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Improvement of waste-fed bioelectrochemical system performance by selected electro-active microbes: Process evaluation and a kinetic study

László Koók, Nicolett Kanyó, Fruzsina Dévényi, Péter Bakonyi*, Tamás Rózsenberszki, Katalin Bélafi-Bakó, Nándor Nemestóthy

Research Institute on Bioengineering, Membrane Technology and Energetics, University of Pannonia, Egyetem u. 10, 8200, Veszprém, Hungary

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ABSTRACT

In this work, bioaugmentation strategy was tested to enhance electricity production efficiency from municipal waste liquor feedstock in microbial fuel cells (MFC). During the experiments, MFCs inoculated with a mixed anaerobic consortium were enriched by several pure, electro-active bacterial cultures (such as *Propionibacterium freudenreichii*, *Cupriavidus basilensis* and *Lactococcus lactis*) and behaviours were assessed kinetically. It turned out that energy yield could be enhanced mainly at high substrate loadings. Furthermore, energy production and COD removal rate showed an optimum and could be characterized by a saturation range within the applied COD loadings, which could be elucidated applying the Monod-model for describing intracellular losses. Polarization measurements showed the positive effect of bioaugmentation also on extracellular losses. The data indicated a successful augmentation process for enhancing MFC efficiency, which was utmost in case of augmentation strain of *Propionibacterium freudenreichii*.

1. Introduction

Microbial fuel cell (MFC) technology can be considered as a rapidly developing alternative for generating electricity using electro-active microorganisms from the chemical energy stored in organic substrates [1–3]. As various research works demonstrated, besides easy-degradable materials, waste streams may also be utilized in MFCs as feedstock for electricity production [4,5] e.g. synthetic human blackwater [6], industrial wastewaters [7–9], landfill leachate [10] or municipal solid waste [11]. Although in practice MFCs are typically operated with a mixed consortium in the anode chamber, a considerable number of pure cultures have been also tested including different Gram-negative/Gram-positive bacteria, yeasts and algae [12,13]. In general, such single-strain MFCs are suitable for fundamental research and have limitations for real-field applications due to strict sterility requirements. Nevertheless, they can be viewed as potential candidates for the augmentation of mixed culture MFCs.

Bioaugmentation is a well-known strategy for process enhancement (i.e. aiming at the efficient removal of specific components) and relies on the addition of selected microbial species to an initial – mostly natural – microbial consortia/environment [14,15]. The target compounds to be converted vary widely and can include oil-based contaminations, polycyclic aromatic hydrocarbons (PAHs), phenol, etc. according to the scientific literature [16–18]. Moreover, microbial augmentation can be advantageous not only in terms of specific substrate degradation but also to improve biofuel (e.g. biogas or biohydrogen) formation as well as integrated applications designed by coupling fermentation and bioelectrochemical treatment [19,20]. The bioaugmentation in microbiologically-assisted electrochemical systems has been demonstrated with success (i.e. to utilize corn stover [21] or synthetic wastewater [22]) by exploiting specific syntrophic processes and hierarchical structures present in such systems in order to boost electricity generation [23]. So far, electro-kinetic analysis of MFCs augmented with *Shewanella haliotis* [22] showed the positive effect of this technique on the grounds of power output and substrate biodegradation. The observed benefits could be mainly attributed to lower activation losses and enhanced shuttling between redox intermediates [22]. In another paper applying electro-active *Pseudomonas aeruginosa* and non-electro-active *Escherichia coli* strains for bioaugmentation

* Corresponding author.
E-mail address: bakonyip@almos.uni-pannon.hu (P. Bakonyi).

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in MFCs, it could be concluded that the bioelectrochemical cells had taken advantage of synergistic species interactions in the mixed consortia, leading to lower polarization resistance and increased power generation capacity [24].

In this work, bioaugmentation of MFCs was carried out by employing pure isolates of electro-active bacteria, namely *Propionibacterium freudenreichii*, *Cupriavidus basilensis* and *Lactococcus lactis*, which to our knowledge, have not been used for this purpose. *P. freudenreichii* is a Gram-positive obligate anaerobic bacteria belonging to the phylum *Actinobacteria* and known as an endogenous mediator-producing strain. Actually, 1,4-dihydroxy-2-naphthoic acid (DHNA) and 2-amino-3-dicarboxy-1,4-naphthoquinone (ACNQ) are reported as electron shuttle molecules, secreted by *P. freudenreichii* [25,26] which allow its application in mediator-less MFC systems [27]. *C. basilensis* is a flagellated Gram-negative, facultative aerobic β-*proteobacteria* [28] and able to the utilize substances e.g. phenol or aliphatic alcohols as substrates [29,30]. The members of this genus are described to be capable of producing endogenous mediators for extracellular electron transfer [30,31]. Since *C. basilensis* is metal-resistant and able to degrade a wide range of materials, its use seems to be promising in wastewater treatment as well as in bioelectrochemical technologies. *L. lactis*, a member of phylum *Firmicutes*, is a Gram-positive, facultative anaerobic bacterium with a potential as a biocatalyst in microbial electrochemical cells because of its self-secreted electron accepting and shuttling agent, ACNQ [32,33]. Furthermore, its important trait is the capability of pursuing electrochemically-modified metabolic pathway besides homolactic fermentation, which leads to the formation of acetate (as by-product) to be consumed by other i.e. exoelectrogenic microorganisms present in an augmented bioelectrochemical reactor [33].

