Bioresource Technology 129 (2013) 599–605

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Spatial uniformity of microbial diversity in a continuous bioelectrochemical system

Paul G. Dennis [a,b], Kun Guo [a], Michael Imelfort [a,b], Paul Jensen [a], Gene W. Tyson [a,b], Korneel Rabaey [a,c,*]

[a] Advanced Water Management Centre, The University of Queensland, Brisbane, Queensland 4072, Australia
[b] Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia
[c] Laboratory of Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

HIGHLIGHTS

- A bioelectrochemical system with exchangeable electrodes was constructed.
- Microbial diversity did not differ between electrodes within a time point.
- Temporal dynamics can be related to community development rather than spatial variation.

ARTICLE INFO

Article history:
Received 30 June 2012
Received in revised form 20 November 2012
Accepted 20 November 2012
Available online 4 December 2012

Keywords:
Bioelectrochemical system
Microbial fuel cell
Microbial communities
Electroactive biofilms
Spatial variation

ABSTRACT

Bioelectrochemical systems (BESs) are emerging as a technology with diverse future applications. Anode-associated microbial diversity and activity are known to change over time, but the consequences of these dynamics on BES functioning are poorly understood. A novel BES with exchangeable anodic electrodes that facilitates characterisation of microbial communities over time was constructed. The BES, received a mixture of volatile fatty acids and produced 0.13 mA cm^{-2} of anodic electrode surface, leading to the removal of 14 g chemical oxygen demand per m^2 electrode per day at a coulombic efficiency of 76%. Pyrosequencing of 16S rRNA genes revealed no differences in the diversity of microbial communities associated with different electrodes within a single time point. This finding validates the design for temporal studies as changes in microbial diversity observed over time can be related to community development rather than spatial variation within the reactor.

1. Introduction

Bioelectrochemical Systems (BESs) exploit microorganisms to catalyse oxidation and/or reduction reactions at anodic and cathodic electrodes, respectively. Key applications for BESs include electrical power generation, *i.e.* microbial fuel cells (Davis and Yarbrough, 1962), bioremediation (Gregory and Lovley, 2005) and production of (bio)chemicals (Hongo and Iwahara, 1979). A wide range of electron donors, including organic compounds associated with wastewater, can be oxidised at the anode and facilitate cathodic production of compounds of interest *e.g.* caustic soda (Rabaey et al., 2010), methane (Clauwaert et al., 2008; Cheng et al., 2009) or acetate (Nevin et al., 2010). While modifications to the architecture of BESs and the materials used in their construction have led to significant advances in BES technologies (Logan et al., 2006; Du et al., 2007), a better understanding of BES microbiology is critical to achieve stable and optimal performance.

A wide range of parameters, including pH, starting inoculum and electrode potential, are known to influence the composition of anode-associated microbial communities (Torres et al., 2009; White et al., 2009; Patil et al., 2011). Nonetheless, little is known about how electrode-associated communities develop over time and influence anodic and/or cathodic processes. Previous studies have demonstrated pH stratification and differential gene expression within *Geobacter sulfurreducens* biofilms (Franks et al., 2010), behavioural changes of *Shewanella oneidensis* MR-1 cells in relation to electron transfer over time (Harris et al., 2010), and temporal structure in the spatial arrangement of different species in co-culture (Read et al., 2010). These studies indicate that electrode-associated microbial communities exhibit differences over time; however, the influence of these changes on the functional stability of BESs is poorly understood. Due to a lack of appropriate BES designs, there are currently no studies that describe in detail the temporal dynamics of electrode-associated microbial communities in detail.

* Corresponding author at: Laboratory of Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.
E-mail address: Korneel.Rabaey@UGent.be (K. Rabaey).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.11.098

To address the above knowledge gap a BES design featuring removable electrodes that facilitate time-course studies of electrode-associated microbial communities was developed. To statistically validate comparisons of diversity over time, three replicate samples should be obtained at each time point. Furthermore, to detect changes in microbial diversity over time it is necessary that variation in community structure between electrodes/samples is minimal. In this study, the diversity of microbial communities sampled at multiple positions on each of 15 electrodes by 16S rRNA gene amplicon pyrosequencing was characterised. The data were used to determine if anode-associated microbial diversity differed between and within electrodes fixed at different positions within the BES.

