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The performance of microbial anodes in municipal wastewater: Pre-grown multispecies biofilm vs. natural inocula

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HIGHLIGHTS

- Comparison of inoculation strategies for continuously operated microbial anodes.
- Inoculation with sludge or plain municipal wastewater yielded similar results.
- Also a pre-grown biofilm of exoelectrogens did not yield higher currents .
- 99% of the pre-grown biofilm was detached after 20 days of operation with wastewater.

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ABSTRACT

In this study, different inoculation strategies for continuously operated microbial anodes are analyzed and compared. After 20 days of operation with municipal wastewater anodes pre-incubated with a biofilm of the exoelectrogenic species Geobacter and Shewanella showed current densities of (65 ± 8) $\mu A/cm^2$. This is comparable to the current densities of non-inoculated anodes and anodes inoculated with sewage sludge. Analysis of the barcoded pre-grown multispecies biofilms reveal that 99% of the original biofilm was detached after 20 days of operation with municipal wastewater. This is in contrast to previous experiments where a pre-grown biofilm of exoelectrogens was operated in batch mode. To implement pre-grown biofilms in continuous systems it will thus be necessary to reveal a window of process parameters in which typical exoelectrogenic microorganisms including model organisms can be kept and/or enriched on anodes.

1. Introduction

The established treatment process for domestic wastewater involves active aeration to achieve oxidation of the organic carbon fraction to CO_2 by aerobic bacteria, which consumes considerable amounts of electricity. To aerate domestic wastewater approx. 18–26 kWh per year and inhabitant can be estimated, from which only approximately 35% of the overall oxygen demand are required for the elimination of ammonia (by a nitrification/denitrification step) (Sperling, 2007).

Microbial fuel cells are considered to be a promising alternative to reduce the energy demand of wastewater treatment since they enable elimination of organic carbon while generating useful electricity from its chemical energy content (Oh et al., 2010).

Calculations based on a 45 L pilot microbial fuel cell operated with municipal wastewater have shown that an electricity production of 2.91 kW per year and inhabitant can be achieved with the microbial fuel cell. Overall, total energy savings of 23% can be expected, including the savings for aeration and sludge treatment as well as the reduced biogas energy production (Hiegemann et al., 2016).

Key components of microbial fuel cells are the electroactive bacteria that oxidize organic matter and transfer the respiratory electrons to the anode of the fuel cell, which acts as a solid phase electron acceptor. From the anode, the electrons flow as an electrical current through an external load circuit to the cathode where oxygen is reduced. Commonly, microbial fuel cell anodes intended for practical application in wastewater treatment are inoculated

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e.g. with sludge from a conventional treatment plant or an acclimated consortium of operating fuel cells (Wang et al., 2010).

In literature, a number of studies using fed-batch systems and synthetic media containing acetate or glucose (Liu et al., 2008; Jiang et al., 2010; Vázquez-Larios et al., 2011; Yu et al., 2014) report that in the most cases, inoculation highly affects the performance. It is shown, that specialized exoelectrogenic consortia or single strains, like *Geobacter sulfurreducens* (Jiang et al., 2010) or a sulphate reducing inoculum (Vázquez-Larios et al., 2011) show better performances than wastewater and soil or aerobic sludge and a methanogenic consortium, respectively. (Yu et al., 2014) in contrast show, that inoculating microbial fuel cells with activated sludge or anaerobic sludge do not lead to significantly different power outputs.

Only few studies are available that compare different inoculation strategies in realistic environments with real and unsterile wastewater. (Dolch et al., 2016) could show that a model biofilm composed of the exoelectrogenic model organisms *Shewanella oneidensis*, *G. sulfurreducens* and *G. metallireducens* shows surprising resilience even in non-axenic systems. More than 50% of the anode community was comprised by organisms of the initial inoculum even after 14 days of operation. Still, this and most other studies analyze batch or fed-batch systems (Liu and Li, 2007; Mathuriya, 2013). Consequently, the continuous outflow of slow growing organisms is not considered. The only study in a continuously fed reactor is (Ismail and Jaeel, 2013), but even here, the dimensions are chosen so that the hydraulic retention time results in 383 h (ca. 16 days, calculation based on the reactor dimensions indicated in the paper).

The aim of the present study was to analyze the impact of different inoculation strategies on bioelectrochemical systems that were continuously fed with domestic wastewater as carbon and energy source at a realistic hydraulic retention time.

Our results revealed that inoculation strategies have under the here described conditions–if at all–only a minor impact on the performance of the bioelectrochemical systems.

2. Materials and methods

2.1. Experiment design

An overview of the different inoculation strategies investigated in this work is given in Fig. 1. In a first run of experiments, a multispecies model biofilm with *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Shewanella oneidensis* was pre-incubated on the anode surface under anoxic conditions with carbonate buffered medium containing lactate (12.5 mM) and propionate (5 mM). After one week of operation, the grown multispecies biofilm was analyzed with qPCR and FISH to reveal its composition.

