaction, *Nat. Mater.* 10 (2011) 780–786.
- [35] C.K. Stover, X.Q. Pham, A.L. Erwin, S.D. Mizoguchi, P. Warrenner, M.J. Hickey, F.S.L. Brinkman, W.O. Hufnagle, D.J. Kowalik, M. Lagrou, R.L. Garber, L. Goltry, E. Tolentino, S. Westbrook-Wadman, Y. Yuan, L.L. Brody, S.N. Coulter, K.R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G.K.S. Wong, Z. Wu, I.T. Paulsenk, J. Reizer, M. H. Saier, R.E.W. Hancock, S. Lory,

M.V. Olson, Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen, *Nature* 406 (2000) 959–964.

[36] J. Xia, C. Yang, D. Xu, D. Sun, L. Nan, Z. Sun, Q. Li, T. Gu, K. Yang. Laboratory investigation of the microbiologically influenced corrosion (MIC) resistance of a novel Cubearing 2205 duplex stainless steel in the presence of an aerobic marine *Pseudomonas aeruginosa* biofilm, *Biofouling* 31 (2015) 481-492; DOI: 10.1080/08927014.2015.1062089.

[37] M. Cooper, G.R. Tavankar, H.D. Williams, Regulation of expression of the cyanide-insensitive terminal oxidase in *Pseudomonas aeruginosa*, *Microbiology* (UK) 149 (2003) 1275–1284.

[38] J.W. Schertzer, S.A. Brown, M. Whiteley, Oxygen levels rapidly modulate *Pseudomonas aeruginosa* social behaviors via substrate limitation of Pqs, *Mol. Microbiol.* 77 (2010) 1527–1538.

[39] M.T. Madigan, J.M. Martinko, D.A. Stahl, D.P. Clark, *Brock biology of microorganisms*, fourteenth ed., Pearson Education Inc., Illinois, 2015, pp. 157-158

[40] V.V. Phelan, J. Fang, P.C. Dorrestein, Mass spectrometry analysis of *Pseudomonas aeruginosa* treated with azithromycin, *J. Am. Soc. Mass Spectrom.* (2015); DOI: 10.1007/s13361-015-1101-6

[41] P. Clauwaert, D. Ha, N. Boon, K. Verbeken, M. Verhaege, K. Rabaey, W. Verstraete, Open air biocathode enables effective electricity generation with microbial fuel cells, *Environ. Sci. Technol.* 41 (2007) 7564–7569.

- [42] L. Huang, X. Chai, X. Quan, B.E. Logan, G. Chen, Reductive dechlorination and mineralization of pentachlorophenol in biocathode microbial fuel cells, *Bioresource Technol.* 111 (2012) 167–174.
- [43] A. Cournet, M. Bergé, C. Roques, A. Bergel, M.L. Délia, Electrochemical reduction of oxygen catalyzed by *Pseudomonas aeruginosa*, *Electrochim. Acta* 55 (2010) 4902–4908.
- [44] Z. Ren, R.P. Ramasamy, S.R. Cloud-Owen, H. Yan, M.M. Mench, J.M. Regan, Time-course correlation of biofilm properties and electrochemical performance in single-chamber microbial fuel cells, *Bioresource Technol.* 102 (2011) 416–421.
- [45] S. Beyhan, N.E. Şahin, S. Pronier, J.-M. Léger, F. Kadirgan, Comparison of oxygen reduction reaction on Pt/C, Pt-Sn/C, Pt-Ni/C, and Pt-Sn-Ni/C catalysts prepared by Bönemann method: a rotating ring disk electrode study, *Electrochim. Acta* 151 (2015) 565–573.
- [46] W. Chen, X. Y. Liu, C. Qian, X.N. Song, W.W. Li, H.Q. Yu, An UV–vis. spectroelectrochemical approach for rapid detection of phenazines and exploration of their redox characteristics, *Biosens. Bioelectron.* 64 (2015) 25–29.
- [47] B. Briard, P. Bomme, B.E. Lechner, G.L.A. Mislin, V. Lair, M.C. Prévost, J.P. Latgé, H. Haas, A. Beauvais, *Pseudomonas aeruginosa* manipulates redox and iron homeostasis of its microbiota partner *Aspergillus fumigatus* via phenazines, *Sci. Rep.* 5 (2015) 8220–8233; DOI: 10.1038/srep08220.
- [48] C.M. Waters, B.L. Bassler, Quorum sensing: cell-to-cell communication in bacteria, *Annu. Rev. Cell Dev. Biol.* 21 (2005) 319–346.

- [49] P.V. Dantas, S. Peres, G.M. Campos-Takaki, C.E.L. Rotta, Utilization of raw glycerol for pyocyanin production from *Pseudomonas aeruginosa* in half-microbial fuel cells: evaluation of two electrochemical approaches, J. Electrochem. Soc. 160 (2013) G142–G148.
- [50] A. Managò, K.A. Becker, A. Carpinteiro, B. Wilker, M. Soddemann, A.P. Seitz, M.J. Edwards, H. Grassmé, I. Szabò, E. Gulbins, *Pseudomonas aeruginosa* pyocyanin induces neutrophil death via mitochondrial reactive oxygen species and mitochondrial acid sphingomyelinase, Antioxid. Redox Signaling 22 (2015) 1097–1110.
- [51] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($\cdot\text{OH}/\cdot\text{O}^-$) in aqueous solution, J. Phys. Chem. Ref. Data 17 (1988) 513–886.
- [52] A. Asghar, A.A.A. Raman, W.M.A.W. Daud, Advanced oxidation processes for in-situ production of hydrogen peroxide/hydroxyl radical for textile wastewater treatment: a review, J. Clean Prod. 87 (2015) 826–838.
- [53] M.S. Verma, P.Z. Chen, L. Jones, F.X. Gu, Controlling “chemical nose” biosensor characteristics by modulating gold nanoparticle shape and concentration, Sens. Biosensing Res. 5 (2015) 13–18.
- [54] J.B. Bulitta, N.S. Ly, C.B. Landersdorfer, N.A. Wanigaratne, T. Velkov, R. Yadav, A. Oliver, L. Martin, B.S. Shin, A. Forrest, B.T. Tsuji, Two mechanisms of killing of *Pseudomonas aeruginosa* by tobramycin assessed at multiple inocula

via mechanism-based modeling, *Antimicrob. Agents Chemother.* 59 (2015) 2315–2327.

