Sustainable Energy & Fuels

Electronic Supplementary Information

Investigating Extracellular Electron Transfer of *Rikenella microfusus*: a Recurring Bacterium in Mixed-Species Biofilms

M. Grattieri,[a] K. Hasan,[a] R.D. Milton,[a] S. Abdellaoui,[a] M. Suvira[b] , B. Alkotaini,[a] and S. D. Minteer*[a]

Received 28th May 2017,
Accepted 28th June 2017

DOI: 10.1039/c7se00270j

rsc.li/sustainable-energy

Materials

R. microfusus culture

R. microfusus was obtained from ATCC® (ATCC® 29728TM), strain designation Q-1 [NCTC 11190]. The bacterial cells were transferred and inoculated in anaerobic conditions using an Atmosbag glove bag (Sigma Aldrich) purged with Argon gas that had been scrubbed of O_2 over a heated Cu catalyst. The cells were initially grown at 37°C in a 5 mL broth medium and following transferred to a 75 mL broth medium. The broth medium composition (per Liter of de-ionized water) was: 10 g beef extract, 10 g peptone, 3 g yeast extract, 5 g glucose, 5 g sodium chloride, 1 g soluble starch, 3 g sodium acetate, 0.5 g cysteine, and 0.5 g agar. All chemicals where from Sigma-Aldrich except for peptone and agar, which where from Becton, Dickinson and Co., sodium chloride from VWR and sodium acetate from Macron Chemicals. Prior to inoculation, the broth medium was autoclaved at 121°C for 15 minutes and the Atmosbag glove bag was sterilized wiping with ethanol and by exposure to UV light for at least 30 minutes. The growth curve was monitored by optical density analysis at 600 nm (OD, Thermo Scientific, Evolution 260 bio UV-Vis spectrophotometer). When the measured OD was higher than 1, the sample was diluted and the OD calculated from the diluted solution. The electrochemical cells were inoculated with bacterial cells in the early-stationary phase at 40 hours of the bacterial growth.

Electrodes preparation

The electrodes used for electrochemical characterization were prepared by connecting a carbon cloth sheet of 1.5 x 1.5 cm (non-wet proofed, E-TEK) to nickel wire (24 BNC, Consolidated Electronic Wire & Cable). All of the electrodes were sterilized at 121°C for 25 minutes. For the experiments performed with pre-acclimated electrodes, the carbon cloth electrodes were placed in the growth medium before inoculation and maintained in the medium during the growth. After 40 hours of growth, the electrode was anaerobically transferred to the electrochemical cell. For the experiments performed without pre-acclimation of the electrodes in the growth medium, the electrodes were directly transferred to the electrochemical cell.

[a] *Department of Chemistry, University of Utah, 315 S 1400 E Room 2020, Salt Lake City, Utah 84112 USA*
[b] *Department of Chemistry and Biochemistry, Loyola University Chicago, 1032 W Sheridan Rd, Chicago, IL 60660 USA.*
* Corresponding Author E-mail: minteer@chem.utah.edu

Methods

Electrochemical Characterization

The electrochemical experiments were performed with a number of biological replicates of three, under continuous purging of nitrogen gas to ensure anaerobic conditions. A three-electrode setup was used, where the carbon cloth sheet pre-acclimated in the bacterial growth was used as the working electrode, a platinum mesh was the counter electrode and a Ag|AgCl (KCl sat.) electrode was the reference electrode (RE). All potentials used in this paper are versus this RE. The platinum mesh was sterilized in nitric acid; the reference electrode was sterilized with ethanol. The electrochemical cells and rubber stoppers were sterilized at 121°C for 25 minutes. The electrolyte for the electrochemical experiments was 34 mL of 0.1 M phosphate buffer solution (PBS) + 0.1 M KCl (pH 7) including 4 mL of cell inoculum grown at the early stationary phase (40 h). The PBS was previously sterilized at 121°C for 25 minutes. Dilution of the cell growth was performed to ensure optimal solution conductivity and avoid noise in the electrochemical response due to the complex growth medium. The use of PBS solution in the study of bioelectrochemical systems is beneficial since the increase in solution conductivity might facilitate an increase in current generation, particularly when the solution resistance plays a significant role, and it has been reported for different microbial fuel cell setups.[1-3] In order to investigate the capability of *R. microfusus* to utilize a redox mediator to facilitate the EET, one set of experiments was performed by adding 0.5 mL of a 500 μM RB 5'-monophosphate sodium salt hydrate (Sigma) solution into the electrolyte. Cyclic voltammetric tests (CVs) were performed using a VSP Bio-Logic Instruments potentiostat, at a sweep rate of 1 mV s^{-1}. Five CVs were performed for each measurement, to ensure a stable electrochemical response. The last cycle was used for representation throughout the manuscript. Chronoamperometric experiments (CA, VSP Bio-Logic Instruments) were performed after the CV by continuously polarizing the WE at a potential of +0.2 V *vs.* Ag|AgCl (KCl sat.) for 70 hours. Additions of 2 mL of a 1 M glucose stock solution were performed to provide the substrate for the bacterial cells (final concentration 50 mM). The electrochemical potential for CAs was selected from the results obtained by CVs, ensuring a sufficient overpotential (η) for the anodic reaction (η= 0.28V). Control (negative) experiments, to confirm the role of *R. microfusus* in the bioelectrocatalytic process, were performed utilizing a sterilized medium and a working electrode pre-acclimated in the sterile broth media without bacterial cells. At the end of the electrochemical tests, the electrodes were dried in sterilized petri dishes at room temperature (20 ± 2°C) before performing scanning electron microscopy (SEM). The SEM micrographs were obtained using a FEI Quanta 600 FEG.

