**Final Report**

*Project Title*: Bioelectrochemically-assisted downstream processing for advanced biohydrogen production

*Project ID*: PD 115640

*Duration*: 1st of February, 2016 – 31st of January, 2020

*Principal Investigator*: Péter Bakonyi, Senior Research Fellow (University of Pannonia, Hungary)

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## Summary of key-results and findings

As it was assessed and found in this PD 115640 project by completing the work plan, that various feedstocks such as algae biomass [1], municipal waste liquor [2,3] and industrial by-product e.g. cheese whey [4] could be applied as starting materials for bioelectrochemical applications, such as microbial fuel (MFC)- and electrohydrogenesis/electrolysis (MEC) cells to recover energy in the form of electricity and hydrogen gas, respectively. However, the electro-active microbiome working in the anode chamber of these bioelectrochemical systems may be unable to directly and fully convert complex (recalcitrant) organic matter. Hence, a pre-digestion stage, particularly based on dark fermentative $H_2$ production (DF) could be suggested. The DF, to produce biohydrogen, involves usually a pretreatment to facilitate the conversion by increasing the accessibility to the target regions in the raw material [5]. Subsequent to pretreatment, hydrolysis should be applied to deliver substances i.e. sugars such as glucose readily consumable in the bioprocess [6]. Therefore, microbial electrochemical technologies such as MEC can potentially harvest more $H_2$ in multi-stage systems and thus, MECs can be viewed as downstream units to be attached after fermentations for the utilization of more easily consumable, residual organic components (metabolic products) such as acetic acid, butyric acid, etc. [7]. Taken into consideration the most essential cornerstones of MEC design, start-up and operation (that we evaluated in [8]), it was experimentally confirmed in our investigations that the properties of underlying microbial community in MEC has a crucial and governing role on the quality of gaseous products and it could be difficult to prevent the appearance of methane in the headspace of single-chamber devices [3,4]. Although we demonstrated an electrically-stimulated process for the purposeful generation of methane [9], in most bioelectrochemical systems aiming at the production of $H_2$, the appearance of $CH_4$ is best avoided to assist $H_2$ recovery and purification. Anyhow, once gaseous impurities are present, their separation from the mixture (to purify $H_2$) can be attempted by gas separation, for which purpose we reported on the behavior of innovative enzymatic membranes with enhanced affinity to $CO_2$ [10,11]. As it could be also deduced in our literature analysis, membrane separators (placed between the anode and cathode electrodes) may show an impact on the efficiency of MFCs in line with their (e.g. ion, substrate, oxygen transport) properties [12]. This information can have relevance for MECs, since it is a widely-accepted strategy to start-up MECs in MFC mode (for the acclimation and maturation of anode-surface electro-active biofilm before switching to hydrogen gas production mode) and therefore, the design and testing of various membrane separators in bioelectrochemical systems were important parts of the research [13,14]. Besides that, the deployment of various electro-active bacteria for the enrichment and microbial augmentation of bioelectrochemical systems was proposed. In fact, the effectiveness of bioaugmentation was studied employing cultures of exoelectrogenic microorganisms, namely *Propionibacterium freudenreichii, Cupriavidus basilensis and Lactococcus lactis* [15]. Depending on the operating conditions i.e. organic matter loading, it was found that the anode-surface normalized current density, power density and energy yield could be increased when *P. freudenreichii* was applied as a part of a mixed culture inoculum, [15]. Accordingly, given that the type of inoculum has a significant effect, in a separate study, it was examined what impact the history/origin of the seed source could have on the start-up and stabilization of bioelectrochemical system [16]. As a matter of fact, it was concluded that the inoculum should be chosen depending on the properties of the substrate e.g. its complexity in order to accelerate the development of the process. For instance, the use of anaerobic digester sludge treating municipal wastewater as inoculum led to considerably better utilization of municipal landfill leachate in the early stages of the process (compared to effluent of biogas fermenter from sugar industry) because of faster adaptation [16]. To further study responses of mixed culture anodic biofilms (e.g. in terms of the enrichment of electro-active microbes e.g. *Geobacter* spp.), we have carried out biohydrogen production measurements in MECs equipped with novel anion-exchange membrane separators (AEMs) using simple (acetate) and complex (model dark fermentation effluent comprising of acetic, butyric and propionic acids) substrates [17]. It was found that the MECs installed with the PSEBS CM DBC AEM ensured the highest $H_2$ yield and lowest internal losses compared to the two other separators and has the potential to improve MECs.

**Scientific outputs**

In total, besides a couple of items (1 article, 1 book chapter) in Hungarian, the below listed 17 papers have been delivered from the project and published mostly in D1/Q1 ranked, prestigious journals with a cumulative impact factor of >88 (in average approx. 5.2 impact factor/article). The number of independent received to these articles is >180, as of January, 2020. Hence, in the opinion of the principal investigator, the goals of the postdoctoral project have been achieved well. The importance of findings seems to be justified by the quality, quantity of publications as well as the notable number of independent citations. All publications contain the acknowledgement dedicated to the funding agency (National Research, Development and Innovation Office, NKFIH, Hungary) for supporting the project under grant number PD 115640. The copies of all articles/manuscripts have been made publicly accessible in REAL repository of the Hungarian Academy of Sciences. For the interested readers, the supporting direct REAL links are found below along with the other details of the papers.

**List of publications delivered from the project**

Péter Bakonyi, the principal investigator of PD 115640 project, has contributed to 2 articles as first author and to 5 articles as corresponding author (✉ denotes corresponding authorship).


[1] R.G. Saratale, C. Kuppam, A. Mudhoo, G.D. Saratale, S. Periyasamy, G. Zhen, L. Koók, **P. Bakonyi**, N. Nemestóthy, G. Kumar, Bioelectrochemical systems using microalgae – A concise research update, Chemosphere. 177 (2017) 35–43. https://doi.org/10.1016/j.chemosphere.2017.02.132
REAL: http://real.mtak.hu/id/eprint/106147

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REAL: http://real.mtak.hu/id/eprint/106148

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REAL: http://real.mtak.hu/id/eprint/106152

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REAL: http://real.mtak.hu/id/eprint/106153

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REAL: http://real.mtak.hu/id/eprint/106155

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REAL: http://real.mtak.hu/id/eprint/106157

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REAL: http://real.mtak.hu/id/eprint/106196