To our best knowledge, no comparative study has been done yet with these microbes to investigate bioaugmentation process in MFCs that involves a kinetic approach for the assessment of system behaviour in the course of waste utilization. Therefore, the results demonstrated may have novelty and added-value to support the better understanding of bioaugmentation in MFCs and expand the perspectives of such bioelectrochemical cells.

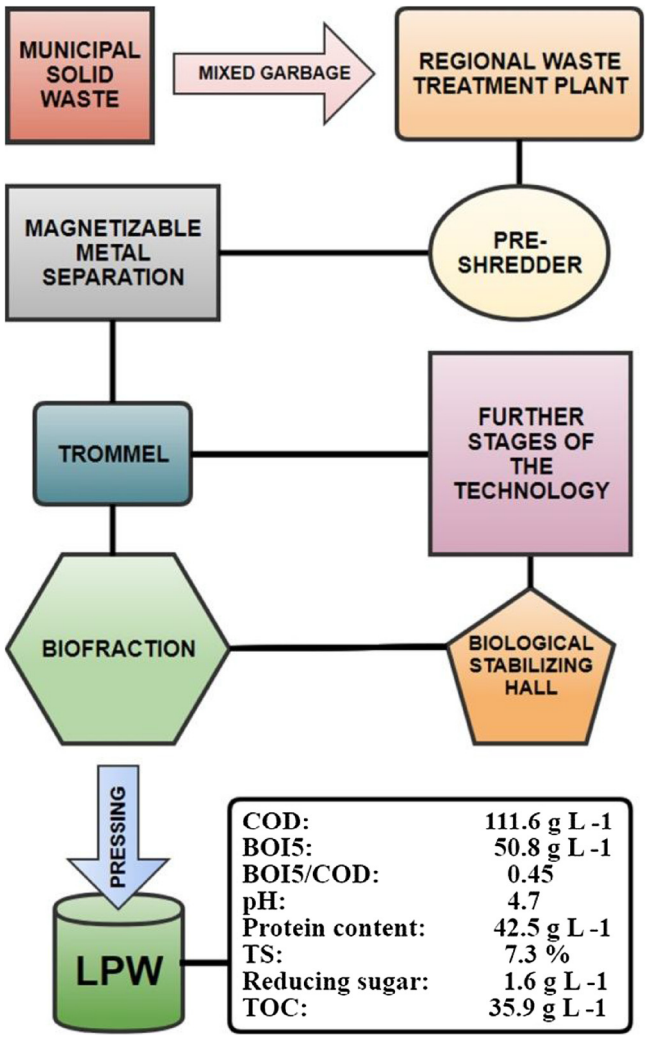

Fig. 1. Process flow diagram of LPW preparation.

2. Materials and methods

2.1. Seed source and substrates

For MFC inoculation, seed source was collected from beet pulp utilizing biogas fermentation unit of Hungarian sugar factory, located at Kaposvár, with an initial microbial community structure demonstrated in our recent work [34]. The anaerobic sludge was pretreated (starved) in a laboratory-scale reactor before use for one week at 37 °C. Its main characteristics were the followings: COD content: 12 g L^{-1}, pH = 7.8, Total solids: 6.7%. As for substrate, pressed fraction of municipal solid waste (LPW) was used. Characteristics of LPW can be found in previous publications [11,35–37]. The most important parameters of the substrate and the flow diagram of its preparation process can be seen in Fig. 1.

2.2. Preparation of pure cultures of selected electro-active microbes for bioaugmentation

The pure cultures of selected microbes were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). The broth media compositions were the followings: *Lactococcus lactis* (DSMZ-20481) broth – casein peptone (pancreatic digest) 17 g L^{-1}, K_2HPO_4 2.5 g L^{-1}, glucose 2.5 g L^{-1}, NaCl 2.5 g L^{-1}, soy peptone (papaic digest) 3 g L^{-1}, yeast extract 3 g L^{-1}, agar 20 g L^{-1} (pH = 7); *Cupriavidus basilensis* (DSMZ-11853) broth – peptone 5 g L^{-1}, meat extract 3 g L^{-1}, agar 20 g L^{-1} (pH = 7); *Propionibacterium freudenreichii* (DSMZ-20271) broth – casein peptone (tryptic digest) 10 g L^{-1}, yeast extract 5 g L^{-1}, Na-lactate 10 g L^{-1}, agar 20 g L^{-1} (pH = 7).

The cultures were incubated on agar plates – and in stab agar in case of *P. freudenreichii* – for two days at 37 °C. Thereafter, colonies were harvested and transferred to liquid media (50 mL, without agar) and incubated for two more days under the same conditions. Before use in MFCs, the cell concentration of liquid cultures was determined by Bürker's chamber.

2.3. MFC design and setup

The design of dual-chamber microbial fuel cells was adopted from our previous work [38]. In this MFC construction, anode and cathode compartments (with 60 mL total volume) were equipped with carbon cloth (Zoltek Corp., USA) and Pt-C (0.3 mg cm^2 Pt content, FuelCellsEtc, USA) electrodes (64 cm^2 apparent surface area), respectively. The anode and cathode were connected by Ti wire (Sigma-Aldrich, USA) to the external circuit, containing a 100 Ω resistor. The chambers were separated by Nafion 115 proton exchange membrane (Sigma-Aldrich, USA) with diameter of 4.5 cm. Before use, the membrane was activated as described elsewhere [38]. In order to maintain aerobic conditions, air was continuously supplemented to the cathode compartment.

The anode side of MFCs was filled with 50 mL of mesophilic sludge (pH adjusted to 7) and 5 mL of individual, pure strain