Gel Electrophoresis analysis

The bacterial cells in the early-stationary phase at 40 hours of growth were centrifuged using Amicon Ultra 10K filter units for 20 min at 15000 rpm (Allegra X-15R, Beckman Coulter). The supernatant containing proteins above 10KDa was collected and centrifuged again at 13000 rpm for 5 min (Eppendorf™ 5424R). Gel electrophoresis (Thermo Scientific Owl® EC3000XL) was performed on a pre-cast gel (Tris-Glycine NN 4-20%, NuSep) using a Tris-Glycine sodium dodecyl sulfate (SDS) buffer (25 mM Tris, 0.19 M Glycine, 0.1 % SDS). The sample was prepared by adding 2 μL of 50% glycerol and 6 μL of Pierce™ lane marker reducing sample buffer (Thermo Scientific) to 22 μL of the supernatant previously obtained. Proteomics mass spectrometry analysis was performed at the Mass Spectrometry and Proteomics Core Facility at the University of Utah. Mass spectrometry equipment was obtained through NCRR Shared Instrumentation Grant # 1 S10 RR020883-01 and 1 S10 RR025532-01A1. The protein sequences were analyzed by Mascot™ database.

Supplementary Figures, Results and Discussion

Figure S1 shows the bacterial growth curve. The early-stationary phase was reached after 40 hours of growth. This time was selected to harvest the cells for electrochemical experiments.

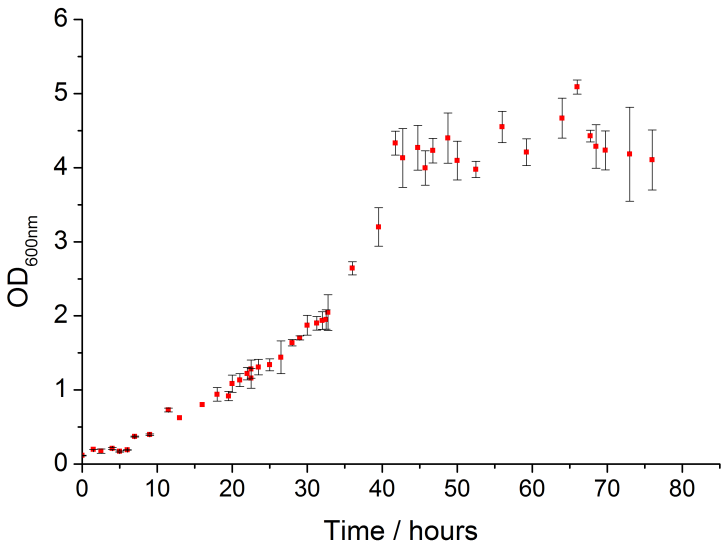

Figure S1 – The growth curve of *R. microfusus* (OD = 600 nm)

Figure S2 shows the CVs for the three replicates tests where carbon cloth electrodes previously exposed to bacterial cells for 40 hours were utilized for the analysis.

