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Start-up and Performance of an Activated Sludge Bioanode in Microbial Electrolysis Cells

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Microbial electrolysis cells (MECs) are a promising technology for wastewater treatment and simultaneous production of reduced value added compounds. The MEC performance strongly depends on the activity and efficiency of the anode and cathode (bio)catalysts. Here, the use of an activated sludge as inoculum of a MEC bioanode has been investigated. The bioanode was operated both in batch and continuous-flow regime and its performance evaluated in terms of substrate removal efficiency and current generation. The transient response of the bioanode to changes in the applied organic load rate (from 0 (g COD)/Ld to 1.08 (g COD)/Ld) was also assessed. Overall, the activated sludge turned out to be an excellent source of 'electro-active' bacteria and a good inoculum to start-up a MEC bioanode.

1. Introduction

Bioelectrochemical systems (BESs) are an innovative and attractive technology that combines bacterial metabolism and electrochemistry, for wastewater treatment. In a BES, 'electro-active' bacteria engage in extracellular electron transfer reactions with solid solid-state electrodes, which serve as electron acceptors or donors in their energy metabolism (Villano et al., 2010).
A promising type of BES is the microbial electrolysis cell (MEC), which exploits the catalytic activity of microorganisms to convert organic substrates, including those contained in wastewater, into valuable products such as fuels (i.e., hydrogen and methane) or chemicals (Nevin et al., 2010; Wagner et al., 2009). MECs typically consist of an anode, where the oxidation of the organic substrates takes place, and a cathode, where the generation of reduced value-added products takes place, with the two electrodic compartments being physically separated by an ion exchange membrane. MECs require the potential generated from microbial substrate oxidation at the anode to be boosted with an external power supply in order to drive the cathodic reaction at high rates.
Compared to conventional fermentation processes, the physical separation of the (anodic) electrons-releasing reaction from the (cathodic) electrons-consuming reaction, as well as the possibility to finely tune the potential of individual electrodes, provide MECs (and more in general BESs) with an unmatched versatility and selectivity and a high degree of control over the bioprocess. In spite of that, however, BESs still suffer from higher capital costs and complex scalability (Logan, 2010).
Moreover, essential to a BES is the presence, both at the anode and at the cathode, of cheap, robust, and effective catalysts which allow converting the organic substrates into the desired products at high rates and yields. *Geobacter* species are among the most studied 'electro-active' microorganisms for

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their unique extracellular electron transfer abilities and are frequently found to play a key role in substrate oxidation at BES anodes (especially those fed with volatile fatty acids) (Kiely et al., 2011). Recently, there is also an increasing interest in using microorganisms as cathode catalysts (so called biocathodes), having, at least in principle, several potential advantages over chemical catalysts such as the low-cost, the ability to self-regenerate, and the resistance to corrosion (Rosenbaum et al., 2011). In a previous study (Villano et al., 2011) we developed and characterized a fully biological methane-producing MEC, consisting of a *Geobacter sulfurreducens* bioanode and an hydrogenophilic methanogenic biocathode. The cell was initially operated at a controlled cathode potential of −0.850 V (vs. standard hydrogen electrode, SHE) in order to develop a methanogenic biofilm capable of reducing carbon dioxide to methane gas using abiotically produced hydrogen gas or directly the polarized electrode as electron donors. Subsequently, *G. sulfurreducens* was inoculated at the anode and the MEC was operated at a controlled anode potential of +0.500 V (vs. SHE), with acetate serving as electron donor. In spite of the repeated inoculations of *G. sulfurreducens* at the anode, the performance of the MEC was found to be primarily limited by the slow kinetics of acetate oxidation.
In order to overcome this limitation, in the present study we have explored the use of a more concentrated inoculum consisting of activated sludge from a municipal wastewater treatment plant as an alternative anode biocatalyst. Moreover, differently from the previous study the anode was operated in continuous-flow mode in order to prevent the possible accumulation of metabolic by-products (including H^+) which could hinder the biocatalytic activity.