[55] Y.-C. Chung, Ya-P. Su, C.-C. Chen, G. Jia, H.-L. Wang, J. C. G. Wu, J.-G. Lin, Relationship between antibacterial activity of chitosan and surface characteristics of cell wall, *Acta Pharmacol. Sin.* 25 (2004) 932–936.

[56] A. Barkleit, H. Foerstendorf, B. Li, A. Rossberg, H. Moll, G. Bernhard, Coordination of uranium(VI) with functional groups of bacterial lipopolysaccharide studied by EXAFS and FT-IR spectroscopy, *Dalton Trans.* 40 (2011) 9868–9876.

[57] N. Singh, S.M. Verma, S.K. Singh, P.R.P. Verma, Evidence for bactericidal activities of lipidic nanoemulsions against *Pseudomonas aeruginosa*, *Antonie Leeuwenhoek*, 107(2015) 1555–1568; DOI: 10.1007/s10482-015-0449-8.

[58] A.J. Bard, L.R. Faulkner, *Electrochemical methods: fundamentals and applications*, John Wiley, New York, 2001.

[59] M. Lashgari, M. Osanloo, Mechanistic studies of the synergistic effect: copper corrosion in HCl medium in the presence of a synthetic/eco-friendly inhibitor, *IREBIC* 2 (2011) 1–8.

[60] Z. He, F. Mansfeld, Exploring the use of electrochemical impedance spectroscopy (EIS) in microbial fuel cell studies, *Energy Environ. Sci.* 2 (2009) 215–219.

[61] A. Heijne, O. Schaetzle, S. Gimenez, L. Navarro, B. Hamelers, F. Fabregat-Santiago, Analysis of bio-anode performance through electrochemical impedance spectroscopy, *Bioelectrochemistry* 106 (2015) 64–72.

- [62] K.D. Young, The selective value of bacterial shape, *Microbiol. Mol. Biol. Rev.* 70 (2006) 660–703.
- [63] A.-C. Chien, N.S. Hill, P.A. Levin, Cell size control in bacteria, *Curr. Biol.* 22 (2012) R340–R349; DOI: 10.1016/j.cub.2012.02.032
- [64] M. Lashgari, Theoretical challenges in understanding the inhibition mechanism of aluminum corrosion in basic media in the presence of some p-phenol derivatives, *Electrochim. Acta* 56 (2011) 3322–3327.

FIGURE CAPTIONS

Figure1. Schematic representation of bacterial respiration (a) and microorganism interaction with electrode surface under cathodic (b) and anodic (c) polarization regimes: electrode in the case of (b) acts as electron source (food/substrate) for the respiration process. In the case of (c), however, the electrode plays as final electron recipient (oxidant) and furnishes a suitable place for the respiration of bacterial cells.

Figure 2. Cathodic polarization behavior of the electrode in the absence of bacteria plotted at low (a) and high (b) overvoltage domains. Solid and dashed lines denote to oxygen ($OCP = -200 \text{ mV}_{SCE}$) and argon ($OCP = -146 \text{ mV}_{SCE}$) purged electrolytes, respectively.

Figure 3. Cathodic polarization behavior of the electrode in oxygen containing electrolytes, in the absence (dashed line; $OCP = -200 \text{ mV}_{SCE}$) and presence of bacteria (solid line; $OCP = -244 \text{ mV}_{SCE}$): (a) and (b) are standing for low and high overvoltage domains.

Figure 4. CS effect on ORR in the presence of bacteria depicted in two low (a) and high (b) overvoltage domains. Dashed and solid lines denote to the presence ($OCP = -244 \text{ mV}_{SCE}$) and absence ($OCP = -153 \text{ mV}_{SCE}$) of CS, respectively.

Figure 5. The electrocatalytic response of the biocathode after anodic (solid line; $OCP = -219 \text{ mV}_{SCE}$) and cathodic (dashed line; $OCP = -220 \text{ mV}_{SCE}$) prepolarization ($|\eta| = 10 \text{ mV}$) of the electrode, recorded in the presence and absence of CS, respectively: (a) and (b) are standing for low and high overvoltage domains.

Figure 6. Impedance spectra recorded for the electrode in the absence (a) and presence of bacteria (b and c), before (a, b) and after prepolarization (c). The inset

is the expansion of spectra replotted at the circle marked zone (the high frequency domain). Values of OCP for the spectra a, b and c are -200 , -244 and $-219 \text{ mV}_{\text{SCE}}$, respectively.

Figure 7. Microscopic images of *P. aeruginosa* taken from the planktonic bacteria in the electrolyte solution (a: optical, $1000\times$) and immobilized bacteria on the electrode surface (b: SEM).

Figure 8. SEM image of the immobilized, massive cells (see Fig. 7b), taken at higher magnification.

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)

Figure(s)