The microbial electrochemical Peltier heat: an energetic burden and engineering chance for primary microbial electrochemical technologies

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Bioelectrocalorimetry allows assessing the heat (enthalpy) balance and revealing the microbial electrochemical Peltier heat of biofilm electrodes. This effect accounts for a heat formation of $27 \pm 6 \text{kJ mol}^{-1}$ of electrons transferred and represents a significant energy loss for primary microbial electrochemical technologies.

Primary microbial electrochemical technologies (METs) are based on electroactive microorganisms capable of performing extracellular electron transfer (EET) to electrodes.\(^1\) Harnessing electroactive microorganisms in primary METs holds great promises and offers a plethora of applications, including electricity production from wastewater, water desalination and production of chemicals.\(^2\)–\(^4\) Research on METs can be divided into two main approaches: bioelectrochemical engineering focusing on the improvement of components like the electrode material and geometry and the entire bioelectrochemical system (BES).\(^5\)\(^,\)\(^6\) The second research scope is devoted to the electroactive microorganisms and their different EET mechanisms, including mediated EET as well as direct EET. Electroactive microorganisms are studied using microbial electrodes, \textit{i.e.} electron conductors interacting with microorganisms, and more particularly biofilm electrodes.\(^7\) Biofilm electrodes are composed of the electrode material and attached microorganisms embedded in their extracellular matrix performing direct EET.\(^8\) However, the incompletely elucidated mechanisms behind EET, not fully-developed primary MET devices and the associated low energy efficiencies have prevented the wide-ranging application of METs so far.

Abiotic electrochemical systems, \textit{e.g.} fuel cells and electrolytic cells, had to overcome similar efficiency and engineering hurdles. One major energy loss in electrochemical systems is the electrochemical Peltier heat (\textit{II}) first described in the 19th century by Mill and extensively studied, so far only on abiotic redox systems, by Lange.\(^9\)\(^,\)\(^10\) A theoretical description was derived later by Vetter.\(^11\) It accounts for an energy loss of up to 20\% for batteries, fuel cells and electrolysis cells.\(^12\)–\(^14\) The electrochemical Peltier heat is defined as the reversible heat effect of an electrochemical reaction at the electrolyte/electrode interface. It can be clearly distinguished from irreversible heat fluxes like overpotential heating and Joule heating of the electrolyte.\(^15\) Two effects contribute to the \textit{II}. The first is the entropy change of the electron transfer reaction. Whereas an electron possesses a restricted probability of presence in the orbitals of a soluble molecule, it is completely delocalized in a certain Fermi level of the solid electron conductor. As a consequence, \textit{e.g.} during an anodic oxidation, the entropy of the electron increases when it is transferred to the electrode, which is compensated by heat release. Second, the movement of ions in solution due to migration induced by the electrode reaction, \textit{i.e.} Eastman entropy,\(^16\) leads to a reversible heat flux. Therefore, the electrochemical Peltier heat is determined by the composition of the electrochemical half-cell, \textit{i.e.} electrode material, redox species and electrolyte.\(^16\) Depending on the direction of electron transfer the electrochemical Peltier...
heat can be exo- or endothermic and by definition, it is considered to be positive for an exothermic anodic reaction.