2. Materials and methods

2.1 The bioelectrochemical reactor: experimental set-up and operation

The bioelectrochemical reactor employed in this study was a MEC consisting of two compartments (i.e., the anode and the cathode), each compartment having a total empty volume of 0.86 L and separated by a Nafion® 117 proton exchange membrane (PEM). Both the anode and cathode compartments were filled with graphite granules (diameter between 2 and 6 mm) giving a bed porosity of 0.48 and a specific electrodic surface area of about 1290 m^2/m^3. Prior to being used, both the PEM and the graphite granules were pretreated as described elsewhere (Villano et al., 2011). A graphite rod current collector and a KCl saturated Ag/AgCl reference electrode (+0.199 V vs. SHE) were also placed in each compartment, in order to guarantee the external electric connection and to measure or control the potential of each electrode, respectively. The reactor was operated by controlling the anode potential at +0.200 V (vs. SHE) by means of a potentiostat. Before being connected to the potentiostat, the anode compartment was inoculated with 0.2 L of activated sludge (having a volatile suspended solids concentration of 1.99 ± 0.06 g/L) from the "Roma-Nord" full-scale municipal treatment plant and then flushed with a N_2/CO_2 (70:30 v/v) gas mixture, in order to establish anaerobic conditions and allow microorganisms to use the electrode as the sole electron acceptor for the organic matter oxidation.
One day after the inoculation, the reactor was connected to the potentiostat and initially operated in batch mode with the liquid phase being continuously recirculated at a flow rate of 30 mL/min, and by carrying out two successive acetate additions (each one corresponding to a final concentration of about 0.40 (g COD)/L). Starting from day 6, the flow regime at the anode was switched from batch to continuous-flow mode. The inlet acetate concentration was 0.64 (g COD)/L and the flow rate 1.44 L/d, resulting in an hydraulic retention time (HRT) and an organic load rate (OLR) of 14.33 h and 1.08 (g COD)/Ld, respectively (both referred to the empty volume of the anode compartment). As for the cathode compartment, it was operated in batch mode with the liquid phase being continuously recirculated at a flow rate of 30 mL/min. Both the liquid phase at the cathode and the feeding solution at the anode contained anaerobic basal medium prepared as describes previously (Villano et al., 2011).

2.2 Tracer experiment

To characterize the hydrodynamic behavior of the anode compartment of the bioelectrochemical reactor, a tracer test was carried out. Before performing the test, the bioanode was fed with a solution containing only mineral medium, in order to let current drop down to the baseline value. For the tracer experiment, the inlet feed solution was modified by adding, to the usual acetate-containing medium, a

conservative tracer (KBr) at a concentration (C_0) of 0.5 g/L (as Br^-). The solution was fed into the anode in a step-input mode at the usual flow rate of 1.44 L/d. The response of the reactor to the step-input of the tracer was calculated from the non-dimensional F(*t*) curve, obtained by plotting $C(t)/C_0$ as a function of time (where C(*t*) is the Br^- concentration at time (*t)*). The time profile of electric current generated from the oxidation of the acetate contained in the modified feed solution was also acquired during the tracer test and qualitatively compared to the F(*t*) curve by plotting $i(t)/i_{MAX}$, where i(*t*) and i_{MAX} represent the current value at time *t* and the maximum value of current achieved at the applied OLR.

2.3 Analytical measurements and calculations

The inlet and outlet acetate concentration in the anode compartment was analyzed by gas chromatography on filtered (0.22 μm porosity) liquid samples. The Br^- tracer concentration in the outlet was analyzed by ion chromatography on filtered liquid samples collected at regular intervals.

The transferred cumulative electric charge (as milliequivalents) was calculated by integrating the current over time and dividing by the Faraday's constant (F = 96485 C/equivalent). The cumulative equivalents released from acetate oxidation were calculated from the measured amount of acetate consumed, considering the corresponding molar conversion factor of 8 milliequivalents/$mmol_{ACETATE}$.

3. Results and discussion

3.1 Start-up and performance of the bioanode

Figure 1A shows the time course of the electric current during the different operational phases of the bioanode. One day after the inoculation with activated sludge, the anode potential was potentiostatically controlled at an oxidizing value (+0.200 V vs. SHE) in order to enhance the bioanode start-up. On day 2, acetate was spiked to a final concentration of 0.40 (g COD)/L and the electric current immediately began to increase from 1.8 mA up to around 12 mA. The absence of a marked lag-phase suggests the presence in the inoculated activated sludge of 'electro-active' microorganisms able to rapidly switch from using oxygen, to using the insoluble graphite anode as terminal electron acceptor in their metabolism. The electric current remained nearly constant until acetate exhaustion, when it suddenly dropped down to around 2 mA. The current, however, rapidly resumed following a new acetate spike, reaching a value of around 20 mA, prior to dropping again in correspondence to a further substrate depletion (day 5). It is also interesting to note that the maximum values of electric current obtained with the activated sludge bioanode are substantially higher than those obtained, under very similar conditions, with a *Geobacter sulfurreducens* bioanode, (i.e., around 9 mA) (Villano et al., 2011). This finding points again to the presence of 'electro-active' bacteria in the activated sludge used as inoculum.

