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Strategic improvement of *Shewanella oneidensis* for biocatalysis: Approach to media refinement and scalable application in a microbial electrochemical system

Nikolai Juergensen [1,6], Janek R. Weiler [2,6], Melanie T. Knoll [3,6], Johannes Gescher [4], Miriam Edel [*,5]

Institute of Technical Microbiology, Hamburg University of Technology, Hamburg 21073, Germany

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ABSTRACT

Microbial electrochemical systems offer a sustainable method for the conversion of chemical energy into electrical energy or hydrogen and the production of valuable compounds, contributing to the development of a bio-based economy. This study aimed to enhance the performance of anodic bioelectrochemical systems by improving the current density of *Shewanella oneidensis* as a biocatalyst through strain modification and medium refinement. The genetic modification, combining the prophage deletion and overexpression of the *speC* gene, resulted in a 4.2-fold increase in current density compared to the wild type. Furthermore, medium refinement and incorporating riboflavin, led to an additional 5.7-fold increase in current density. The application of the modified strain and medium in a scalable microbial electrolysis cell resulted in a current density of 1.2 A m^{-2}, similar to what was achieved previously with an *S. oneidensis* and *Geobacter sulfurreducens* co-culture, substantiating the substantial performance increase for a pure culture of *S. oneidensis*. Furthermore, *S. oneidensis* was shown to grow in medium containing up to 500 mM sodium chloride and increasing the salt concentration to 400 mM had a minor influence on growth but significantly lowered the cell voltage of the MEC system.

Introduction

The rising energy demand and mounting environmental challenges point to the importance of alternative energy sources and the transition to a bio-based economy through new sustainable technologies [1]. One promising approach to produce bioenergy and industrially valuable chemicals from biomass are bioelectrochemical systems (BES) [2,3]. In these systems, exoelectrogenic microorganisms can be used as biocatalysts at the anode to convert chemical substrates into electrical energy or hydrogen and platform chemicals [4,5]. In order to transfer catabolic electrons to a solid anode, microorganisms have developed different strategies [6,7]. One strategy is a direct electron transfer which is dependent on the contact of the microorganisms to the electrode surface. Here, the electrons are transferred directly from outer membrane cytochromes or other electron transferring proteins on the cell surface onto the insoluble electron acceptor [8]. A version of this electron transfer strategy is applied when organisms build conductive cell appendages such as pili and nanowires in order to realize electron transfer beyond the dimensions of a single cell [9]. These cell appendages enable microorganisms to grow multilayer conductive biofilms of more than 40 μm on top of the anode surfaces [10]. Another strategy for bacterial cells to transfer electrons onto insoluble electron acceptors is via electron shuttles. These shuttles are reduced by the microorganisms and are abiotically reoxidized at the anode surface [9]. An example of such an electron shuttle is riboflavin, which is produced by *Shewanella oneidensis* cells [11]. *S. oneidensis* is the best understood model organism

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* Corresponding author.
E-mail address: miriam.edel@tuhh.de (M. Edel).

[1] ORCID: 0009-0007-8879-4077

[2] ORCID: 0000-0003-4930-5764

[3] ORCID: 0000-0003-1009-3055

[4] ORCID: 0000-0002-1625-8810

[5] ORCID: 0009-0008-6283-0006

[6] All authors contributed equally to this work

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regarding extracellular electron transfer so far. Besides shuttled electron transfer, *S. oneidensis* is also capable of transferring electrons directly onto insoluble electron acceptors. However, *S. oneidensis* is unable to form conductive pili which is most likely the main reason for it to only grow in thin biofilms on anodic surfaces. This, in turn, leads to rather low current densities achieved by the organism compared to other exoelectrogens such as *Geobacter sulfurreducens* [12,13]. However, in contrast to *G. sulfurreducens*, *S. oneidensis* is easily genetically accessible and its facultatively anaerobic metabolism enables fast pregrowth and easy handling [7,13]. Therefore, a lot of effort is put in producing thicker and more conductive biofilms using *S. oneidensis* to achieve higher metabolic turnover rates and consequently higher productivities [6]. In this work, a broader approach was pursued which not only considered biofilm formation but also the composition and refinement of the medium. By combining the approaches of overexpression of *speC*, a gene which was shown to have a positive influence on biofilm formation [14], and the deletion of λ prophage genes according to Bursac et al. [15], a strain was developed in this study, which produced significantly more current compared to the individual predecessor strains. However, optimization and productivity in lab-scale reactors is only the first step towards sustainable industrial processes. To be industrially relevant, such optimizations must also be transferable to larger reactors. So far, various bioelectrochemical reactor systems have been developed to study anodic biofilms for sustainable hydrogen production and the production of valuable chemicals [14–16]. Nevertheless, the scalability of these systems is often constrained by higher internal resistances compared to small scale systems. In this context a scalable rotating disc bioelectrochemical reactor (RDBER) was presented previously and its potential application as a microbial electrolysis cell (MEC) has been proven [17]. One of the key benefits of the described RDBER is the possibility to adjust the surface size of the working electrode based on the number of used graphite discs. Hence, the RDBER was used in this study to facilitate the transfer of the before investigated results and the here presented strain and the subsequent medium adaptions into a new system with a larger volume. For further improvement the effect of increased conductivity of the electrolyte on the system was investigated. Low medium conductivity has been identified as an important reason for the suboptimal performance of larger scale BES and represents a significant challenge for future developments of this technology[18,19]. Overall, this study showed that current density catalyzed by *S. oneidensis* could be increased by an overall factor of 23.8 as a result of strategic strain improvement and medium adaption and resulted in large scale experiments with current densities of 1.2 A m^{-2}. These current densities are comparable to previously observed current densities using a co-culture of *S. oneidensis* and *G. sulfurreducens* [20].