Here we present the first description of the microbial electrochemical Peltier heat ($H_{\text{Peltier}}$) representing an energy barrier for electroactive microorganisms performing EET at electrodes. The underlying analysis is based on the development of a bioelectrocalorimeter, i.e. a device that allows the monitoring of heat and electron fluxes of biofilm electrodes in situ, and the subsequent assessment of the enthalpy (or heat) balance of microbial direct EET. The followed approach is widely used for describing microbial systems but has not been applied for electroactive microorganisms, yet.\textsuperscript{17} Fig. 1 shows a simplified sketch of the bioelectrocalorimeter (see ESI,\textsuperscript{†} 3.1, for details) and summarizes the different heat fluxes. In the bioelectrocalorimeter the electrode compartments are spatially separated, while still being ionically connected. The heat flux at the working electrode can thus be monitored, while the heat flux at the counter electrode does not contribute to the calorimetric signal (ESI,\textsuperscript{†} 2.3). Due to the partial positioning of the counter electrode compartment within the reactor vessel (Fig. 1), it was necessary to identify the extent of the ionic path that contributed to the Joule heating, i.e. heat flux due to ion migration in solution, measured by the bioelectrocalorimeter. Therefore, an additional reference electrode was introduced to the counter electrode compartment and its position was calibrated during chronoamperometry experiments (ESI,\textsuperscript{†} 2.4). Furthermore, electrochemical experiments with the inorganic model redox pair ferrocyanide/ ferricyanide were performed to validate the experimental setup. The obtained values of $H_{\text{Oxidation}} = -47 \pm 11$ kJ mol$^{-1}$ and $H_{\text{Reduction}} = 46 \pm 12$ kJ mol$^{-1}$ for the electrochemical Peltier heat of $K_d[\text{Fe(CN)}_6]^{3-}/K_d[\text{Fe(CN)}_6]^{-}$ at a platinum electrode were in accordance with the literature value of $H_{\text{Ox/Red}} = \pm 45$ kJ mol$^{-1}$ (see Fig. 2 and the ESI,\textsuperscript{†} 2.2, for more details) and clearly validate the experimental setup.

Based on an established procedure, secondary biofilms were cultivated in fed-batch mode at the working electrode of the bioelectrocalorimeter at a potential ($E_{\text{WE}}$) of 0.37 $\pm$ 0.10 V (reported like all potentials in this article vs. standard hydrogen electrode (SHE)) with 5 mmol L$^{-1}$ acetate serving as the substrate. As confirmed by genetic analysis and cyclic voltammetry, the biofilm anodes were dominated by Geobacter spec. and the formal potential of the EET-site was $E^0_{\text{EET}} = -0.14 \pm 0.01$ V (ESI,\textsuperscript{†} 5.1 and 5.2). Fig. 3 shows the geometric current density ($j$) and the heat production rate ($P_{\text{Heat}}$), with a negative value corresponding to an exothermic reaction and thus heat production, of a first batch cycle. After a lag phase of about 60 h the microbial oxidization of the substrate acetate and subsequent transfer of electrons to the anode by direct EET can be monitored as an electric current flow. The lag phase and the quasi-exponential increase of heat and current production are almost identical. Afterwards a delay between $j$ and $P_{\text{Heat}}$ becomes apparent. Probably, this can be assigned to the high rates of the endothermic acetate oxidation (ESI,\textsuperscript{†} 4.4) partly compensating for the exothermic effects. For a detailed analysis of the contribution of different heat sinks and sources, the following workflow was applied. First, mature Geobacter spec. anodes, characterised by a reproducible performance (ESI,\textsuperscript{†} 5.3), were cultivated in the bioelectrocalorimeter. In order to analyze steady state biofilms, at least four growth cycles were conducted to yield mature, i.e. steady state, biofilms. Subsequently, these steady state biofilms, characterized by minimized anabolic heat flux ($P_{\text{Ana}} = 0, E_{\text{SI}, \text{†} 4.5}$), were analyzed in detail. During the peak current production of a growth cycle of a mature biofilm, i.e. assuming only minimal metabolic changes, redox titrations were performed. Therefore, different $E_{\text{WE}}$ values were applied stepwise and the current and heat responses were recorded until steady state at each potential. Consecutive potential steps were interrupted by open circuit, i.e. $j = 0$, to reach the baseline level of heat production rate. Fig. 4a shows the example of a redox titration from $E_{\text{WE}} = -0.15$ V to $+0.04$ V. With more positive potentials, i.e. higher driving force for EET and coupled catabolism, both the current density and heat production rate increased. The current density reached a saturation level of 0.38 $\pm$ 0.01 mA cm$^{-2}$ at $E_{\text{WE}} = -0.03$ V, indicating that EET had already taken place at the maximum rate at this anode potential and other processes like counter ion transport or acetate metabolism may limit further increase in current production. In contrast, the heat production rate continuously increased with more positive potentials.
This further increase in heat production was attributed to overpotential heating due to the higher applied potentials and was not of biological origin. Fig. 4B shows an analysis of the different heat fluxes contributing to the measured heat production rate on the example at $E_{\text{WE}} = 0.12$ V. In addition to the measured heat production rate ($P_{\text{Heat}}$), the heat flux of Joule heating ($P_{\text{Joule}}$), overpotential heating ($P_{\text{Over}}$) and catabolic endothermic acetate oxidation ($P_{\text{Cat}}$) were calculated (see the ESI, † 4.2–4.4) with the latter three being summed up in $P_{\text{Theory}}$ (ESI, † 4.6). A comparison of $P_{\text{Theory}}$ and $P_{\text{Heat}}$ clearly shows a gap of ca. 2 mW ($\approx 40\%$ from $P_{\text{Heat}}$) during steady state (Fig. 4B). A detailed analysis of
all performed redox titrations of different biological and technical replicates (see ESI† 4.8, for a statistical analysis of the performed experiments) confirmed this discrepancy. This unconsidered heat flux is the microbial electrochemical Peltier heat ($\Pi_{\text{m}}$). In order to reveal the flux of the microbial electrochemical Peltier heat, an established and above-introduced algorithm was followed (Fig. 2). To adapt this procedure for the determination of the biological $\Pi_{\text{m}}$, $P_{\text{Cat}}$ was subtracted from $P_{\text{Heat}}$ to gain $P_{\text{Electro; summarizing all electrochemical heat fluxes (eqn (1)):