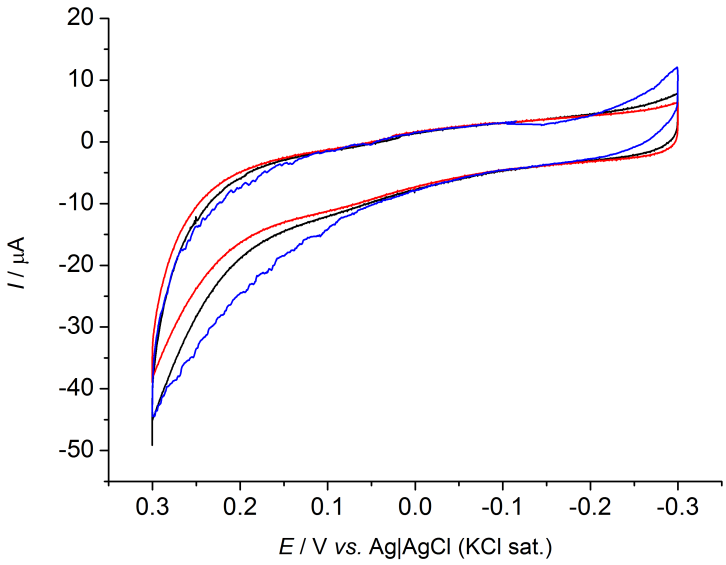

Figure S2 - Cyclic voltammograms for three replicate tests with addition of exogenous RB. Scan rate: 1 mV s^{-1}.

Figure S3 reports the chronoamperometric traces, performed at +0.2 V (*vs.* Ag|AgCl (KCl sat.)) after the cyclic voltammetric analysis reported in Figure 1. During the initial 45 hours of polarization, the current response in the presence (red) and absence (blue) of exogenous RB was comparable. After 45 hours, 2 mL of a 1 M glucose solution was added (to a final concentration of 50mM) and a significant increase of the catalytic current output was obtained only for the experiments that contained additional exogenous RB. The higher current output lasted until 52 hours. Interestingly, the addition of glucose to *R. microfusus* that did not contain additional RB only yield an small increase in the oxidative current up to 47 hours, when the baseline current was restored.

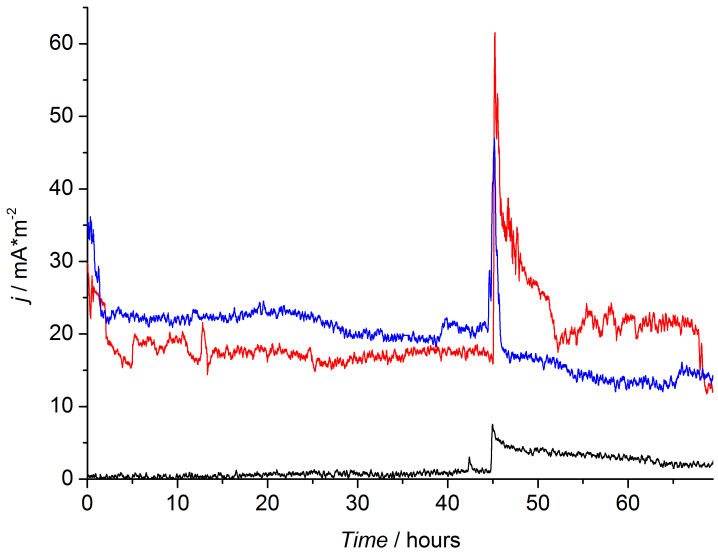

Figure S3 - Chronoamperometric analysis of *R. microfusus* with (red) and without (blue) exogenous RB. Control experiment comprising sterilized electrode and electrolyte (black). Glucose added after 45 hours of continuous operation (final concentration 50mM). Applied potential, E_{APP} = +0.2 V *vs.* Ag|AgCl (KCl sat.). Current densities are calculated based on the projected surface of the carbon cloth anodes.

Figure S4 shows the CVs for the pre-acclimated electrodes after the chronoamperometric tests of Figure S3 (CA, 70 hours with an applied potential of +0.2 V *vs.* Ag|AgCl (KCl sat.)). After the CAs the maximum current achieved was decreased to 20 and 16 μA with and without RB, respectively. However, the onset for the oxidation of glucose is shifted to more negative potentials, at approximately -0.21 V (vs. Ag|AgCl (KCl sat.)). The shift could indicate an enhanced communication between bacterial cells and electrodes, which may be due to increased concentration of redox mediators with a more negative redox potential secreted by the microorganisms.

