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Toxicity Response of Electroactive Microbial Biofilms—A Decisive Feature for Potential Biosensor and Power Source Applications

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Herein, we investigate the effect of exemplary biocides on wastewater-derived electroactive microbial biofilms. We show that the current response of these biofilms as a measure of their bioelectrocatalytic performance is not affected by the presence of antimicrobial compounds such as the sulfonamide-based antibiotics sulfamethaxozole and sulfadiazin, the disinfectant chloramine B and the metal ions Cu^{2+}, Ag^{+}, Pb^{2+}, and Hg^{2+}, even at concentrations an order of magnitude higher than average concentrations of these compounds in wastewaters. In contrast to the electroactive biofilms, planktonic cells of the same origin, studied in a mediator-based microbial fuel cell, are massively affected by the presence of the antimicrobial agents.

Introduction

Microbial bioelectrochemical systems (BES) such as microbial fuel cells are on the threshold from the lab bench to technological realization. This promising development is the result of the recent establishment of appealing application goals, above all the implementation of BES technology in wastewater treatment[1–4]—even combined with the production of chemicals like hydrogen.[5–7] The key elements of the great majority of recent microbial BES are electroactive microbial biofilms, which are responsible for the anodic bioelectrocatalytic oxidation of the substrate (Figure 1).[8–11]

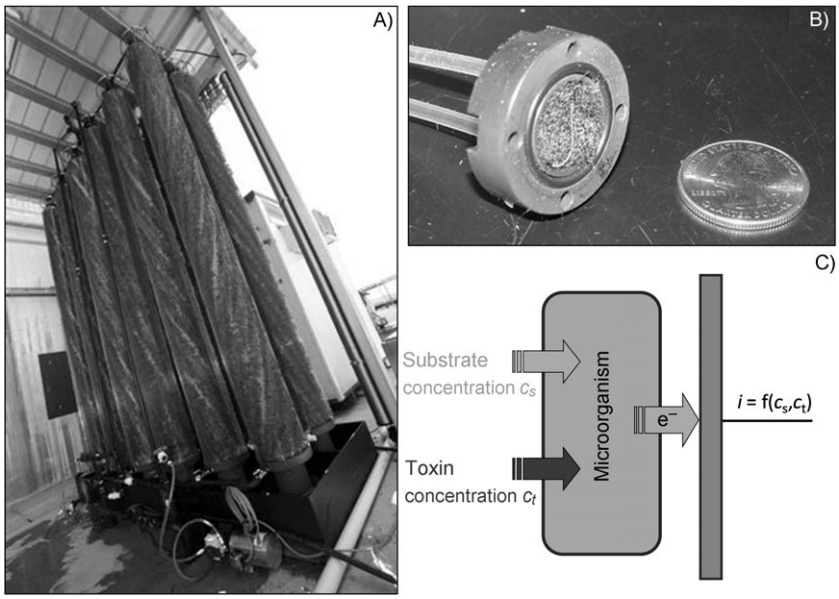

Figure 1. A) Prototype of an up-scaled MFC for wastewater treatment (photograph courtesy of Jeremy Patten, University of Queensland). B) Miniaturized MFC, suitable for online biosensing applications.[12] C) Common, underlying MFC anode elements: microbial electrocatalysis and extracellular electron transfer.

The robustness and longevity of these biofilms will be one of the main factors determining the success of microbial BES. As we have recently shown, anodic electroactive microbial biofilms are astonishingly robust against temperature variation.[13] A key question that is, however, largely unaddressed, is the susceptibility of these systems against toxins. Thus, a typical hazard for a wastewater-treating BES would be an irregular contamination of the incoming wastewater stream by antimicrobial agents such as heavy metals, disinfectants or antibiotics, from any municipal, agricultural or industrial source. A deterioration of the BES performance would lead to a decreasing treatment capacity. Consequently, a BES should ideally not be affected by incoming con-

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taminations. In the worst case, irreversible losses may occur that would put the BES out of operation for a long period of time.

