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Electroactive biofilms as sensor for volatile fatty acids: Cross sensitivity, response dynamics, latency and stability

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ABSTRACT

Microbial electrochemical sensors are an evolving technology platform based on electroactive microorganisms. Sensors based on anodic biofilms that oxidize organic substrates like acetate as living recognition element are promising for online monitoring of anaerobic digestion (AD), wastewater treatment as well as other processes. Essential for future engineering of microbial electrochemical sensors is the detailed characterization of its cross sensitivity as well as response behavior and latency. These parameters were examined on the example of a microbial electrochemical acetate sensor build in a 100 mL continuously stirred tank reactor. Furthermore, the ability of the sensor to recover after different periods (5–10 days) of shut down (i.e. open cell potential (OCP)) was studied. The sensor showed cross sensitivity towards propionate and butyrate that can be described as a baseline sum signal (0.040 ± 0.008 mA cm^{-2}) irrespective of the applied concentration. The sensor also revealed biphasic response behavior towards dynamic changes in acetate concentration shown to be strongly dependent on prior exposure to low acetate concentrations. This behavior is discussed by means of the metabolic state of the microbial cells forming the recognition element. Furthermore, the sensor revealed full recovery of activity after three consecutive OCP periods showing that sensor shutdown is not a limiting factor. The dynamic response behavior and the cross sensitivity of the sensor are discussed as challenges for engineering of future applications.

1. Introduction

Microbial electrochemical technologies (MET) are a promising technology platform of increasing interest. Primary MET are based on the ability of electroactive microorganisms to use electrodes as electron acceptors or donors [1,2]. This interaction can either be based on direct [3–6] or mediated [4,7] extracellular electron transfer. The microbial fuel cell (MFC), an electric power producing device, is the archetype of MET. In a MFC an anodic biofilm composed of electroactive microorganisms oxidizes organic compounds of, e.g. wastewater, to CO_2 and protons while transferring electrons to the electrode. Other applications comprise microbial electrochemical sensors. In microbial electrochemical sensors the permanent connection of the microbial metabolism and the electrode is used for generating an analytical signal, e.g. using the electric current as measure of cellular respiration, and hence, as measure of concentration of the microbial substrate. Most promising are sensors for the detection of volatile fatty acids (VFA) in biotechnological processes like wastewater treatment or anaerobic digestion (AD) for biogas production [8–13]. As there are no cost efficient sensors for monitoring VFA in AD plants available, this study focuses on the latter. During AD macromolecules are broken down to methane by four microbiological steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis [14]. Acetogenic bacteria that perform acetogenesis live in a strong syntrophic relationship with the methanogenic archaea, as they consume the H_2 produced by the acetogens whereby acetate production is made energetically feasible [15,16]. Process imbalances leading to the inhibition of methanogenesis result in an accumulation of VFA, most important acetate that in turn inhibits the involved microorganisms and can hence cause process breakdown [17–20]. Running anaerobic digestion at high or varying loading rates, e.g., for flexible biogas production as a tool of power grid management [21–23], requires monitoring VFA concentrations to avoid the risk of process acidification and breakdown. Here we will demonstrate that the required highly time resolved VFA measurement can be accom-

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plished by a microbial electrochemical sensor. The sensor is based on a naturally grown anodic *Geobacter* sp. dominated biofilm as recognition element, i.e. receptor that has been demonstrated to deliver an amperometric biosensor signal (oxidation current correlating to the concentration of acetate [13]).

Transferring such a technology concept from the laboratory scale to real application is challenging. One key challenge is related to different and especially changing environmental conditions affecting the involved electroactive microorganisms [24–28]. It was shown that changing salinity, pH, temperature as well as substrate concentrations influences the activity and stability of electroactive biofilms [29–33]. Fluctuations in the environmental conditions, however, contradict with the strong need for specificity, reproducibility and stability of the sensor. Thus, a stable or at least predictable performance while facing changing environmental conditions is a basic prerequisite.

For a better understanding of the response behavior of the sensor in the light of its proposed application for AD-monitoring, this work describes the following exemplary key challenges. When moving from an acetate based laboratory setup to a real application it is of particular interest to examine: I) the cross sensitivity towards different VFA, II) the response dynamics and latency (*i.e.*the delay in time of the measured sensor signal compared to a signal that occurs without any lag of time) caused by different acetate provision rates and incubation times at low acetate concentration, and III) the stability of the biofilm when shutting down the sensor, i.e. incubation of the electroactive biofilms at open cell potential (OCP), and its effect on sensor functionality.

2. Material and methods

All chemicals were of analytical or biochemical grade and purchased from Carl Roth GmbH (Germany) and ChemSolute (Th. Geyer GmbH & Co. KG, Germany). If not stated otherwise, all potentials provided in this article refer to the Ag/AgCl reference electrode (sat. KCl, 0.197 V vs. standard hydrogen electrode (SHE)).