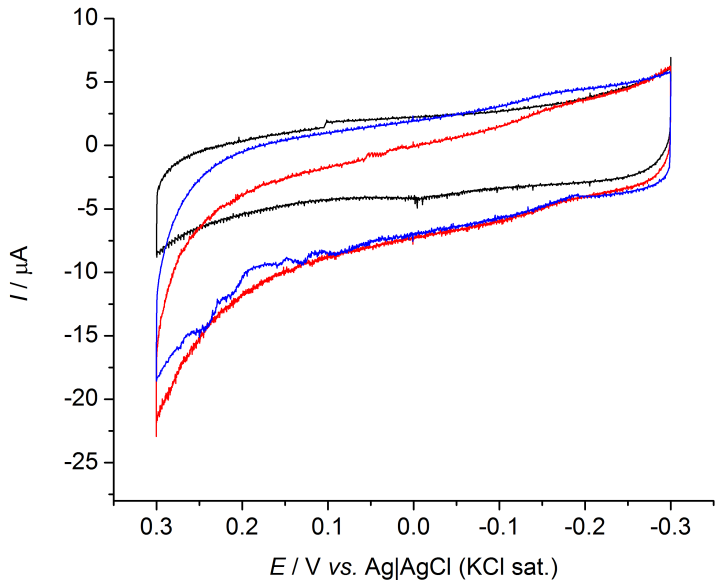

Figure S4 - Cyclic voltammograms with (red), and without (blue) addition of exogenous RB conducted after the chronoamperometric tests of Figure S3. Control experiments were carried out with sterile electrodes and electrolyte (black) (50mM glucose). Scan rate: 1 mV s^{-1}.

Scanning electron microscopy (SEM)

Figure S5 shows the SEM analysis for control (negative) experiments, where no bacterial cells where present in the electrolyte (sterile electrolyte).

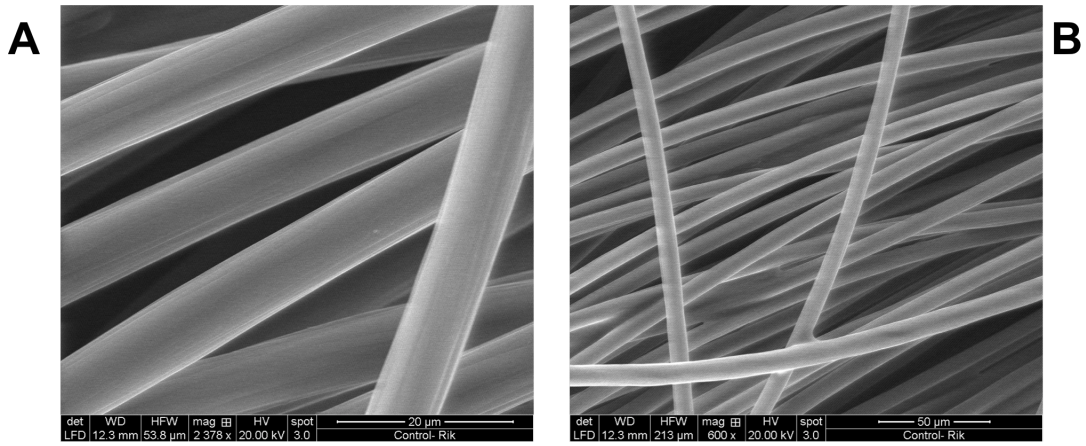

Figure S5 - Control experiment. SEM images of carbon cloth electrode in sterile electrolyte (absence of *R. microfusus*).

Figure S6 shows the widespread colonization of the carbon cloth electrode obtained when exogenous riboflavin was added to the electrolyte.

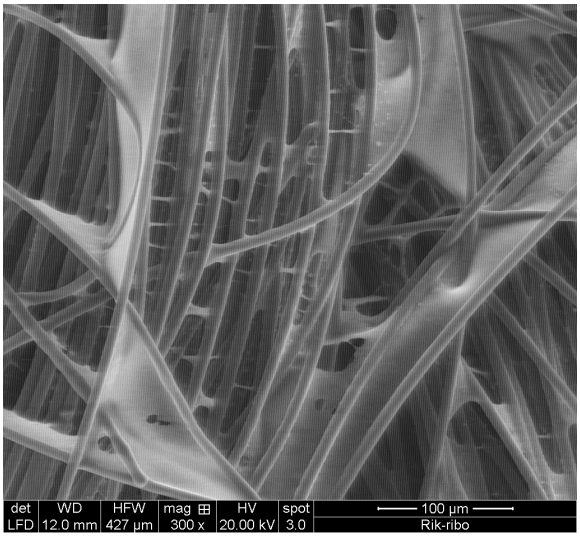

Figure S6 - SEM images of possible extracellular polymeric substances secreted from *R. microfusus* with addition of 6.25 mM exogenous riboflavin.