There is however, a different view on this matter—the view from the biosensing perspective: Microbial fuel cells (MFCs) have often been proposed as biosensors for, for example, biochemical oxygen demand (BOD)[14–16] or toxicity monitoring.[15,17,18] For this application, the response of the MFC should be a function of the respective analyte. In the case of an amperometric toxicity sensor, the current should be a function of the presence and the concentration of an antimicrobial agent.

Herein, we have examined the effect of exemplary antimicrobial agents on the electrocatalytic performance of wastewater-derived, mixed-culture electroactive microbial biofilms. In relation to the application of MFC technology in wastewater treatment and as sensors, commonly found biocides and disinfectants were selected for this study. These include antibiotics, heavy metals and a disinfectant. The behavior of the wastewater-derived electroactive biofilms was compared to that of planktonic cells of the same origin, studied in a mediator-based MFC.

Experimental Section

Growth Medium and Microbial Inoculum: The bacterial growth medium contained NH_4Cl (0.31 g L^{-1}), KCl (0.13 g L^{-1}), $NaH_2PO_4{\cdot}H_2O$ (2.69 g L^{-1}), Na_2HPO_4 (4.33 g L^{-1}), trace metal (12.5 mL) and vitamin (12.5 mL) solutions.[19,20] Acetate (10 mM) served as substrate throughout this study. The pH of the medium was adjusted to 6.8. The growth medium was purged with nitrogen for $\geq$20 min before using it to ensure anoxic conditions. The inoculum used was primary wastewater obtained from wastewater treatment plant Steinhof (Braunschweig).

Electrochemical Experimental Setup and Equipment: All experiments in this study were conducted under strictly anoxic conditions at room temperature, either as half-cell experiments under potentiostatic control (PGSTAT 30 Autolab systems, Ecochemie, Netherlands or EG & G Princeton Applied Research Model 263A, USA), or as fuel-cell experiments in assembled MFCs. The half-cell experiments were carried out using a conventional three-electrode arrangement. Unless stated otherwise, the working electrode was a polycrystalline graphite rod (CP Graphite GmbH, Germany) with a projected surface area of 8 cm^2. Similar graphite rods were used as counter electrodes, whereas the reference electrode was a Ag/AgCl electrode (sat. KCl, Sensortechnik Meinsberg, Germany, 0.195 V vs. standard hydrogen electrode, SHE). All potentials provided herein refer to this electrode.

The MFC was composed of two chambers (respective volume of 120 mL). A 100 mM $K_3Fe(CN)_6$ solution in 50 mM phosphate buffer (pH 7) was used as cathode solution replacing an oxygen cathode. A Fumasep cation-exchange membrane of 6 cm diameter was clamped between the anode and cathode compartments. Current and potential measurements were carried out using a data-acquisition system comprised of an Integra 2700 digital multimeter equipped with a 7700 multiplexer (Keithley Instruments, Inc., USA) and interfaced to a personal computer. For the recording of potential and current output, a resistance of 100 Ω was used as the external load. Alternatively, the current was monitored with a potentiostat.

Preparation of Electroactive Biofilms and Planktonic-Cell-Based MFCs: The formation of electroactive microbial biofilms was carried out under potentiostatic control. The procedure was followed as described by Liu et al.[21] Primary waste water (4 mL) was inoculated into the sealed electrochemical cell with substrate solution (120 mL) and a constant potential of 0.2 V was applied to the working electrode to facilitate the biofilm formation. The biofilm growth was monitored by measuring the bioelectrocatalytic oxidation current. The substrate (acetate) level was monitored by high-performance liquid chromatography (HPLC) analysis. The exhausted substrate solutions were replenished regularly. After acclimatization of the biofilm-covered electrode to a reproducible maximum bioelectrocatalytic current generation within at least three fed-batch cycles, the biofilm was employed for the biocide experiments.