Materials and methods

Media and growth conditions

All strains used in this study are listed in Table 1. The tested *S. oneidensis* strains were precultured under oxic conditions in lysogeny broth (LB) (Lennox) medium with kanamycin (50 μg mL^{-1}) for 14 h at 30 °C. For the strain development experiments, M4 minimal medium supplemented with 70 mM lactate was used. The composition of the M4 minimal medium was described previously [21].

LB-medium was used to investigate approaches to maximize the electron transfer and the overall measured current with *S. oneidensis*, as it exhibits positive influences on growth of prokaryotic cells due to its high content of yeast extracts, peptides and amino acids [22]. To further develop this approach, the medium was substituted with 50 mM HEPES buffer to ensure stable pH over the time of the experiments. To increase the available substrate for the cells 70 mM lactate was additionally added. This altered medium was then used in experiments with the before established, improved strain. As riboflavin has been shown to act as an electron shuttle and as a cofactor of outer membrane cytochromes [23,24], 160 μM of riboflavin was added in an additional comparative experiment. For plasmid preservation 50 μg mL^{-1} kanamycin and for induction 0.1 mM arabinose were added to all experiments. Precultures for BES experiments in the RDBER were grown with optimized LB medium containing 20 mM lactate.

Table 1
Overview of strains used in this study.

Strain	Source
S. oneidensis WT + pBAD*empty*	[14]
S. oneidensis Δλ + pBAD*empty*	[15]
S. oneidensis Δλ + pBAD*speC*	This study

Bioelectrochemical systems

Small scale setup

The strains were cultured in a single chamber BES with a working volume of 270 mL and a projected anode area of 36 cm^2 that was described previously [14]. Before inoculation of the BES, the cells were harvested from the preculture via centrifugation at 6000 g for 7 min and washed three times with M4 minimal medium without electron donor and electron acceptor. After autoclaving the BES, 270 mL of the respective media were added and the initial OD_{600} was adjusted to 0.07. The working electrode was set to 0 mV vs SHE and current was monitored for 46 h. To ensure anoxic conditions, the setup was continuously gassed with N_2. The medium was continuously stirred to ensure thorough mixing. Flavin quantification was performed using HPLC measurements as described before [14,24].

Large scale setup

Furthermore, the most effective of the aforementioned strains and the modified LB medium were evaluated in experiments utilizing a 10 L RDBER with a three-electrode configuration. This methodology allowed for the verification of the initial findings in a larger volume system, with the potential for further scalability. The configuration of the RDBER, comprising a total anode area of 0.5 m^2 and a total volume of 12.6 L including the periphery, was recently described in detail [20]. The operation of the RDBER was carried out with the λ prophage deficient *S. oneidensis* strain containing a copy of the *speC* gene on a pBAD plasmid. The plasmid was induced using 0.1 mM arabinose. LB medium containing 50 mM HEPES buffer, 20 mM lactate and 160 μM riboflavin was used as growth substrate as described above. During the operational phase of the RDBER and converse to the small scale systems, the system was not flushed with N_2/CO_2 as the produced hydrogen and CO_2 were considered enough to ensure anoxic conditions throughout the experiments. The initial OD_{600} in the system was adjusted to 0.07. After 24 h the batch system was switched to fed-batch conditions by starting a refined LB medium feed with 50 mM HEPES and 6 M lactate with a rate of 2.1 mL h^{-1}. In a second approach, the conductivity of the refined LB medium was increased using the addition of sodium chloride, the conductivity was measured using the EL30 device from Mettler Toledo AG (Switzerland) and after 54 h the batch system was changed to fed-batch conditions by adding the refined LB medium containing 2 M lactate with a rate of 1.05 mL h^{-1}.