From Figure S7, the typical rod shape of *R. microfusus* cell can be recognized on the carbon fibers.

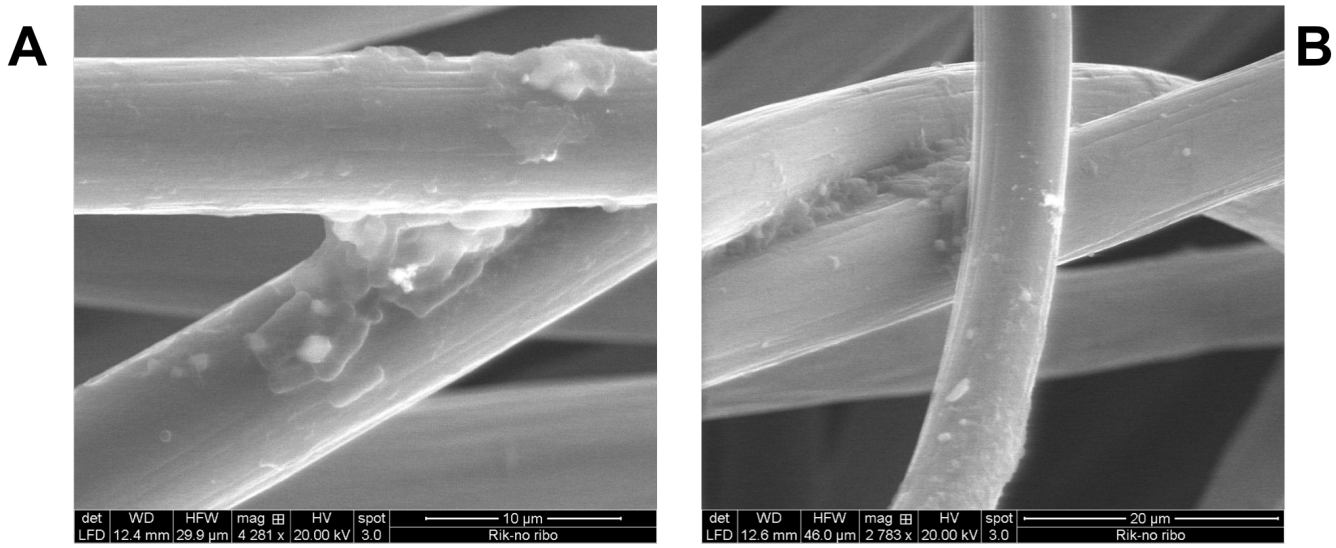

Figure S7 - SEM images of *R. microfusus* cells attached to the carbon fiber cloth. Scale bar of 10 and 20 mm for A and B respectively.

Table S1 reports the complete results of the proteomic analysis, including peptides sequences matching proteins with a low score.

Table S1. Proteomic data analysis of peptides sequences including proteins with low score from gel electrophoresis of *R. microfusus* growth medium.

NCBI Accession no.	Mass	Score	Protein	Detected Peptides sequences
gi | 488469612	45773	71	Phosphopyruvate hydratase	K. IQIVGDDLFVTNPK. R R. AAVPSGASTGQFEAVELR. D R. IEEELGDSAEYAGASAFPR. F K. VNQIGSLSETIDAVELAHR. N R. AAVPSGASTGQFEAVELR. D
gi | 85681833	48565	161	Protease IV	R. GGLYGGPSYCGAPTSQR. N R. AAPLAPKPGTPLQVGVGLK. T K. YSQGNVSAVGVTYDGHTALTR. V K. KYSQGNVSAVGVTYDGHTALTR. V
gi | 50840727	32154	33	Lysozyme M1 precursor	K. FATNETATPR. H K. ATEGTSYQNPFYASQYNGSQSAGLIR. G
gi | 282582005	37331	24	Ser/Thr phosphatase family protein	R. IATGANLFTPVVEAR. N

References

1. D. Sun, D. Call, A. Wang, S. Cheng and B. E. Logan, *Environ. Microbiol. Rep.*, 2014, **6**, 723-729.
2. C. Santoro, S. Babanova, P. Atanassov, B. Li, I. Ieropoulos and P. Cristiani, *J. Electrochem. Soc.*, 2013, **160**, H720-H726.
3. A. Kumlanghan, J. Liu, P. Thavarungkul, P. Kanatharana and B. Mattiasson, *Biosens. Bioelectron.*, 2007, **22**, 2939-2944.