

Supplementary information I

1

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4 **S1.** Sediment core liner, water enclosure and Electric Potential measuring set-up

5 **S2.** NapA phylogeny

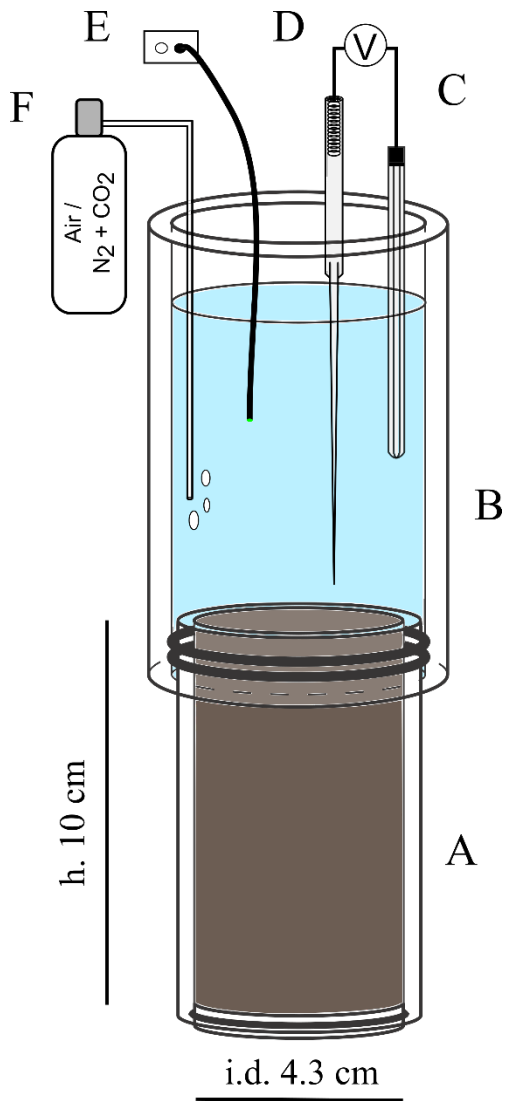
6 **S3.** NapD phylogeny

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8 **S5.** pOCC extended phylogeny