On day 6, the bioanode was switched from batch to a continuous-flow mode, with an influent acetate concentration of around 0.64 (g COD)/L. This resulted in a nearly exponential increase of the electric current which, after reaching a peak value of approximately 140 mA (on day 8), stabilized at around 100 mA. The remarkable increase of the electric current, and in turn of the acetate oxidation rate, upon switching the anode from batch to continuous-flow mode is a main finding of this study which indicates that the feeding regime has a major impact on the bioanode performance. More specifically, an acclimation and/or growth of 'electro-active' microorganisms seem to have occurred during this phase. On day 14, the anode started to be fed with a solution lacking of acetate, causing the current to sharply drop to approximately 8 mA. This value is substantially higher than that (2 mA) observed in the absence of acetate during the batch mode operation, indicating that some current continued to be generated from the endogenous microbial metabolism and/or from some residual acetate adsorbed onto the biofilm.

However, as soon as the OLR was restored to 1.08 (g COD)/Ld, the electric current promptly resumed to the previous value of around 100 mA, despite the two days of starvation. Finally, a further drop to around 8 mA was observed, on day 22, when the bioanode was again fed with a solution lacking acetate. These latter current variations, quicker than that previously observed upon switching from batch to continuous-flow regime, provide a further indication that 'electro-active' microorganisms capable to quickly adapt to changes in the influent acetate concentration had established in the

system. Moreover, the reproducible values of current reached in the presence of acetate, in spite of acetate feeding interruptions, indicate a highly reproducible performance of the bioanode.
Importantly, throughout the period of continuous-flow operation, the average acetate removal efficiency was around 92 % and the coulombic efficiency was approximately 85 %. This latter represents the acetate recovery into electric current and is calculated as the ratio between the electric charge transferred to the electrode and the acetate removed (both as milliequivalents), as reported in Figure 1B. The very high value of the coulombic efficiency indicates that only a little fraction of the removed acetate was diverted into new biomass and that no other metabolisms, such as acetoclastic methanogenesis occurred (Sleutels et al., 2011).
Finally, during the continuous-flow operational period at an applied OLR of 1.08 (g COD)/Ld, the cathode potential stabilized at values in the range between -0.9 V and -1.1 V (vs. SHE), with hydrogen and methane being the main cathodic products. A detailed characterization of the cathode performance was not carried out here, not being the main focus of the present study.