For the study of suspended (planktonic) cells, an MFC setup with anthraquinone-2-sulfonate (500 μM) as a mediator was used. The substrate used was acetate (10 mM) and the other conditions (including growth medium and inoculum) were the same as those mentioned above. These mediator-based experiments were performed at a considerably shorter timescale (7 days maximum) to prevent the formation of electroactive biofilms.

Conduction of the Biocide Experiments: The biocide stock solutions were prepared in water (using additional 1% of methanol to solubilize sulfamethaxozole). The details on the used biocides and their concentration range are presented in Table 1. The concentrations of some of the used biocides for this study were orders of magnitude higher than the common concentrations in municipal wastewater and higher than the concentration inhibitory to the growth of bacteria in suspensions and mostly employed for disinfection purposes.[22]

The effect of biocides on the bioelectrocatalytic performance of electroactive microbial biofilms was studied as follows: Two plastic tanks (10 L each) served as reservoirs for the substrate and buffer solutions. The flow rates of both solutions were maintained at 0.42 mL min−1 using a peristaltic pump (Ismatec, Reglo Analog, Switzerland/Germany). After establishing a continuous current gen-

Table 1. Details of the different biocides and concentrations used in this study. The typical concentrations in wastewater and the toxicity concentrations are also shown.

Biocide	Concentrations used in this study	Concentration ranges in wastewaters	Toxicity/inhibitory/disinfection concentrations
sulfamethaxozole	0.05–1000 μg L^{-1}	few ng L^{-1}–8 μg L^{-1} [23]	2 μg L^{-1}–256 mg L^{-1} [24]
sulfadiazin	0.01–1000 μg L^{-1}	few ng L^{-1} to 5 μg L^{-1} [25]	21.7–57.8 mg L^{-1} [26]
chloramine B	0.16–3.96 mg L^{-1}	present as decomposition products	0.8–3.0 mg L^{-1};[22] 0.5–2.0 mg L^{-1} [27]
Cu^{2+}	0.01–6.0 mg L^{-1}	0.01–100 μg L^{-1} [28]	0.04–0.4 mg L^{-1};[22] 7.08 ± 0.352 μg L^{-1} [29]
Ag^{+}	0.02–1.0 mg L^{-1}	0.01–1.1 mg L^{-1} [30]	0.02–0.1 mg L^{-1};[22] 7.92 ± 0.667 μg L^{-1} [29]
Pb^{2+}	0.41–12.48 mg L^{-1}	0.0017–5 μg L^{-1};[28] 16–84 μg L^{-1} [31]	669 ± 95.7 μg L^{-1} [29]
Hg^{2+}	0.83–8.33 mg L^{-1}	0.08-3.5 ppm[28]	33.8 ± 1.99 μg L^{-1} [29]

eration with the biofilm for at least 12 h, the addition of individual biocides with stepwise increase in its concentration was executed. The continuous flow operation was stopped during the respective biocides exposure. Unless specific exposure times were known from literature, the exposure time of the biocides was fixed to 24 h, according to standard biological procedures.[22] Thus, the microbial systems were exposed to the sulfonamides, Pb^{2+} and Hg^{2+} for 24 h, to Ag^{+} for 10 h, to Cu^{2+} for 2 h and to chloramine B for a minimum of 10 min. The experiments were performed with three independent biofilms and each biocide concentration was applied at least two times.

The impact of biocides on suspended cells in the mediator-based setup was performed under batch conditions. Once the current of a batch experiment was stabilized, the biocides were added into the reactor with stepwise increases in concentration. The substrate (acetate) concentration in the reactor was analyzed to confirm that the current decrease was not due to substrate depletion by HPLC analysis as described before.[21]

Results and Discussion

Figure 2 illustrates, on the example of copper(II) ions and chloramine B, the effect of biocides on the electrocatalytic performance of wastewater-based electroactive microbial biofilms and of planktonic cells (also based on wastewater inoculum). The figure very clearly shows that the microbial biofilm was completely unaffected by the presence of both biocides. Even at concentrations of 6 mg L^{-1} (Cu^{2+}) and 4 mg L^{-1} (chloramine B), no effect was observed (data not shown). The planktonic cells (also based on the same primary wastewater inoculi), however, showed a strong decrease of the performance, even at lower biocide concentrations. These results are supported by metabolite analyses. These clearly showed that in presence of the biocides, the acetate degradation was not affected at electroactive biofilms, whereas the planktonic cells did not degrade the complete acetate.