Growth experiments at different salt concentrations

Investigation of growth of *S. oneidensis* Δλ + pBAD*speC* was performed using an Infinite200Pro plate reader (Tecan Trading AG, Switzerland) over 18 h until the stationary phase was reached. The preculture was grown overnight in LB medium at 30 °C and 180 rpm. Experiments were performed in a 96-well plate at 30 °C in optimized LB medium containing different sodium chloride concentrations ranging from 85 mM to 600 mM to evaluate the influence of different salt concentrations on growth. Optical density at 600 nm (OD_{600}) was measured every 30 min, with a two-minute pre-phase of shaking at 180 rpm, followed by a one-minute settling phase. The wells were filled with 200 µL of medium. The experiments were started with an initial OD_{600} of 0.08.

High-performance liquid chromatography

Organic acids, lactate and acetate, were analyzed using a high-performance liquid chromatography (HPLC) system (Dionex UltiMate3000 SD HPLC system; Thermo Scientific, Waltham, MA, US) with an integrated Refractive Index (RI) Detector RefractoMax520 (ERC, Riemerling, Germany) and a Hi-Plex H column (Agilent, Santa Clara, CA, US). The RI was set to a polarisation of 500 mV. The samples were prefiltered with a 0.2 µm polyethyltetrafluoroethylene (PTFE) filter (VWR, Darmstadt, Germany) and 150 µL of samples were mixed with 15 µL of a 0.5 mol L^{-1} sulfuric acid solution prior to analysis. As eluent a 5 mmol L^{-1} sulfuric acid solution was used with a flowrate at 0.5 mL min^{-1} and the column temperature was set to 60 °C.

Total organic carbon measurements

The total organic carbon (TOC) content of the medium was quantified using a DIMATOC® 2100 device from DIMATEC, Analysentechnik GmbH. All samples were diluted at a 1:5 ratio with dionized water, filtered with a 0.2 µm polyethyltetrafluoroethylene (PTFE) filter (VWR, Darmstadt, Germany), measured in triplicate, and the results were automatically calculated.

Calculation of the specific growth rate

The specific growth rate µ [h^{-1}] was calculated in the exponential growth phase by the change of the measured OD_{600} over a 8-hour time period according to the following Eq. 1. Hereby, the natural logarithm values of $OD_{600\ T1}$ and $OD_{600\ T0}$ were employed for the designated time T_1 and T_0, respectively.

$$\mu = \frac{\ln(OD_{600\quad T1}) - \ln(OD_{600\quad T0})}{T_1 - T_0} \tag{1}$$

Calculation of the coulombic efficiency

To quantify the performance of the large-scale BES the coulombic efficiency (CE) was calculated based on measured HPLC and TOC values. CE is the ratio of the electrons measured as current to the metabolically released electrons. To calculate this, the total current was first determined by integrating the produced current (I) over the operating time (t) and divided by the total number of metabolically released electrons, as described in Eq. 2. The number was determined by the consumed amount of substrate according to the TOC measurement (ΔTOC), the reactor volume ($V_R = 12.6$ L), the number of electrons released per mole of carbon metabolized ($z_{e,carbon} = 4.2$ [25]) and the Faraday constant (F = 96485 C mol^{-1}). The CE values were initially calculated based on lactate consumption. However, to ensure an accurate determination, the consumption of organic carbon and the electrons released from the yeast extract in the LB medium must also be considered. For this purpose, the consumed TOC of the medium was measured at the sampling points and by taking the supply of carbon through feeding of yeast extract, organic buffer, riboflavin and lactate into account.

$$CE = \frac{\int_{t_0}^{t} I\,dt}{\Delta TOC \bullet V_R \bullet z_e \bullet F} \tag{2}$$

Results

Improved current production via genetic engineering

As already shown by Bursac and colleagues, the deletion of the λ prophage from the *S. oneidensis* genome leads to an increase in current density [15]. This increase was not influenced by the addition of an empty pBAD plasmid, as shown in Fig. 1. The empty plasmid was included in the experiment to confirm that the observed effect resulting from the expression of *speC* was not related to the addition of the empty plasmid or the corresponding antibiotic in the medium. The mean current density that was achieved over 46 h was 2.5-fold higher (0.28 ± 0.028 A m^{-2}) than the mean current density achieved by the wildtype with the empty pBAD plasmid (0.11 ± 0.008 A m^{-2}). The additional overexpression of the gene for the ornithine-decarboxylase *speC* further increased the current density 1.6-fold (compared to the λ-deletion mutant) to a mean current density of 0.46 ± 0.023 Am^{-2}. This observation corroborates observations made by Edel *et al.* [14], where the overexpression of *speC* led to a 2-fold increase in current density compared to wildtype *S. oneidensis.* Overall, the combination of these two approaches led to an increase of current density by a factor of 4.2.