$$P_{\text{Electro}} = [P_{\text{Heat}} - P_{\text{Cat}}] = [P_{\text{Joule;}} + [P_{\text{Over}}] + [P_{\text{neePb}}]$$

As $P_{\text{Joule;}}$, $P_{\text{Over}}$ and $P_{\text{neePb}}$ were exothermic processes they possess negative values. Further, as only oxidation takes place at biofilm anodes only positive currents and overpotentials are needed to be considered in the following equations:

$$P_{\text{Electro}} = -I^2R_{\text{Joule}} - \eta I - \frac{\Pi_{\text{m}}}{2F}$$

Transforming eqn (2) by dividing by the current $I$, a linear relationship can be obtained:

$$\frac{P_{\text{Electro}}}{I} + \eta = -IR_{\text{Joule}} - \frac{\Pi_{\text{m}}}{2F}$$

In this linear equation the electrolyte resistance ($R_{\text{Joule}}$) represents the slope, and the intercept allows the calculation of $\Pi_{\text{m}}$, using the Faraday constant ($F$) and the number of transferred electrons ($z$) as described in Fig. 2 and the ESI† 2.2.2. An analysis of 34 potential steps ($E_{\text{WEC}} \leq -0.05 \text{V}$) from 13 redox titrations derived from 5 independent biological replicates is shown in Fig. 5. A molar microbial electrochemical Peltier heat, $\Pi_{\text{m}}$, i.e. the microbial electrochemical Peltier heat per transferred electron, of $27 \pm 6 \text{kJ mol}^{-1}$ for Geobacter spec. (i.e. their EET cytochromes) at a graphite electrode in the cultivation medium was derived. This value is in the same order of magnitude as the $\Pi$ of different inorganic redox systems.20

As redox reactions at an electrolyte/electrode interface are accompanied by isobaric reaction conditions, the enthalpy and entropy of a reaction of an electron transfer to a solid electron acceptor are linked and related to the electrochemical Peltier heat (ESI† 2.2.1). As mentioned for fuel and electrolytic cells, the electrochemical Peltier heat represents an energy barrier for transferring electrons across the electrode interface and thus lowers the efficiency of converting chemical into electric energy and vice versa. Biofilm electrodes in primary METs should be influenced in a similar manner. The $\Pi_{\text{m}}$ represents an energetic hurdle that needs an energy investment, being measured as heat flux. The estimated value of $\Pi_{\text{m}} = 27 \pm 6 \text{kJ mol}^{-1}$ of electrons is in the range of the standard Gibbs energy of the hydrolysis of one mole ATP to ADP and therefore indicates that the electroactive bacteria need one molecule of ATP for transferring one electron at the cytochrome/electrode interface for the present cultivation conditions (i.e. graphite electrode, ion composition of the medium). This amount of energy is composed of the entropy change of the terminal redox reaction of the electron and the Eastman entropy, i.e. heat flux due to ion migration. It is noteworthy that the Eastman entropy might also include the contribution of the H$^+$-tunnelling associated with the electron transfer across cellular membranes (redox-Bohr effect).21