2. Methods

2.1. Reactor design

A lamellar type reactor consisting of two end plates and three paired anode–cathode compartments was constructed from acrylic sheeting (Fig. 1; Supplementary information). Each anode frame (inner volume of 0.56 l) housed three $4 \times 30 \times 150$ mm IGS-743 granite electrodes (Morgan Industrial Carbon, Revesby, Australia) mounted in acrylic blocks with rubber o-rings. Due to the insertion of the electrodes into the acrylic blocks and the 20 mm wall thickness of the Perspex frames, only 120 mm of electrode length was exposed to the medium. As there were nine electrodes in total, the total electrode surface area was 745 cm^2 (324 cm^2 projected relative to the membrane). The anode and cathode compartments were separated by a cation exchange membrane (Ultrex CMI7000, Membranes International Inc., USA) sandwiched between two 2-mm thick rubber frames with the same dimensions as the electrode frames. Each cathodic electrode consisted of a stainless steel wire mesh (316 SS, size 300, Locker, Australia) within a stainless steel frame (Fig. 1; Supplementary information). Anode–cathode pairs were separated by a sheet of impermeable rubber. When all of the layers were correctly positioned, the end plates were fastened in place and the reactor was connected to recirculation, buffer vessels and feed circuits (Supplementary information). Both the anode and cathode fluids were recirculated at a rate of 20.78 l h^{-1}. The total liquid volumes of the anode and cathode circuits (*i.e.* the frames, recirculation bottles and tubing) were 1.8 l and 1.9 l, respectively.

The electrodes were connected in parallel to a PAR VMP-3 Potentiostat (Princeton Applied Research, USA). A Ag/AgCl electrode (assumed 0.197 V vs. Standard Hydrogen Electrode) filled with 3 M KCl was fitted to the middle anode compartment via a glass luggin capillary. A pH electrode was fitted to the recirculation circuit of the anode and attached to a control module that activated a 1 M NaOH dosing pump. This system maintained the anode at pH 6.5 for the duration of the experiment.

2.2. Reactor operation

The reactor was inoculated with a mixed microbial community isolated from the uppermost 5 cm of sediments in a lake at The University of Queensland by gentle agitation in sterile phosphate-buffered saline solution (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4). These sediments are generally suboxic and have been successfully used to initialise BESs

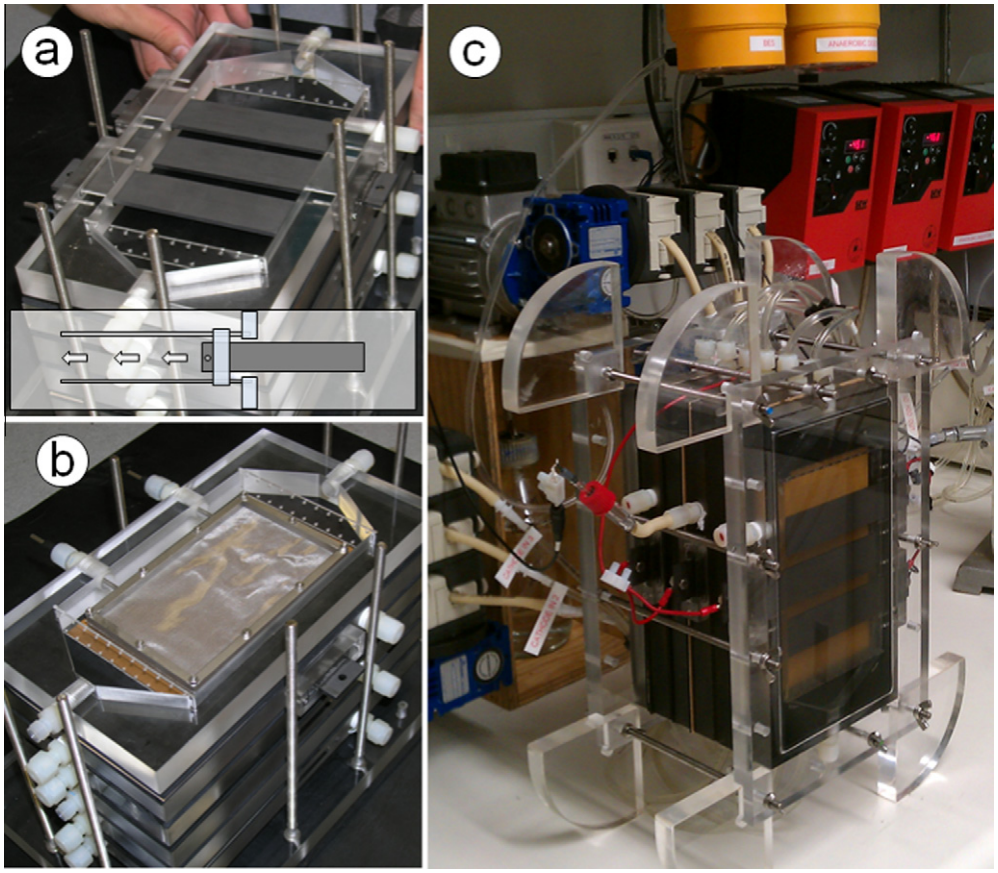

Fig. 1. Photographs of the reactor setup: (a) an anode frame fitted with three exchangeable anodic electrodes; (b) a cathode frame fitted with an assembled cathodic electrode (c) an overview of the assembled reactor showing pH control and recirculation pumps.