Improved current production via medium optimization

To further improve the performance of the *S. oneidensis* Δλ + pBAD*speC* strain, the medium was adjusted (Fig. 2). To improve growth and metabolic turnover rate of the strain the minimal medium was switched to LB medium containing 50 mM HEPES buffer and 70 mM lactate. The new complex medium led to a mean current density of 0.7 A m^{-2} and thus a 1.5-fold improvement in current density. To further improve the performance of the strain, riboflavin was added to the complex medium to increase the contribution of shuttle-based electron transfer and support cofactor acquisition of outer membrane cytochromes. The addition of 160 µM riboflavin resulted in a 3.7-fold increase in current density compared to the complex medium without riboflavin. Overall, the current density could be improved by a factor of 23.8 by strain engineering and medium alterations to a total mean current density within a 46 h time frame of 2.62 A m^{-2}.

Current production in a larger scale MEC system

After the modification of the medium, the genetically engineered strain *S. oneidensis* Δλ + pBAD*speC* was analyzed regarding its performance in a scalable MEC setup to evaluate its produced current densities compared to current densities observed in a co-culture of *S. oneidensis* and *G. sulfurreducens* in the RDBER [20]. The current density was measured for 7 days of operation using a RDBER which is shown in Fig. 3. Upon initial inoculation of the refined LB medium with the advanced *S. oneidensis* strain, a rapid increase in measured current density was observed, reaching 0.96 A m^{-2} within the first day of operation. After 1 day the current density further increased up to 1.28 A m^{-2} reaching a plateau after 2.5 days. During this plateau phase an average current density of 1.2 A m^{-2} with a maximum of 1.4 A m^{-2} was reached. The average cell voltage after the first day until the end of the operation was 0.91 V. OD_{600} measurements showed optical density increasing with a mean value of 0.085 per day and a maximum at the end of the experiment of 0.73. The CE was calculated based on the metabolically released electrons per mole of carbon related to the total lactate and yeast extract consumption, including the supplied carbon. This resulted in a CE of 36.4 %.

In order to lower cell voltage, we aimed to improve the conductivity of the optimized LB medium by increasing its sodium chloride concentration. Hence, the influence of increased salt concentrations from 85 mM up to 600 mM on the growth rate and the maximum OD_{600} of *S. oneidensis* Δλ + pBAD*speC* was tested over 18 h in a microplate assay, which is shown in Fig. 4. No significant effect on the cell growth was observed up to 150 mM NaCl. Concentrations of 200 mM and 250 mM showed a slightly delayed growth but still resulted in comparable maximum OD_{600} values with the control conducted with 85 mM NaCl. The specific growth rates were observed to be 7.8 % and 4.7 % lower, respectively. At higher salt concentrations from 300 mM up to 450 mM