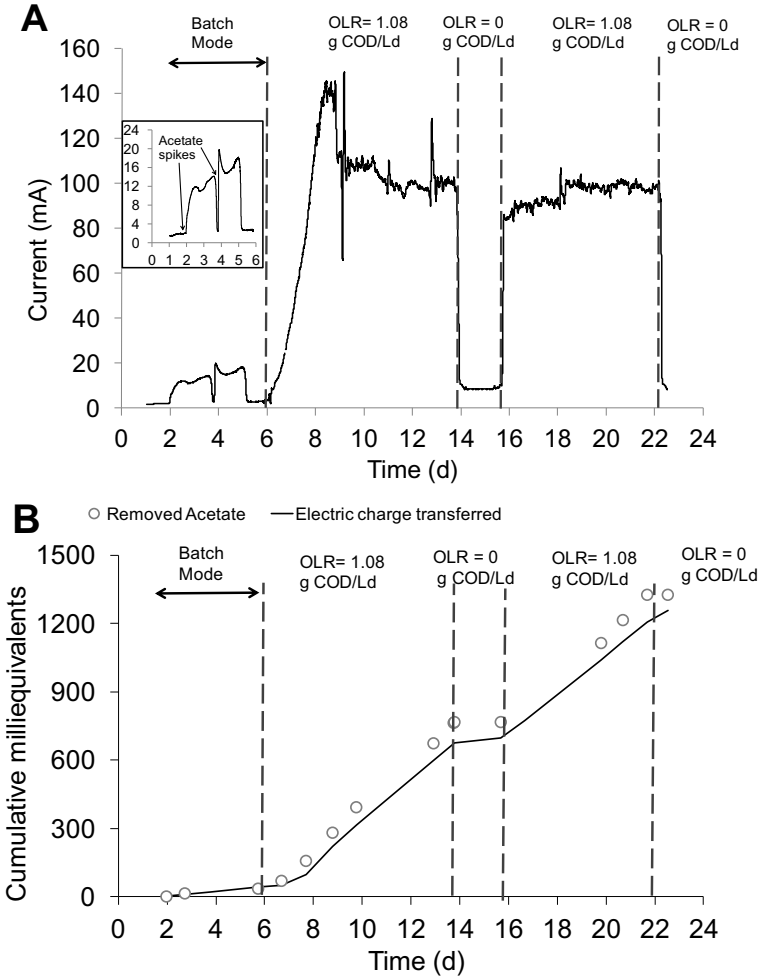

Figure 1: current generation (A) and cumulative amounts of removed acetate and electric charge transferred (B) during the different operational phases of the bioanode

3.2 Hydrodynamic characterization of the bioanode
During the last part of this study, a tracer experiment was carried out in order to characterize the hydrodynamic behavior of the bioanode. For this test, a conservative tracer (KBr) was added to the feed solution and was introduced into the bioanode in a step-input mode. Figure 2 shows the results of

the tracer test in terms of F(t) function and normalized electric current (i.e., i(t)/i_{MAX}). Noteworthy, the trend of both curves is very similar, with the normalized current even anticipating the F(t) function during the initial part of the test. This is a further confirmation of the ability of microorganisms to respond to the presence of acetate by quickly generating electrical current, without any significant lag phase. From the F(t) function, an actual HRT (i.e., based on void volume) of 7.38 h has been calculated (Levenspiel, 1972) and exploited to model the F(t) curve of an ideal CSTR (Figure 2). This latter well fitted the experimental F(t) curve, indicating that the hydrodynamic behavior of the bioanode resembled that of a completely stirred reactor. Finally, from the ratio between the actual HRT and the HRT referred to the total empty volume of the anode compartment, a bed porosity of 0.51 has been determined; which is in good agreement with that measured in a static experiment (i.e., 0.48).

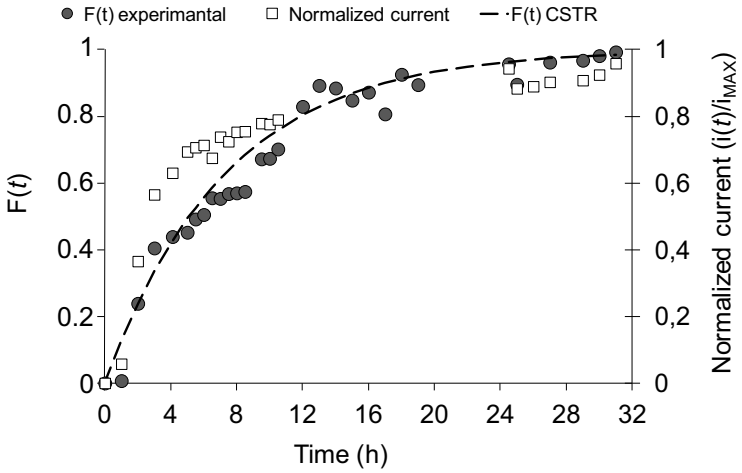

Figure 2: hydrodynamic response of the bioanode to tracer step-input as F(t) function and normalized current generation. The dashed curve represents the F(t) curve of an ideal CSTR having the same hydraulic retention time of the bioanode

4. Conclusions

This study pointed out that activated sludge is an excellent source of 'electro-active' microorganisms and can be used as inoculum for a rapid start-up of a MEC bioanode. When the bioanode was switched for batch to continuous-flow mode a remarkable increase in current generation was observed, suggesting that the feeding regime has a major impact on the bioanode performance.
Most likely, the continuous-flow mode favored the acclimation and/or growth of the 'electro-active' microorganisms.
Notably, at the applied OLR of 1.08 (g COD)/Ld, the substrate removal efficiency was over 90 % with a coulombic efficiency of 85 %. Finally, the activated sludge bioanode quickly responded, in terms of current generation, to changes in the acetate influent concentration, in a highly reproducible way.

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