2.1. General experimental setup and operation

All working and counter electrodes, with a geometric surface area of 3.34 cm^2 and 4.12 cm^2, respectively, were made from carbon rods with a diameter of 0.5 cm (CP-Handels-GmbH, Germany).

Each experiment was conducted in a flow cell setup consisting of a 100 mL continuously stirred tank reactor (CSTR) type vessel (n = 3) possessing three ports for electrodes (working, counter and reference electrode) as well as inlet and outlet for substrate feeding and removal, respectively [13]. The growth medium was based on 50 mM phosphate buffer supplemented with trace elements and vitamins as described elsewhere [34,35]. It differed in concentration and type of the analyte that also represents the sole carbon source (see specific descriptions of the single experiments below). The medium was provided with a peristaltic pump (TU 200, Medorex e.K., Germany) and air-tight Tygon tubes (E 3603, Saint – Gobain Performance Plastics, France) at different flow rates.

All experiments were conducted at 38 °C (Incubator Hood TH 15, Edmund Bühler GmbH, Germany) at constant stirrer speed of 250 rpm using a magnetic stirrer. The headspace of the 5 L storage tank was continuously flushed with nitrogen. For the experiments described below secondary, electroactive anodic biofilms [36] were grown in batch mode according to [35] using acetate as sole substrate. Once the first batch of substrate was depleted the experimental setup was switched to continuous flow mode [13]. Only for the examination of the biofilms cross sensitivity towards different VFA, an equimolar mixture of butyrate, propionate and acetate was used for the biofilm formation (see Section "VFA cross sensitivity and structure of the microbial community"). The term "secondary biofilms" implies the use of established primary biofilms from wastewater as inoculum for the biofilm formation and represents an established enrichment method for electroactive microorganisms [36].

Electrochemical measurements were always performed with a multipotentiostat (PARSTAT MC, AMETEK Inc., USA) using the following electrochemical techniques: chronoamperometry (CA) at 0.2 V and cyclic voltammetry (CV) with a scan rate of 1 mV s^{-1} and vertex potentials at 0.3 V and −0.5 V. In case of CV measurements, three cycles were performed with only the last cycle being used for data analysis. The use of CV measurements allows a detailed monitoring of the dynamic current response of the biofilms at different potential whereas CA measurement provides the current density at a fixed potential, i.e. electrochemical driving force.

2.2. VFA cross sensitivity and structure of the microbial community

For this set of experiments, the formation of the secondary electroactive anodic biofilms was conducted with a 5 mmol L^{-1} equimolar mixture of acetate, propionate and butyrate. To subsequently examine the cross sensitivity of the biofilms, acetate, propionate and butyrate were applied as single analyte as well as in equimolar mixture. Altogether, four different concentration steps (0.5, 1, 2 and 4 mmol L^{-1}) were each applied for ~25 h at a constant flow rate of 1 mL min^{-1} (hydraulic retention time: 100 min). CA measurements were performed for 23 h followed by CV. Biofilm samples were taken at the end of the experiments with a spatula and stored at −18 °C until microbial community analysis (T-RFLP and sequencing, see Section "Microbial community analysis") was performed.

2.3. Analysis of response behavior and latency

For this set of experiments, the biofilms were grown with 10 mmol L^{-1} acetate as sole substrate. To characterize the response behavior of the biofilms, two experimental settings were investigated: I) response to different acetate concentrations (2 and 3 mmol L^{-1}) pumped with varying flow rates and II) response to a stable final acetate concentration (4 mmol L^{-1}) pumped with a stable flow rate but varying initial incubation times at defined lower acetate concentrations (1 and 2 mmol L^{-1}).

For the first setting, 2 and 3 mmol L^{-1} acetate was added to the experimental setup with different flow rates (1, 2, 3, 4, or 5 mL min^{-1}) each. At the start of the experiment the growth medium in the experimental setup contained no acetate. The biofilms were always provided with 5 L of growth medium per experimental run. The flow rate in combination with the 5 L volume storage tank defined different experiment durations ranging from 5.5 h for 5 mL min^{-1} to 27.7 h for 1 mL min^{-1}. The biofilm response was measured with CA at 0.2 V.

For the second experimental setting, the flow rate remained stable at 3 mL min^{-1} and the sensor was set to a low initial acetate concentration (1 and 2 mmol L^{-1}) for two defined periods (3 and 24 h). The final acetate concentration was raised to 4 mmol L^{-1} acetate to keep the concentration difference stable compared to the fist experimental setup. For data acquisition, CA measurement at 0.2 V was performed. To detect any deviation from an ideal and latency free response of the biofilms, the current density (sensor signal) measured in the experiments was compared to the calculated acetate concentration for an ideal CSTR [13]. Furthermore, the t_{95} for the experimental setup, i.e. the time needed to reach 95 % of the maximum current density, and the sensor latency was determined (see Section "Data and statistical analysis").