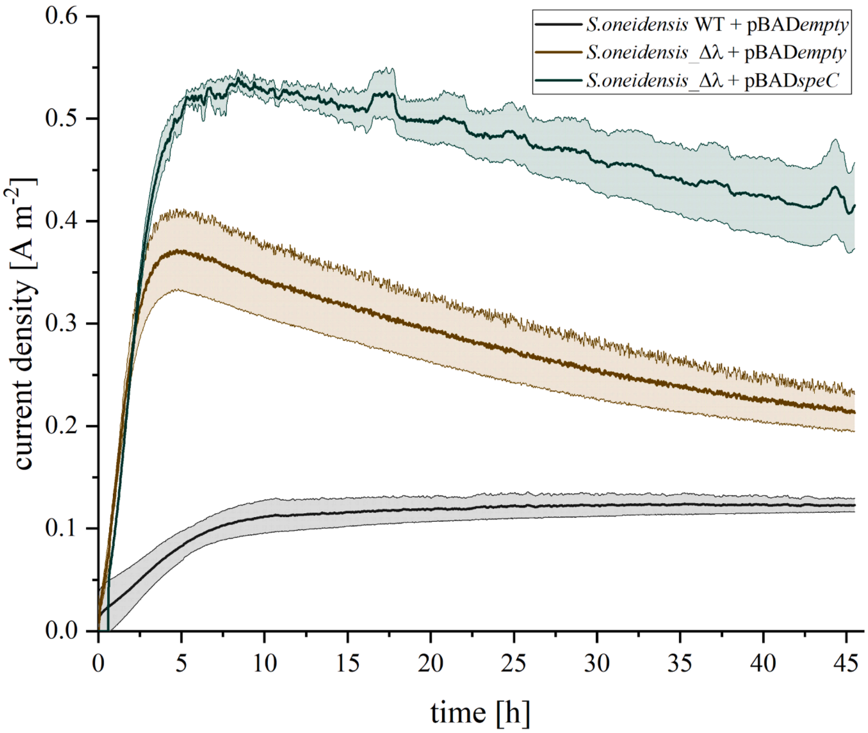

Fig. 1. Current density of *S. oneidensis* WT + pBAD*empty*, *S. oneidensis* Δ*λ* pBAD*empty* and *S. oneidensis* Δ*λ* + pBAD*speC* in 46 h using M4 minimal medium. The deletion of the *λ* phage results in an increased current density of 2–3-fold. Furthermore, the additional overexpression of the ornithine-decarboxylase *speC* increases the current density 4.2-fold. Error bars represent the standard deviation of individual replicates (n = 3).

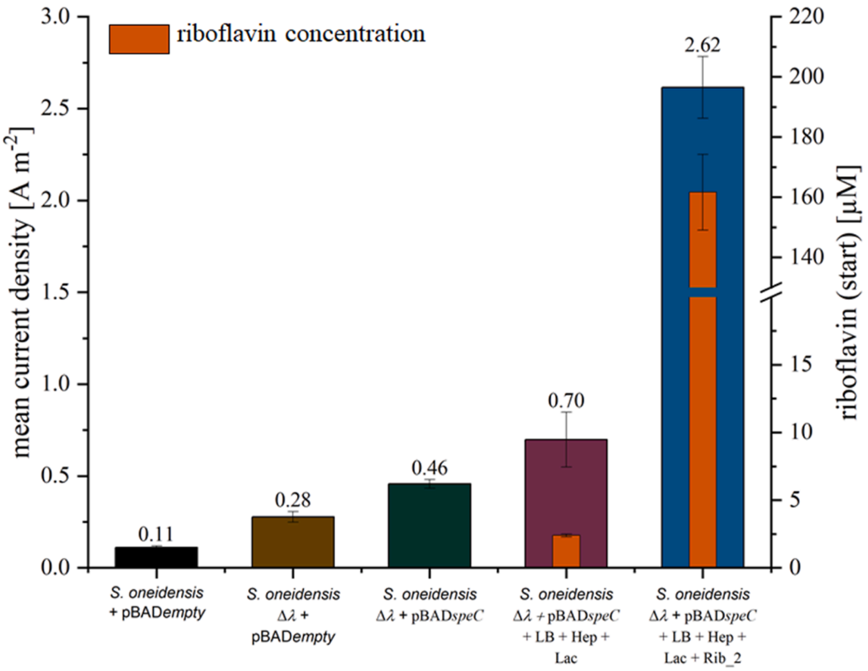

Fig. 2. Effect of complex media on mean current density of the improved *S. oneidensis* Δ*λ* + pBAD*speC*. By using complex LB medium with 50 mM HEPES and 70 mM lactate the current density increased 1.5-fold. The further addition of 160 µM riboflavin led to an increase in current density of 3.7-fold.

the growth period was prolonged obtaining 11.6–47.3 % lower specific growth rates and a lower maximum OD_{600} after 18 h of observation. At 400 mM the OD_{600} resulted in roughly half of the maximum OD_{600} observed at 85 mM NaCl, whereas the specific growth rate was 35.3 % lower. The lag phase at 500 mM NaCl was prolonged to 10 h compared to the regular concentration of 85 mM NaCl and demonstrated a lower specific growth rate of 54 %. No growth was detected during the 18 h time period with 600 mM NaCl.

Within the tested salt concentrations *S. oneidensis* Δ*λ* + pBAD*speC* did show moderate growth at 400 mM NaCl. Additionally, this salt concentration drastically improved the conductivity of the electrolyte to 37 mS cm^{-1}, while the refined LB medium exhibited an electrical