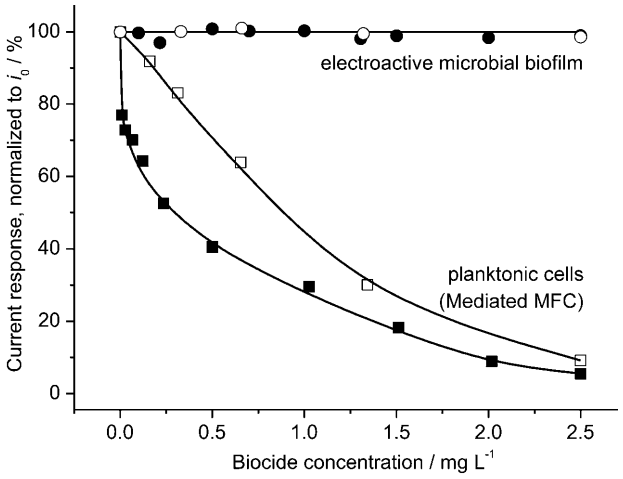

Figure 2. Effect of different concentrations of copper(II) (full symbols) and chloramine B (open symbols) on the electrocatalytic performance of wastewater-based electroactive microbial biofilms (circles) and of planktonic cells of the same origin (squares). In the latter case, anthraquinone-2-sulfonate (500 μM) served as redox mediator.

The biofilm results of Figure 2 apply to all the biocides and concentration ranges tested herein (see Table 1). In all experiments (data not shown), the effect of the biocides on the bioelectrocatalytic currents and MFC potentials was negligible and within the error of the experiments.

Although the extent of the robustness was astonishing to us, the principle effect is not surprising. Thus, microbial biofilms are generally acknowledged to possess a great resistivity against biocide attacks, which especially in medicine causes severe problems; see, for example, refs. [32] and [33]. In accordance with our results, this leads to the conclusion that also anaerobic, electroactive microbial biofilms are strongly resistant against biocides. From a toxicity-biosensing perspective, it thus seems more reasonable to use either suspended or immobilized cells (with artificially mediated electron transfer) as recognition elements rather than natural biofilms—as illustrated, for example, in refs. [34–36]. From a BOD-sensor viewpoint, on the other hand, these findings indicate a low cross-selectivity of potential biofilm-based BOD sensors against toxins.

The results of our study may appear contradictory to the findings of Kim and co-workers,[15] who observed antibiotic effects on electroactive biofilms using several toxins (heavy metal ions and organophosphorous compounds). This apparent contradiction, however, points out that many variables may influence the behavior of biofilms and much further research, including a broader screening of antibiotics is still necessary.

Conclusions

This study clearly shows that electroactive microbial biofilms show a high degree of resistance against the presence of antimicrobial agents. No remarkable deterioration of the bioelectrocatalytic performance was observed in any of the experiments. Only very drastic conditions, as the presence of hydrogen peroxide, lead to the inactivation of the biofilms. These results are highly promising for an application of microbial fuel cells or electrolysis cells as a water-treatment technology. Here, the results indicate that BES may possess a lower susceptibility to toxins than anaerobic digestion.[37] This assumption, however, needs to be confirmed in real wastewater experiments.

On the other hand, the results also mean that it is unlikely that electroactive biofilms are suitable as sensing elements for microbial toxicity sensors. For this purpose, either planktonic cells or artificially immobilized cells are more promising.

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