In a second run of experiments the inflow was changed to wastewater after one week of preincubation with the model biofilm community. As a control, parallel experiments were conducted without preincubation with exoelectrogenic model organisms.

In run 3 (see Fig. 1) reactors inoculated with sludge were compared with non-inoculated reactors. To inoculate the anodes (anode chamber volume: 25 ml) 1 ml of sludge was used. The inoculation sludge was a mixture of activated sludge and anaerobic sludge in the volume ratio 1:4 from the same municipal treatment plant. After inoculation, the cells were run without flow for 24 h before starting the pump.

The inflow in run 2 and 3 was real municipal wastewater of the "Verbandskläranlage Untere Elz" in Teningen, sampled after the sand trap. The wastewater was collected weekly and stored at 4 °C (the different batches are marked in the results). The wastewater showed very low COD concentrations with a maximum of 97.5 mg/l COD during the time the experiments were carried out, due to dilution with rain water. To achieve a municipal wastewater with higher carbon content, the water was complemented with 0.323 g/l peptone (from soybean, Carl Roth, Germany) and 0.226 g/l meat extract (Carl Roth, Germany) to COD concentrations of (715 ± 38) mg/l COD. The complementation was conducted right before connecting a new wastewater subbatch to the experimental setup.

2.2. Growth of a synthetic multispecies biofilms

Shewanella oneidensis MR-1, *Geobacter sulfurreducens* PCA and *Geobacter metallireducens* GS-15 were routinely cultured anaerobically at 30 °C in a medium that was developed according to (Dolch et al., 2014) as blueprint. All three strains were genetically modified to contain a genomic barcode for qPCR based quantification (Dolch et al., 2016). The growth medium contained 0.42 g/l KH_2PO_4, 0.22 g/l K_2HPO_4, 0.2 g/l NH_4Cl, 0.38 g/l KCl, 0.36 g/l NaCl, 0.21 g/l $MgCl_2$ $6H_2O$, 1.8 g/l $NaHCO_3$, 0.5 g/l Na_2CO_3, 60 mg/l $CaCl_2$ $2H_2O$, 2 g/l casitone, and 1.0 ml/l of selenite-tungstate solution (0.5 g/l NaOH, 3 mg/l Na_2SeO_3, 4 mg/l Na_2WO_4 $2H_2O$). The medium was further supplemented with 10 ml/l NB trace mineral solution (Coppi et al., 2001), 10 ml/l vitamin solution (German Type Culture Collection, DSMZ, media 141), 0.2 mM sodium ascorbate, 1.0 mM cysteine, 0.2% (w/v) yeast extract and 50 mM ferric citrate as electron acceptor. Sodium lactate (12.5 mM), sodium acetate (6.25 mM) and sodium propionate (5 mM) were used as electron donors. Medium pH was adjusted to 7.2. The optical density during anaerobic growth on ferric citrate was measured at a wavelength of 655 nm. The addition of ferric citrate was omitted in the bioelectrochemical cell experiments. Here, the working electrode served as sole electron acceptor.

2.3. Continuous flow reactors

The experiments were conducted in two chambered microbial fuel cells made from polycarbonate as described elsewhere (Dolch et al., 2014). Both, anode and cathode compartment have a volume of ca. 45 ml each and were filled with 25 ml. A proton exchange membrane (Fumapem F-950, 50 μm, FumaTech, Germany) was used to separate the anode compartment from the counter electrode as well as the reference electrode compartment. Activated carbon cloth (CTex13; MAST Carbon, UK) with a geometric size of 2.25 cm^2 exposed to the anolyte and catholyte was used as material for the working and counter electrode. The electrodes were connected to a potentiostat (1470E, Solartron Analytical, Farnborough, UK) using platinum wires (0.1 mm; Chempur, Germany).

The synthetic medium was stored in a sterile container without cooling while wastewater was held at 4 °C. In both cases the storage tank was continuously sparged with nitrogen to ensure anaerobic conditions. The container was connected in parallel to the inflow of all anode chambers using viton tubings (LEZ-VIT 70, Lézaud, Germany). The anode compartments were fed using a peristaltic pump (Reglo Digital, Ismatec, Germany) with Fluran tubes (HCA, F-5500-A, Ismatec, Germany) with an inner diameter of 0.51 mm. The HRT was between 3 and 4 h (flow rate of 0.22–0.17 ml/min). Before introduction into the anode chamber the feed flow was acclimated to 30 °C.

All parallel experiments were fed from the same tank with wastewater to eliminate the effect of fluctuations in the wastewater composition. The wastewater was renewed weekly. The cathode compartment was filled with medium once and not exchanged during the operation.