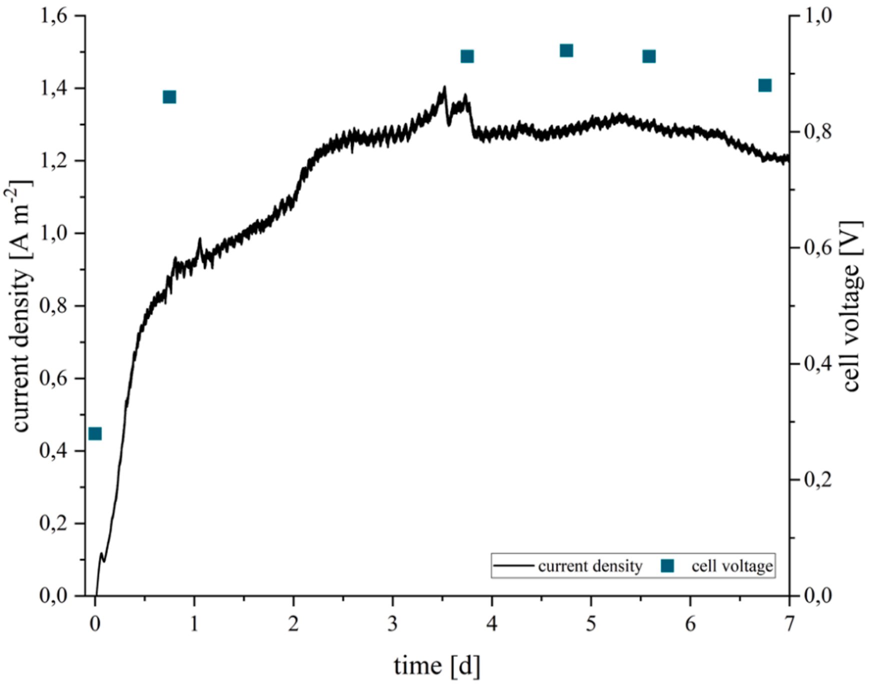

Fig. 3. Current density of *S. oneidensis* $\Delta\lambda$ + pBAD*speC* over 7 days in the RDBER using the optimized LB medium. The average current density after 1 day was 1.2 A m^{-2} with an average cell voltage of 0.91 V. The refined LB medium contained a salt concentration of 85 mM NaCl.

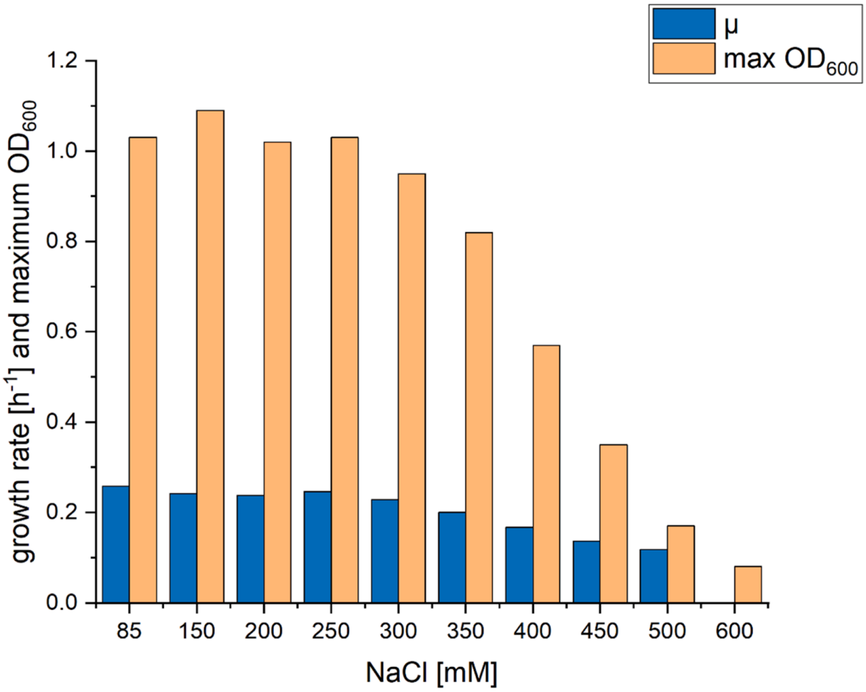

Fig. 4. Growth rate and maximum OD_{600} of *S. oneidensis* $\Delta\lambda$ + pBAD*speC* at different concentrations of NaCl with an initial OD_{600} of 0.08 over 18 h. The salt concentrations were ranging from 85 mM to 600 mM NaCl, whereas 85 mM is the concentration of the refined LB medium. The experiment was conducted as a single test.

conductivity of 11 mS cm^{-1}. Therefore, the genetically engineered strain *S. oneidensis* $\Delta\lambda$ + pBAD*speC* was operated in the RDBER with an increased salt concentration of 400 mM over 6 days and the current density was measured (Fig. 5). After inoculation of the system the current density increased during the first 2 days up to 0.54 A m^{-2} reaching a stable plateau. A further increase of the current density was measured after 3 days resulting in a maximum of 1.07 A m^{-2}. The average cell voltage from day one until the end of operation was 0.77 V with an average current density of 0.70 A m^{-2}. Hence, while cell voltage was decreased by 15 % compared to the experiment conducted with 85 mM NaCl, the final current was decreased by 17 %. OD_{600} measurements showed optical density increasing with a mean value of 0.043 per day with a maximum at the end of the experiment of 0.35.

