Redox Potential Heterogeneity in Fixed-Bed Electrodes Leads to Microbial Stratification and Inhomogeneous Performance

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Supporting Information

SI-1 Performance of bedBES2

Figure SI-1. Chronoamperogram of bedBES2 polarized at +200 mV. 1) Inoculation with secondary *Geobacter* spp. enrichment biofilm, 2) granular sampling under batch operation, 3) change in reactor operation from batch to continuous mode, 4) acetate concentration increase from 10 mM to 20 mM, 5) granular sampling under continuous operation.
**Si-2 Acetate removal and coulombic efficiencies of bedBES**

Table **Si-2.** Acetate removal and coulombic efficiencies of bedBES1 and bedBES2 during batch cycles and under continuous operation. Acetate concentration in the feeding media of both besBES increased from 10 to 20 mM after 7 days of continuous operation.

| Feeding cycle (batch operation) | bedBES 1 | bedBES 2 |
|-------------------------------|----------|----------|
|                               | Total acetate removal (%) | Coulombic efficiency (%) | Total acetate removal (%) | Coulombic efficiency (%) |
| 1 (before inoculation)         | 12.6     | 0        | 10.9     | 0        |
| 2                              | 100      | 98.3     | 100      | 100.2    |
| 3                              | 100      | 86.5     | 100      | 83.5     |
| 4                              | 100      | 113.9    | 100      | 86.7     |
| 5                              | 100      | 115.8    | 100      | 101.4    |
| 6                              | 100      | 109.2    | 100      | 132.6    |
| 7                              | 100      | 106.8    | 100      | 108.8    |
| 8                              | 100      | 133.4    | 100      | 112.7    |
| 9                              | 100      | 103.6    | 100      | 120.1    |
| Continuous operation (10 mM acetate) | 100      | 91.4     | 100      | 88.1     |
| Continuous operation (20 mM acetate) | 89.7      | 56.7     | 95.1      | 46.1     |
SI-3 Cyclic voltammetry experiments performed with bed anode and current collector

Figure SI-3. Turnover cyclic voltammetry (CV) performed with the complete reactor of bedBES1 before and after inoculation (A). Turnover CV performed with the current collector of bedBES1 but without connection to the granule bed (by lifting up the current collector from the fixed bed to the media) before and after inoculation (B). The scan rate for CVs was 1 mV s\(^{-1}\) (3\(^{rd}\) scans are shown). First derivative of the voltammetric curve showing one major redox system at a potential of −340 mV (C).
**SI-4 Evaluating volumetric current densities of bedBES**

According to Table 2 of Altermann et al., an average current of 165.9 ± 37.7 mA was achieved after a cultivation period of 4 weeks in a two-chamber reactor with 0.11 L bed anode volume (granule diameter between 1.5 and 5 mm). It was fed with 6 mM acetate and was polarized to 0 V (vs. Ag/AgCl sat. KCl).[1] The resulting volumetric current density of 1508 mA L⁻¹ is ca. 30 times higher than the 53.6 mA L⁻¹ achieved by bedBES1 during continuous mode with 10 mM acetate by this work.

Furthermore, the mean diameter of the used granules was 3.5 mm resulting in a surface area of 0.0000385 m² per granule assuming granules would be ideal spheres. 1.5 kg granules were used for each bedBES with an individual average dry weight of 0.0465 g (determined by the method described in section 4.7 of the main manuscript) resulting in 32.258 granules. Thus, the bed anode surface area was 0.0000385 m² × 32.258 granules = 1.24 m² considering each individual granule. As the current production achieved by bedBES1 during continuous mode with 10 mM acetate was 53.6 mA L⁻¹, a current density of 53.6 mA L⁻¹ × 1.45 L ÷ 1.24 m² = 62.7 mA m⁻² = 0.00627 mA cm⁻² can be calculated. This current density is ca. 80 to 160 times smaller than usually observed current densities of 0.5-1.0 mA cm⁻² when Geobacter spp.-based biofilms are cultivated at monolithic electrodes.[2–6] Although the porous nature of granules and there contacting surfaces were not considered, these calculations clearly indicate that the obtained current is comparable low suggesting that a considerable share of bed anode volume was not used for current production.
SI-5 Cyclic voltammetry control experiments