9 **S6.** Alignment of the amino acid sequences of the pOCC

10 **S7.** Extended discussion

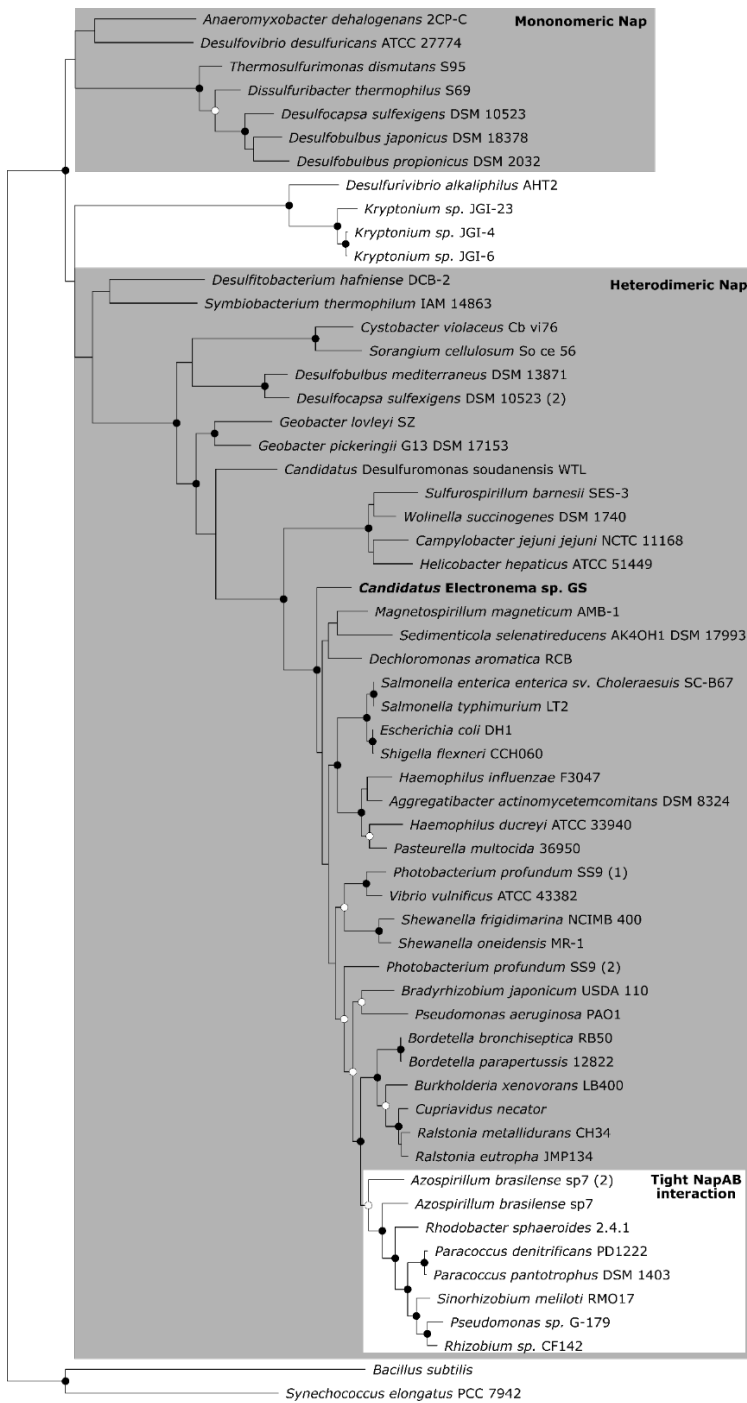


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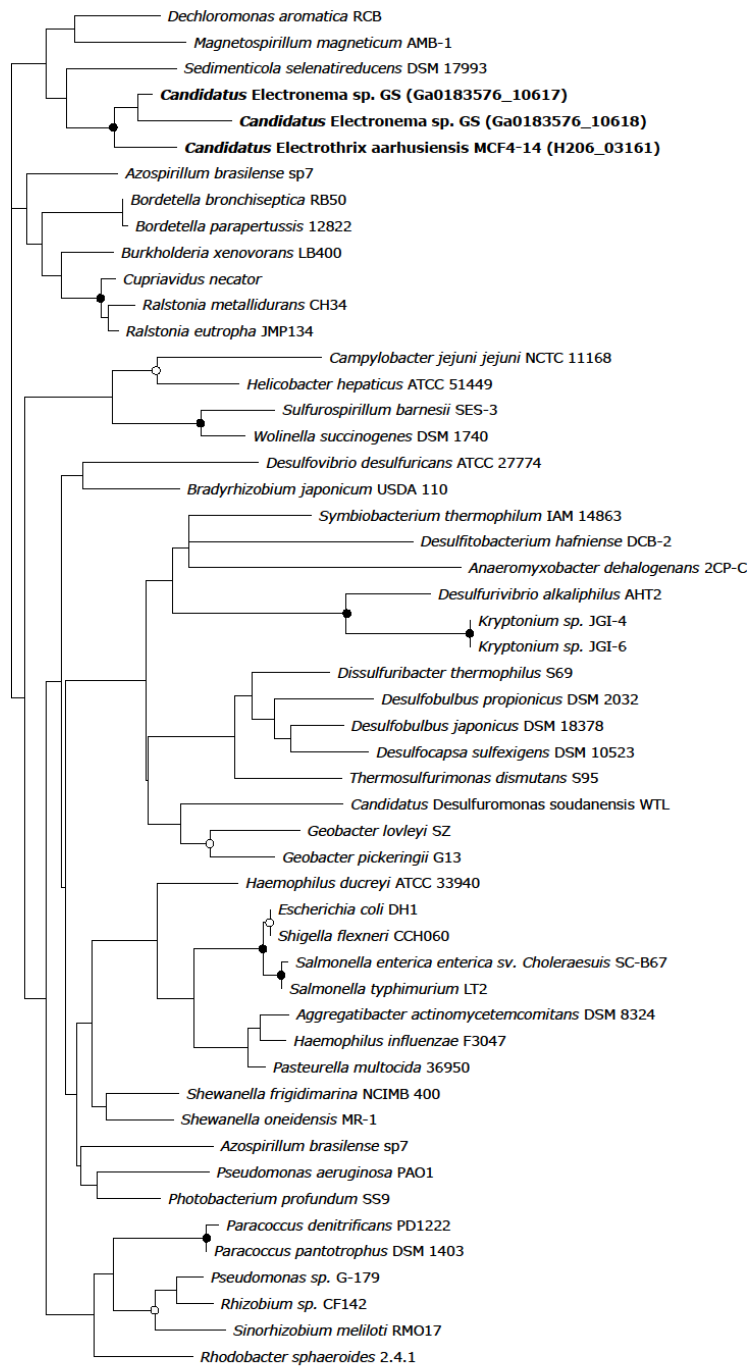
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13 **Figure S1.** Core liner and water enclosure for sediment incubation. Liner for sediment incubation
 14 (A), enclosure for water incubation with o-rings (B), Electric Potential sensor and reference (C),
 15 Oxygen fiber optode (E). Air or N₂ + CO₂ gas mix (F).