The CE was determined by calculating the metabolically released

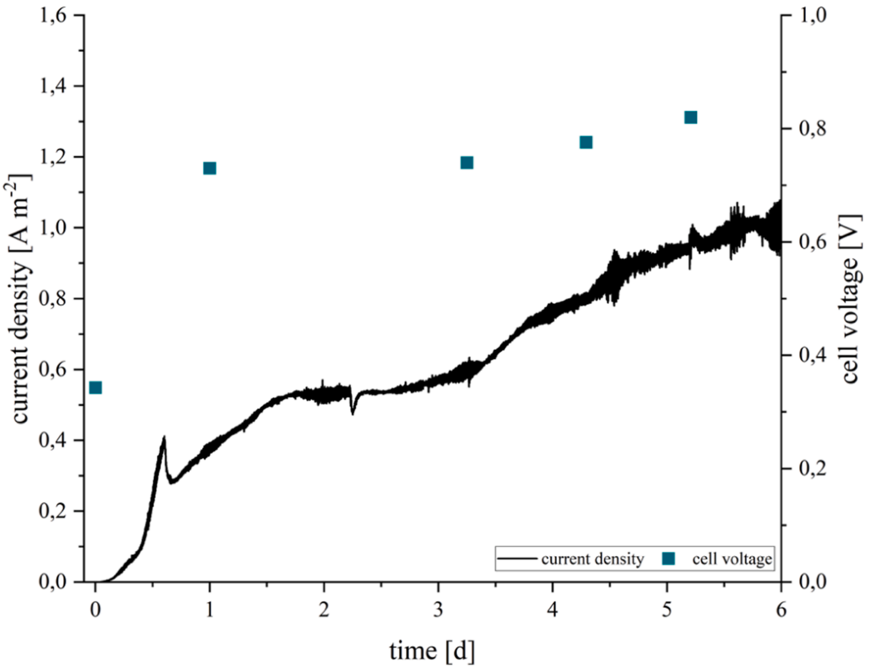

Fig. 5. Current density of *S. oneidensis* Δ*λ* + pBAD*speC* over 6 days in the RDBER using the refined LB medium with an increased salt concentration of 400 mM NaCl. The average current density after 1 day was 0.70 A m^{-2} with an average cell voltage of 0.77 V.

electrons per mole of carbon in relation to the total lactate and yeast extract consumption, which included the carbon supplied through the feed. This calculation yielded a CE of 98.2 %.

Discussion

Improving current production via genetic strain engineering and medium optimization

A frequently used approach to improve productivity in *S. oneidensis*-based BES is to improve biofilm formation. Since the organism natively forms only thin biofilms, there seems to be some potential for improvement using this approach. Bursac *et al.* have already observed an increase in the number of cells on the anode along with an increased current density by deleting the *λ* phage [15]. This effect could also be observed in this study with the further addition of an empty pBAD-plasmid. Edel *et al.* further observed an increase in current density along with an increase of the number of cells on the anode through the expression of the gene for the ornithine decarboxylase *speC* [14]. The latter mimics a quorum sensing response of the cells to riboflavin and might trigger alterations in the chemistry of the extracellular polymeric matrix. The combination of these two genetic modifications in this work led to an increase in the current density of 4.2-fold. Hence, both factors could be combined, and one was not masking the effect of the other. While overexpression of *speC* seems to activate a new kind of quorum sensing mechanism, deletion of the prophage might be connected to two factors. First, it might be stabilizing the cells during initial surface contact, a time point which was shown to be connected to increased prophage activity and consequently cell lysis. Moreover, phage dependent lysis was shown to lead to the release of DNA which seems to be an important factor for oxic biofilm development [26–28]. Nevertheless, increased DNA concentrations between the cells might have an insulating effect as the DNA will prohibit the direct interaction of cell surface exposed *c*-type cytochromes from different cells with each other thereby hampering cell-cell-electron transfer. Along these lines, Gao *et al.* revealed that *S. oneidensis* cells from which EPS was removed were characterized by 30 % higher hematite reduction rates and 3–4-fold higher current densities in BES [29].

A further improvement in current density was achieved by using complex medium. The use of LB medium with added buffer and lactate ensured an optimal nutrient supply to the cells and presumably increased the metabolic turnover rate. Further, the presence of 2.4 µM riboflavin from yeast extract, a component of LB media can help planktonic electron transfer [31]. Taken together, the complex media resulted in an increase in current density by a factor of 1.5 compared to the optimized strain in minimal media. In order to further increase the current density, riboflavin was added to the medium in an additional step. In previous studies, it had already been observed that the addition of small amounts of riboflavin coupled to magnetic beads led to increased biofilm formation and an increased current density [21]. Moreover, flavins are known to act as cofactors of outer membrane cytochromes and can thus increase electron transfer processes in *S. oneidensis* [23]. Furthermore, riboflavin acts as an electron shuttle and can thus also be used by planktonic *S. oneidensis* cells to transfer electrons to the anode [21,32]. This approach showed an increase in current density of 5.7-fold compared to the optimized strain in minimal media.

