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Microbial community dynamics in continuous microbial fuel cells fed with synthetic wastewater and pig slurry

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ABSTRACT

Two-chambered microbial fuel cells (MFCs) operating with synthetic wastewater and pig slurry were assessed. Additionally, the use of 2-bromoethanesulfonate (BES-Inh) was studied. The synthetic wastewater-fed MFC (MFC_{SW}) showed a maximum power density (PD_{max}) of 2138 mW m^{-3}, and the addition of BES-Inh (10 mM) did not show any improvement in its performance (PD_{max} = 2078 mW m^{-3}). When pig slurry was used as feed (MFC_{PS}), PD_{max} increased up to 5623 mW m^{-3}. The microbial community composition was affected by the type of substrate used. While, *Pseudomonadaceae* and *Clostridiaceae* were the most representative families within the acetate-based medium, *Flavobacteriaceae*, *Chitinophagaceae*, *Comamonadaceae* and *Nitrosomonadaceae* were predominant when pig slurry was used as feed. Otherwise, only the Eubacterial microbial community composition was strongly modified when adding BES-Inh, thus leading to an enrichment of the *Bacteroidetes* phylum. Oppositely, the Archaeal community was less affected by the addition of BES-Inh, and *Methanosarcina* sp., arose as the predominant family in both situations. Despite all the differences in microbial communities, 6 operational taxonomic units (OTUs) belonging to *Bacteroidetes* (*Porphyromonadaceae* and *Marinilabiaceae*) and *Firmicutes* (*Clostridiales*) were found to be common to both MFCs, also for different contents of COD and *N*-NH_4^+, and therefore could be considered as the bioanode core microbiome.

1. Introduction

In a microbial fuel cell (MFC), microorganisms can convert the chemical energy present in organic compounds directly into electricity by transferring electrons to an anode [1]. The formation of a biofilm on the anode surface is essential for an efficient electron transfer in a MFC [2]. These microorganisms can use a wide range of substrates from pure and mineral compounds to complex organic compounds present in wastewaters [3]. Hence, depending on the type of substrate used as feed, both the microbial community and the MFC performance might be different.

To date, many studies have investigated the effect of synthetic substrates on MFC performance and microbial diversity; however, few studies have studied the effect of using complex substrates (e.g. pig slurry) on the stability of the anode microbiome [4]. On the other hand, the study of anodic microbial communities is being increasingly investigated in order to better understand potential electron transfer mechanisms and current generation in bioelectrochemical systems [5,6]. For application purposes, mixed cultures are the most appropriate as, in general, pure cultures are not capable of producing the necessary high power, and their resilience and range of metabolizable organic compounds is limited.

Exoelectrogenic bacteria and methanogens share similar growth conditions and actively compete for the organic substrate in the anodic chamber [7,8], thus affecting efficiency in terms of power generation and coulombic efficiency (CE) [9]. Consequently, there is a growing interest in gaining insight into inhibition strategies against methanogenesis. So far, many types of methanogenesis inhibitors have been described [10], where the 2-bromoethanesulfonate ($BrCH_2CH_2SO^-_3$) (BES-Inh) stands out due to its specificity [11,12], being the most efficient to inhibit both acetoclastic and hydrogenotrophic methanogenesis [13]. BES-Inh is a structural analogue of coenzyme M (2-mercaptoethanesulfonic acid, HS-CoM), only present in methanogenic Archaea, and it is directly involved in the final enzymatic steps during methane biosynthesis [14]. BES-Inh bounds to methylreductase, a membrane-bound enzymatic system, thus avoiding methane formation.

Although, effective concentrations of methanogenic inhibitors vary depending on the system applied [10], most of these studies aim solely at enhancing H_2 production in MEC systems by inhibiting hydrogenotrophic methanogenic populations in a single-chamber MFC/MEC [15,16,17]. Thereafter, it is important to emphasise that to date, the effect of BES-Inh addition on the whole microbial community (Eubacteria and Archaea) structure in the biofilm of the anode has not been studied in detail.

