Bioelectrochemistry 121 (2018) 160–168

Contents lists available at ScienceDirect

Bioelectrochemistry

Revealing metabolic storage processes in electrode respiring bacteria by differential electrochemical mass spectrometry

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ARTICLE INFO

Article history:
Received 19 November 2017
Received in revised form 29 January 2018
Accepted 30 January 2018
Available online 8 February 2018

Keywords:
Microbial fuel cell
Charge and carbon storage
Online measurement of CO_2 production
Differential electrochemical mass spectrometry
Mass spectrometric cyclic voltammetry

ABSTRACT

In this work we employ differential electrochemical mass spectrometry (DEMS) in combination with static and dynamic electrochemical techniques for the study of metabolic processes of electrochemically active bacteria. CO_2 production during acetate oxidation by electrode respiring bacteria was measured, in-vivo and online with a sensitivity of $6.5 \cdot 10^{-13}$ mol/s. The correlation of ion current and electrical current provides insight into the interaction of metabolic processes and extra-cellular electron transfer. In low-turnover CVs, two competing potential dependent electron transfer mechanisms were observed and formal potentials of two redox systems that are involved in complete oxidation of acetate to CO_2 were determined. By balancing charge and carbon flows during dynamic measurements, two significant storage mechanisms in electrochemically active bacteria were identified: 1) a charge storage mechanism that allows substrate oxidation to proceed at a constant rate despite of external current flowing in cathodic direction. 2) a carbon storage mechanism that allows the biofilm to take up acetate at an unchanged rate at very low potentials even though the oxidation to CO_2 stops. These storage capabilities allow a limited decoupling of electrical current and CO_2 production rate.

1. Introduction

Gaining deeper knowledge on electron transfer mechanisms and energy conversion in bioelectrochemical systems (BES) is not only of scientific interest but also highly relevant for system design for technical applications. In most BES, anode respiring bacteria (ARB) form a biofilm that is firmly attached to a solid electrode. There are numerous in-situ and ex-situ techniques for studying ARB [1] but most of them focus on the transfer of electrons out of the cell to an electrode. Analysis of metabolic processes in the cell are usually not conducted online, but rather ex-situ [2]. They also require to irreversibly destroy the biofilm. Dynamic techniques are non-destructive and allow to study the interaction of transport processes and kinetics by evaluating typical response signals [3]: Chronoamperometry, impedance spectroscopy and cyclic voltammetry have been used extensively to characterize, analyse, and even improve the oxidation of C-containing fuels; they may be combined with surface and concentration measurements such as mass spectrometry and infrared spectroscopy to obtain further insights [3]. One of the most popular dynamic techniques for studying phenomena related to extracellular electron transfer (EET) between a biofilm and an electrode is cyclic voltammetry (CV). Usually two types of CVs are distinguished in the field of BES [4]: non-turnover and turnover CVs. Non-turnover CVs are recorded using substrate-depleted solutions, i.e. at conditions where there is no substrate available to the biofilm. The current recorded then is only due to capacitive effects and due to the oxidation and reduction of redox systems within the cell. When no substrate oxidation or other irreversible processes take place, the integrals of positive and negative currents over time are equal and the average current is zero. The peaks observed in the CV can be assigned to certain redox systems such as the outer membrane protein OmcB [4,5]. However, as no substrate oxidation takes place under non-turnover conditions, assigning oxidation and reduction of these redox systems to processes observed in the substrate metabolizing biofilm needs to be done with uttermost care. On the other hand, turnover CVs are recorded at high substrate availability. The measured current mainly results from substrate oxidation and often follows an s-shaped curve [4,6,7]. Since substrate oxidation is irreversible, the average current under turnover-conditions is usually

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https://doi.org/10.1016/j.bioelechem.2018.01.014
1567-5394/

positive. Current peaks from oxidation and reduction of inter-cellular redox systems are masked by the large current from substrate oxidation. Thus, it is difficult to clearly identify the role that each redox system plays in substrate oxidation from turnover as well as from non-turnover CVs.

Direct, non-invasive measurement of metabolites or products such as CO_2 during CVs would help to understand the contributions of the redox systems that are visible in CVs to anodic respiration. So far, measurements of gas production in microbial fuel cells or electrolysis cells have been limited to headspace gas analysis by gas chromatography and quantification by measuring the total volume of collected gas [8–10]. The reported approaches did not achieve the time resolution needed to monitor product formation during CV measurements. One reason is that the equilibrium reaction between hydrogen carbonate and CO_2 slows down the transfer of CO_2 from a buffered solution near pH 7 into the gas phase [11] so that even small headspace volumes and intense stirring are unlikely to solve this problem. An alternative technique applied for non-electrochemical reactions is membrane inlet mass spectrometry (MIMS); with this technique, volatile products and intermediates from normal bacteria have been analysed online [12,13]. MIMS yields a high time resolution and sensitivity because volatile substances pervaporate through a gas-permeable membrane into a vacuum system where they are detected by mass spectrometry. It has already been shown that CO_2 production in bioreactors can be monitored and quantified this way [11,14]. Following these studies, we suggest the use of differential electrochemical mass spectrometry (DEMS) in combination with static and dynamic electrochemical techniques for the study of metabolic processes of electrochemically active bacteria. While DEMS has been numerously used to study electrode reactions [15,16], so far no bioelectrochemical reactions have been investigated by DEMS. During DEMS measurements, volatile products and intermediates of electrochemical reactions pervaporate through a PTFE membrane into a differentially pumped vacuum system where they are detected by mass spectrometry. The membrane is placed closely to the electrode to minimize time delays. This online-technique provides a high sensitivity, short response times, and allows quantification. In a recent study, we combined quantitative DEMS experiments and modelling of electrochemical reactions to analyse kinetics of CO oxidation at porous electrodes [17].