16



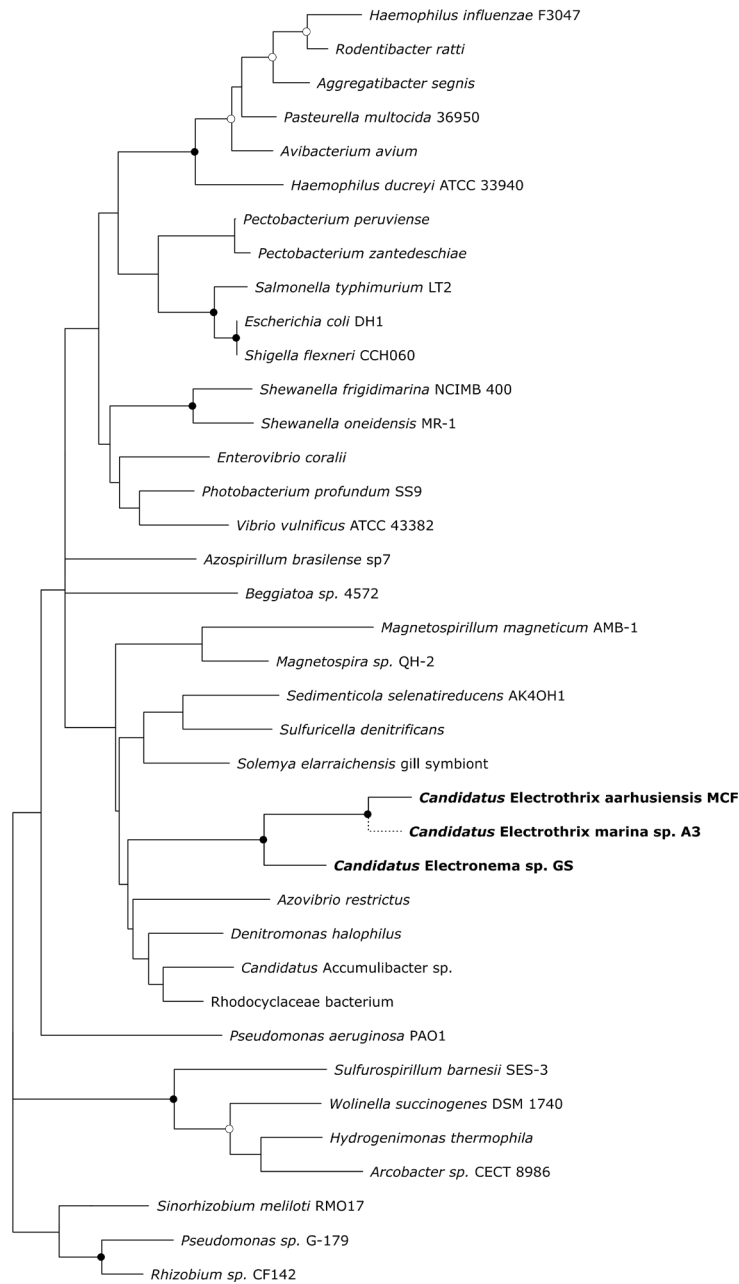
19 **Figure S2.** Phylogeny of the *napA* gene of *Ca. Electronema* sp. GS. Maximum likelihood tree
 20 supported by 1000x bootstrap resampling. Bootstrap values are represented by circles: open >70%,
 21 filled >90%. The scale bar represents 0.3 estimated amino acid substitutions. Tree was rooted with
 22 the nitrate reductase (*nas*) of *Bacillus subtilis*.