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Recently, outstanding studies reporting on syntrophic interactions between methanogenic Archaea (*Methanosaeta* and *Methanosarcina*) and exoelectrogenic Eubacteria such as *Geobacter* species, via direct electron transfer (DIET) in the environment have been described [18,19]. Therefore, it is crucial to take into account the potential presence of such kind of syntrophic interactions in MFC electrodes when an extensive microbial community assessment is performed. Such kinds of complex microbial interactions between eubacteria and archaea populations could also have implications both for the overall anodophilic microbial community and for MFC performance.

The objectives of the present study were: i) to evaluate the performance of two MFCs operating subsequently with different inocula (anaerobic digester inoculum and MFC inoculum), and different substrates (synthetic wastewater and liquid fraction of pig slurry); ii) to study the evolution over time of the microbial community developed on the anode material under these different operational conditions; and iii) to assess the effect of 2-bromoethanesulfonate as inhibitor of methanogenesis, on the microbial community established in the anodic biofilm, aiming to find out putative exoelectrogenic microorganisms hampered by active methanogenic microbial communities.

2. Material and methods

2.1. MFC configuration

Two identical two-chambered MFCs were used in this study. Both MFCs were constructed with methacrylate plates, each chamber with a size of $0.14 \times 0.12 \times 0.02$ m^3, separated by a cation exchange membrane (CEM) (14×12 cm) (Ultrex CMI-7000, Membranes International Inc., Ringwood, NJ, USA). The anodes were made of granular graphite (2 to 6 mm in diameter) (El Carb 100, Graphite Sales Inc., U.S.A.) and carbon felt, 3.18 mm thick, 99.0% (Cymit Química, S.L.), resulting in a respective net anode volume (NAV) of 165 mL for the MFC fed with synthetic wastewater (MFC_{SW}), and 269 mL for the MFC fed with pig slurry (MFC_{PS}). The net cathode volume (NCV) was 250 mL for both MFCs. Cathodes were made of stainless steel mesh in both cases. The anode and cathode were connected to 1000, 500 and 100 Ω external resistors. Prior to their use, both electrodes, granular graphite and carbon felt, were sequentially soaked in 1 M of HCL, and 1 M of NaOH, in each case for 24 h, and finally rinsed in deionised water in order to remove organic and inorganic impurities.

2.2. Start-up and MFC operation

The MFC_{SW} was fed with synthetic wastewater containing (per litre) $CaCl_2$, 0.0147 g; KH_2PO_4, 3 g; Na_2HPO_4, 6 g; $MgSO_4$, 0.246 g; a 1 mL trace element solution per litre prepared as described elsewhere [20], and CH_3COONa and NH_4Cl as a carbon and nitrogen source, respectively. The concentrations of acetate and ammonia were increased along the experiment ranging between 0.46 and 2.91 $g \cdot L^{-1}$ for CH_3COONa, and 0.26–3.82 $g \cdot L^{-1}$ for NH_4Cl. The catholyte used in both MFCs consisted of a buffer phosphate (KH_2PO_4, 3 $g \cdot L^{-1}$ and Na_2HPO_4 6 $g \cdot L^{-1}$). 2-bromoethanesulfonate (BES-Inh) was added to the synthetic medium, in a range between 0.16 and 10 mM, to study its effect on the microbial community and on MFCs performance. The cell (MFC_{SW}) was inoculated with biomass taken from a mesophilic anaerobic digester (bench-scale, continuously stirred tank reactor) fed with slaughterhouse waste (In.AD).