The microbial electrochemical Peltier heat affects virtually every microorganism that performs EET, that are, not only biofilm electrodes in primary METs but also biogeochemical redox cycling, degradation of humic substances and biocorrosion.22–24 Foremost, there is a clear need for engineering electrodes in primary METs for improved energetic efficiency. This research should focus on: (i) engineering of electrode materials towards improved properties for EET reactions, i.e. lower microbial electrochemical Peltier heat. Promising materials are functionalized carbon based materials, but also metals.25,26 Baudler et al. recently reported that electroactive biofilms are thicker and produce higher current at a copper electrode compared to graphite electrodes, indicating a higher metabolic energy gain.5 Here one may speculate that this could be a consequence of a lower microbial electrochemical Peltier heat effect. (ii) Engineering new/improved electroactive bacteria with a different set of terminal direct EET proteins, as these also affect the $\Pi_{\text{m}}$.27 (iii) The electrolyte can also be optimized for minimizing the Eastman entropy as a considerable share of the $\Pi_{\text{m}}$. Certainly, here is a conceivable trade-off because the salinity of the solution also affects the conductivity, i.e. the performance, of the system and the viability of the microorganisms.28,29

A look at nature is advisable as strategies have emerged circumventing the energetic consequences of the microbial electrochemical Peltier heat. For instance $\Pi_{\text{m}}$ does not play a role if the microorganisms exploit soluble electron acceptors (i.e. mediators) because no direct cytochrome/electrode contact is present. However, utilizing mediators in METs for avoiding the $\Pi_{\text{m}}$ effect would raise another problem on the system level.
For the recycling of the mediator there is still energy needed for overcoming the $\Pi_m$ that has to be supplied by the anode, i.e. higher anodic overpotentials. When considering biofilms based on long-range EET solely the cells being in direct contact to the electrode are at a disadvantage by $\Pi_m$ as only for these the state (and entropy) of the electron changes. As a consequence, the energy usable for anabolic processes of the bacteria in the first cell layer of the biofilm is lower since a certain share of the energy from the catabolism has to be invested for overcoming the $\Pi_m$. Another pathway for removing electrons from the metabolism and circumventing the energetic barrier related to $\Pi_m$ might be interspecies electron transfer a potential new level of trophic interaction. Here, electrons are transferred from one microorganism to another. However, when the final receiving partner does perform direct EET to its terminal acceptor, the burden is only passed on. For an overall energetic advantage, the final electron transfer to a terminal acceptor has to be, e.g., based on mediated EET or take place intracellularly.

Conclusions

Based on the development of bioelectrocalorimetry the microbial electrochemical Peltier heat is revealed for the first time. A certain energy share from catabolism is needed to overcome this energetic hurdle at biofilm electrodes. As this energy share is transformed to heat, it represents an energetic loss for primary METs. An established algorithm for inorganic electrochemical systems was followed and adapted for biological extracellular electron transfer processes, hence allowing the quantification of this effect. The derived value for Geobacter spec. anodes of $\Pi_m = 27 \pm 6$ kJ mol$^{-1}$ of electrons is in the order of the energy derived from the hydrolysis of one mole ATP to ADP and thus represents a severe energetic drawback for electroactive microorganisms and microbial electrochemical technologies.

Clearly, further studies are needed for elucidating how, e.g., different electrode materials or solution ionic strengths affect the $\Pi_m$ of one microbial species. It should also be investigated how the $\Pi_m$ differs between microbial species in studies on pure cultures (e.g. Geobacter sulfurreducens and Shewanella oneidensis). Moreover, electrodes inhering microbiomes possess complex food webs and electron transfer mechanisms, which necessitate additional exploration. In this respect the developed bioelectrocalorimetric approach can provide fundamental insights into the role that enthalpy and entropy changes during EET play for microbial electrochemistry in technical systems and natural habitats.

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Notes and references