Current production in a larger scale MEC using a genetically engineered S. oneidensis strain with a refined medium

In order to reveal whether the observed effects could also lead to increased current production in a scalable reactor system, we inoculated the advanced strain in the refined medium in a RDBER and monitored current production over 7 days of operation. In comparison to the average current density of 2.62 A m^2 in the small scale batch system, we could only reach 45.8 % of this value in the RDBER. The lower obtained current density could be due to several reasons. The graphite plates that were used as anode material of the RDBER offer only a smooth surface, whereas the graphite felt which was applied in small-scale experiments offers a 3D structure for cells to attach to. Hence, the larger surface area within the projected surface area will likely contribute to the higher current density achieved with the graphite felt compared to the graphite plates. The achieved average current density of 1.2 A m^{-2} with the genetically engineered strain and refined medium in the RDBER was comparable to the monitored average current density of 0.9 A m^{-2} and 1.2 A m^{-2} achieved with a *S. oneidensis* and *G. sulfurreducens* co-culture grown in minimal media in the same reactor over 9 and 46 days respectively [17,20]. Although the media were different, this comparison shows at least what can be reached with *S. oneidensis* as it seemed so far that this organism alone cannot reach comparable current densities to cultures containing *G. sulfurreducens* [13,33,34]. Coulombic efficiencies calculations of 36.4 % were achieved based on TOC measurements, which improved to 98.2 % following enhancements to the medium's conductivity. The electrons are supplied by lactate and the yeast extract present in the refined LB medium and were included into the calculations by measuring the total organic carbon consumed. However, yeast extract is a complex and undefined mixture of substances composed of various components, lacking a general chemical formula [22], which is why the mean oxidation state of carbon in biomass of −0.2 was assumed [25] enabling the calculation with an approximated value. The discrepancy in CE values can be attributed, at least in part, to the discrepancies in optical density measurements. In accordance with the findings of the growth experiments conducted with increasing salt concentrations, the addition of salts resulted in 50.59 % growth and 47.95 % of the maximum OD_{600} observed in the comparison experiment. Consequently, a greater number of electrons were required to accumulate the biomass, which could explain the lower CE value. To improve the conductivity of the refined medium and reduce ohmic losses in the system we aimed to increase the salt concentration. The impact of different salt concentrations on the growth of the genetically engineered strain showed an increased inhibition of the growth with increasing the salt concentration until no growth was measured. High salinity can cause stress conditions on bacterial growth and a higher energy consumption due to active sodium export out of the cells [30]. At a salt concentration of 400 mM, growth was still possible, reaching 55 % of the maximum OD_{600} in the original refined medium without additional salt. MEC performance can benefit from an increased salt amount, due to a higher ionic strength of the electrolyte resulting in a higher electrical conductivity and a reduced internal resistance [18,19]. The RDBER operation at a high salt concentration of 400 mM reached a maximum current density of 1.07 A m^{-2} which was 76.4 % of the maximum current density at 85 mM NaCl. Nevertheless, the higher ionic strength resulted in 15.4 % lower average cell voltage compared to the experiment with 85 mM NaCl. The experiments show the plasticity of *S. oneidensis* to adapt to increasing salt concentrations and that MEC efficiency can be increased by varying the salt concentration. To this end, future experiments might focus on laboratory evolution of *S. oneidensis* to higher salt concentrations in order to not only decrease cell voltage but at the same time keep current density at its maximum.

Conclusion

The objective of this study was to improve the performance of *S. oneidensis* via genetic engineering and optimize the composition of the growth medium to increase the achievable current density. This goal was reached by the combination of prophage deletion and *spe*C overexpression coupled to media refinement and increased flavin concentration. Furthermore, the developed strain and refined medium were applied to a larger scale MEC. These results led to an average current density of 1.2 A m^{-2}, comparable to the current densities observed in the co-culture of *S. oneidensis* and *G. sulfurreducens* in the RDBER enabling the successful transfer of the engineered strain and refined medium to a larger bioelectrochemical system. Additionally, the conductivity of the electrolyte in the large-scale MEC was enhanced by the addition of salt which led to a lower cell voltage of the system. The results demonstrate that an elevated ionic strength exhibits an influence on the genetically engineered strain as shown by altered CE values and on electron transfer in a larger scale MEC.

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CRediT authorship contribution statement

Johannes Gescher: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Melanie Tabea Knoll:** Investigation, Formal analysis, Data curation. **Miriam Edel:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Janek R. Weiler:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Nikolai Juergensen:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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