The MFC_{PS} was fed with the liquid fraction of pig slurry (PS) collected from a pig farm (Calldetenes, Catalonia). The characterization of the PS used in the experiments, previously centrifuged (at 4991 g for 10 min) before feeding it to the cell, was: pH, 7.72; total and soluble chemical oxygen demand, COD_t (mg O_2 kg^{-1}), 6908; COD_s (mg O_2 kg^{-1}), 3462; total ammonia nitrogen, $N\text{-}NH^+_4$ (mg $N\text{-}NH^+_4$ L^{-1}), 858.15; Kjeldahl-nitrogen, TKN (mg N L^{-1}), 1068.47; total and volatile solids, TS (%), 0.78; VS (%), 0.42; and electric conductivity, EC (mS cm^{-1}), 7.73. In order to obtain the expected concentrations for the different assays, specific dilutions were carried out. The inocula used in the MFC_{PS} was the anode biofilm from the MFC_{SW} after one year in operation, named B_2 MFC_{SW}. A summary of the samples collected for microbial community analysis is presented in Table 1, whereas an overview of the operational conditions throughout the experiments is described in Table 2.

2.3. Chemical and electrochemical measurements

The voltage (V) was measured using a multimeter data acquisition unit (Mod. 34970A, Agilent Technologies, Loveland, CO, USA.) The current density (I) was then calculated using Ohm's Law, and the maximum power density (PD_{max}), the open circuit voltage (OCV) and the internal resistance (R_{int}) were obtained through polarization curves following the procedure described elsewhere [21].

Chemical oxygen demand (COD) was measured following an optimized APHA–AWWA–WPCF Standard Method 5220 [22], filtering the samples through a 0.45 μm pore diameter Nylon syringe filter (Scharlau, S.L.). Coulombic efficiency (CE), defined as the ratio of electrons used as current to the theoretical maximum electron production, was calculated as described elsewhere [1].

Methane was determined by a CP-3800 gas chromatograph (Varian, USA) fitted with a Haysep-Q 80–100 Mesh ($2\ m \times 1/8'' \times 2.0$ mm) column (Varian, USA) and a thermal conductivity detector (TCD). Helium was used as the carrier gas (45 mL min^{-1}) and the temperatures of the injection port, oven, and detector were 50 °C, 150 °C and 180 °C, respectively.

2.4. Anode community characterization

2.4.1. Biofilm morphology

In order to determinate biofilm morphology on the anode, samples from the biofilm were examined with SEM microscopy (model Hitachi S-4100FE). A small portion of granular graphite and carbon felt (both containing microbial biofilms) was carefully removed from the anodic compartment of both MFCs. Biofilms were fixed by immersing samples in a 2% glutaraldehyde buffer at pH 7.4. Then, samples were dried and sputter-coated with a graphite layer. As control, both electrodes without biofilm were also examined.

2.4.2. DNA extraction and PCR-DGGE amplification

Genomic DNA was extracted in triplicate from the granular graphite (MFC_{SW}) and carbon felt (MFC_{PS}) with the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following the manufacturer's instructions. Two primer sets were used, for subsequent DGGE assessment, to amplify total Eubacterial and Archaeal partial-16S rRNA genes. Universal Eubacterial forward F341-GC and reverse R907 primers were used to amplify the hypervariable V3–V5 region with a polymerase chain reaction (PCR) [23]. Regarding Archaeal population, a Nested PCR approach was performed using the primer pairs

Table 1
Microbial samples taken and compared in the present work.

Aim	Set-up	Sample name
Microbial population adaptation over time and under different operational conditions (COD and $N\text{-}NH^+_4$)	MFC_{SW}	In.AD
		B_1 MFC_{SW}
		B_2 MFC_{SW}
		PS
	MFC_{PS}	B_1 MFC_{PS}
		B_2 MFC_{PS}
Effect of different substrates fed	MFC_{SW}/MFC_{PS}	B_1 MFC_{SW}
		B_2 MFC_{SW}
		B_1 MFC_{PS}
		B_2 MFC_{PS}
Effect of BES inhibitor	MFC_{SW}	B_2 MFC_{SW}
		B_3 $MFC_{SW\text{-}BES}$