Figure SI-5. CV analysis of used e-Clamps without granules (i.e., e-Clamp control) and with non-inoculated granules (i.e., abiotic control).
Figure SI-6. Exemplary cyclic voltammograms of granules sampled from bedBES2 longitudinal axis during batch (A) and continuous (B) operation. The CV analyses were performed with the e-Clamp and granules from the middle pocket of the top (black lines), middle (red lines), and bottom sampling unit (blue lines). Scan rate: 1 mV s⁻¹, 3rd scans are shown.
**SI-7 First derivatives of cyclic voltammograms of granules sampled from the longitudinal axis of bedBES1 and bedBES2**

**Figure SI-7.** First derivatives derived from cyclic voltammograms recorded with granules sampled from the middle pocket of the top sampling unit of bedBES1 (corresponding Figure 2 in the main manuscript) and bedBES2 (corresponding to Figure SI-6) during batch (A) and continuous (B) operation.
Figure SI-8. Chronoamperometric cultivation of granules (three replicates, R1-3) sampled from bedBES2 longitudinal axis (top (A), middle (B), and bottom (C) sampling unit, in all cases from the middle pocket) during continuous operation. The granules were polarized at +200 mV for 10 h directly after CV analysis (see Figure SI-6).
SI-9 Cyclic voltammetry performed with sampled granules after 10 h of chronoamperometric cultivation

Figure SI-9. Cyclic voltammograms of a sampled graphite granule after 10 h of chronoamperometric (CA) cultivation at +200 mV (A). First derivatives derived from these cyclic voltammograms (B). $E_f$ shifted to more positive potentials ($E_{f,1} = -346.8 \pm 13.7$ mV) compared to the initial CV ($E_{f,2} = -374.3 \pm 10.7$ mV). The scan rate for CVs was 1 mV s$^{-1}$ (3rd scans are shown).
**SI-10 Chronoamperometric cultivation of granules sampled from transversal axis**

**Figure SI-10.** Chronoamperometric cultivation of granules sampled from bedBES1 transversal transect line (outer (A), middle (B), and inner (C) pocket, in all cases from the top sampling unit) during batch operation. The granules were polarized at +200 mV for 10 h directly after CV analysis (please see Figure 5A in the main manuscript).
**Figure SI-11.** Microbial community composition within bedBES2 determined with TRFLP analysis for the bacterial (A) and the methanogenic community (B). Granules from all pockets of the top (S 1.1, S 1.2, and S 1.3 for inner, middle, and outer sampling pocket, respectively), middle (S 2.1, S 2.2, and S 2.3), and bottom (S 3.1, S 3.2, and S 3.3) sampling unit were analyzed. Sampling was conducted at day 33 and day 62 for obtaining results for batch and continuous operation, respectively. Restriction enzymes *Hae*III and *Mwo*I were used for bacterial and methanogenic community, respectively.
**SI-12 Literature on acetate uptake rates of acetate-oxidizing anaerobic microorganisms**

**Table SI-12. Literature on acetate uptake rates of acetate-oxidizing anaerobic microorganisms**

| Microorganism                 | Electron acceptor | Acetate uptake rate / mmol Ac\(^{-}\) g\(_{DW}\)\(^{-}\) h\(^{-}\) | Reference |
|-------------------------------|-------------------|---------------------------------------------------------------|-----------|
| *Geobacter sulfurreducens*    | Fumarate          | 4.5                                                           | [7]       |
| *Geobacter sulfurreducens*    | Fe(III)-citrate   | 14.8                                                          |           |
| *Desulfobacter postgatei*     | Sulfate           | 3.2                                                           | [8]       |
| *Desulfobacca acetoxidans*    | Sulfate           | 2.6±0.8                                                       |           |
| *Desulforhabdus amnigenus*    | Sulfate           | 1.7±0.4                                                       |           |
| *Methanosaeta soehngenii*     | Carbon dioxide    | 4.6                                                           | [9]       |
| *Methanosaeta concilli*       | Carbon dioxide    | 1.9                                                           |           |

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