Further information on DEMS can be found for instance in two comprehensive reviews by Baltruschat and co-workers [15,16].

In this work we combine DEMS with cyclic voltammetry of electroactive anodic biofilms for the first time. Mass spectrometric cyclic voltammograms (MSCVs) that allow to directly correlate CO_2 production and current at high and low substrate availability are reported, and electron transfer systems that are related to CO_2 production are identified. We demonstrate that DEMS can be employed for quantitative analysis of the CO_2 production rate of electrochemically active bacteria and discuss two intra-cellular storage mechanisms for charge and substrate as well as their implications on the analysis of BES.

2. Experimental

2.1. Electrochemical cell

The experiments were conducted in the cyclone flow DEMS cell which we described and characterized in detail earlier [17]. In brief, a working electrode made from PTFE-free carbon paper ($r = 0.5$ cm, $d = 0.2$ mm, Sigracet GDL 29AA) is mounted at the bottom of a cyclone flow cell that allows to establish defined mass transfer conditions at the electrode. A porous PTFE membrane (Pall Membranes, specified pore size 0.2 µm, thickness 60 µm) is pressed against the bottom of the working electrode separating the working electrode compartment from the mass spectrometer's vacuum system. Through this membrane, volatile substances evaporate into the vacuum where they are detected by a mass spectrometer (Pfeiffer QMG220 M1 quadropole mass spectrometer with secondary ion multiplier). Because of the direct contact of electrode and membrane, volatile species have a short diffusion path into the vacuum system. The ion current at a mass to charge ratio of $m/z = 44$ (CO_2) was recorded throughout the duration of the experiment. The complete setup is depicted in Schematic 1: A peristaltic pump recirculated the substrate solution from the cyclone cell to a glass vessel at 105 ml min^{-1}. This vessel was constantly purged with high purity nitrogen (99.999% Westfalen AG) in order to remove oxygen and to prevent the accumulation of volatile substances in the bulk solution. The volume of the flow cell is 77 ml, the total liquid volume including the recirculation vessel and the tubing is approximately 160 ml. FEP (fluorinated ethylene propylene) tubing was chosen as a compromise between chemical inertness and gas permeability.

The temperature in the recirculation vessel was kept at 36 °C, resulting in a temperature of 35 °C in the working electrode compartment. The calibration of the MS with CO_2 (99.999%, Westfalen AG) was done as described elsewhere [17].

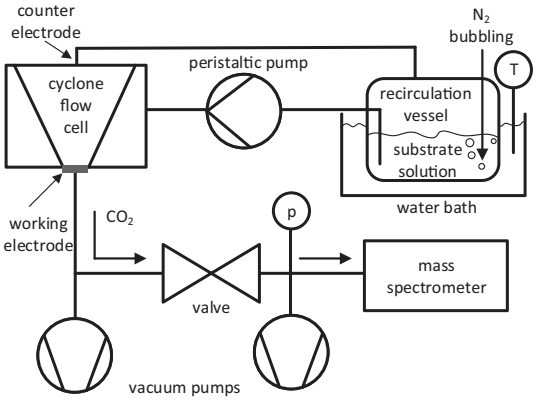
A saturated Silver/Silver-Chloride electrode was used as a reference electrode. The counter electrode was made from a platinum wire. It was placed at the outlet of the cyclone cell and separated from the cell volume by a glass frit.

2.2. Electrochemical measurements

A Gamry Reference 3000 potentiostat was used for electrochemical measurements. All potentials are reported with respect to the saturated Silver/Silver-Chloride Reference Electrode (Meinsberger Elektroden, Germany, +0.197 V vs. SHE). Chronoamperometry (CA) was recorded at a constant potential of 0.2 V, cyclic voltammograms (CV) were recorded from 0.2 V to −0.5 V at the scan rates given below. Two cycles were recorded, they showed only minor deviations. For all CVs, the second cycle is shown. Also potential steps from 0.2 V to −0.5 V and back to 0.2 V were applied.

2.3. Inoculum and media composition

The inoculum was a mixed culture biofilm scraped from an secondary biofilm electrode. No further analysis of the biofilm population was conducted. However, a community analysis was conducted in another recent study [18], where we used waste water from the same source (Wastewater treatment plant Steinhof, Braunschweig, Germany) as initial inoculum, and applied the same growth conditions and cultivation steps. There the biofilm consisted mainly of *Geobacter anodireducens*. Because of the very similar culturing



Schematic 1. Experimental setup including DEMS, cyclone flow cell, and substrate recirculation loop.