23

0.6

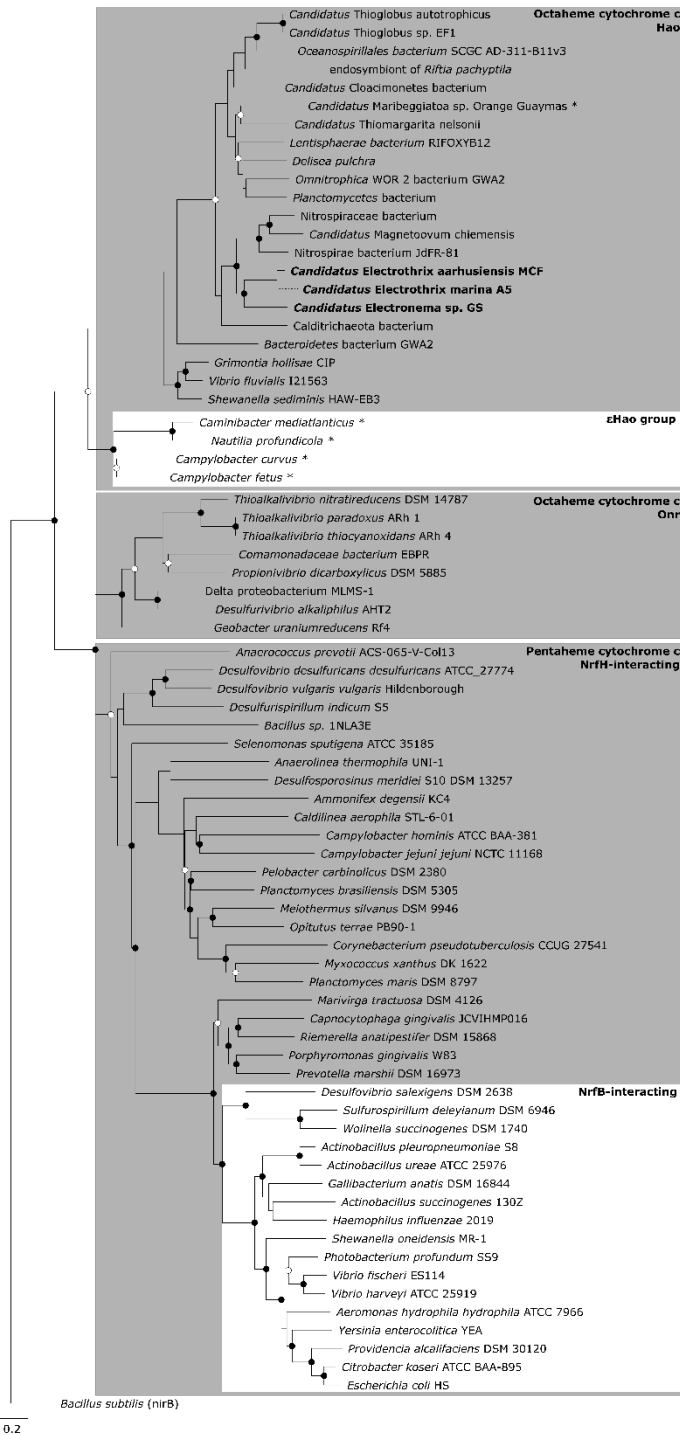
24 **Figure S3.** Phylogeny of the *napD* gene of *Ca. Electronema* sp. GS and *Ca. Electrothrix aarhusiensis*
 25 MCF. Maximum likelihood tree supported by 1000x bootstrap resampling. Bootstrap values are
 26 represented by circles: open >70%, filled >90%. The scale bar represents 0.6 estimated amino acid
 27 substitutions. Locus tags of cable bacteria genes are in parentheses.



28

29 **Figure S4.** Phylogeny of the *napF* gene of *Ca. Electronema* sp. GS, *Ca. Electrothrix aarhusiensis*
 30 MCF and *Ca. Electrothrix marina* A5. Maximum likelihood tree supported by 1000x bootstrap
 31 resampling. Bootstrap values are represented by circles: open >70%, filled >90%. The scale bar
 32 represents 0.3 estimated amino acid substitutions. The phylogenetic position of the short NapF
 33 fragment of *Ca. E. marina* (dotted line) was calculated by the maximum parsimony method in the
 34 program ARB without changing the overall tree topology.

35



36

37 **Figure S5.** Full phylogeny of the pOCC of *Ca. Electronema* sp. GS and *Ca. Electrothrix aarhusiensis*
 38 MCF. Maximum likelihood tree supported by 1000x bootstrap resampling. Bootstrap values are
 39 represented by circles: open >70%, filled >90%. The scale bar represents 0.2 estimated amino acid
 40 substitutions. The phylogenetic position of the short pOCC fragment of *Ca. E. marina* (dotted line)
 41 was calculated by the maximum parsimony method in the program ARB without changing the overall
 42 tree topology. Tree was rooted with the nitrite reductase (nirB) of *Bacillus subtilis*.

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92	H206_03164	QIMGSLDNMLAEVVEGNRGMVT-----ESFPDGI SAAAVNGCWQCHGSKVKIMKDG
93	BOGUAY_0691	RIIGSLDNLLADVVEGNRAMVT-----ESFPEGVSAAVNGCWQCHGSEVKVRKDG
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103	WP_011992073.1	MQMYANPA-IVKLMYHYEG-----ADHPDFKMAPDATGCTQCHGTVIKLDADH
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108	H206_03164	KLDP-ATWPN--SGIGRI-----NPDGSTGS-----CAACHSRHDF-
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193	2601634560	SNK-----IDFIWFELWHHEGRRARMAASMQGPDY-THWEGTYDLGKN--FYTELVPPELK
194	2730372430	AQK-----IDYTWFEIWHHEGRRVRHGAASMMAPDY-TQWHGNYDLAKN--WYSEYIPEIR
195	KJR43854.1	AQK-----IDYTWFEIWHHEGRRVRHGAASMMAPDY-TQWHGNYELARN--WYGEYVTELK
196	RMH60699.1	SDD-----IDFVWFELWHHEGRRARHGAASMMAPDY-THWHGTYDLAKN--FYTEFIPEIK
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198	WP_015902282.1	SDP-----FFKLYYYLWHHEGRRFRHGAAMVMSPDY-AHWHGVFQVMQD-----IREMK
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219	Ga0183576_10612	KEDEADKAERAKGAEFFKAKYAK-----

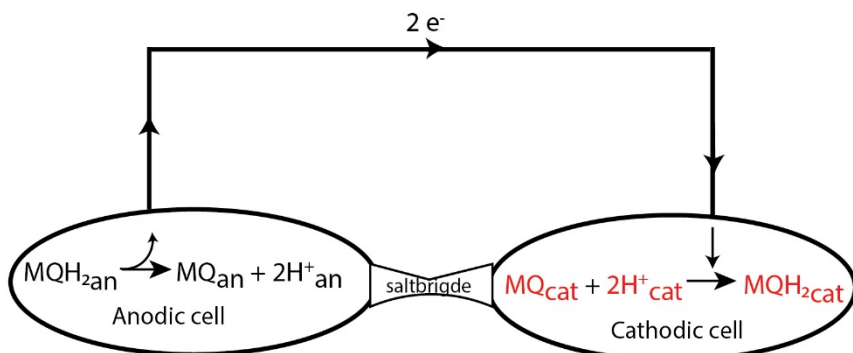
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221	BOGUAY_0691	KMSPEEAKARKESREAFIQRYGEQTDR----
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223	WP_053951924.1	KMSAKQQEIRKKATADFKAQFEK-----
224	2647705483	KMSAKQQEIRKKATADFKAQFEK-----
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227	KJR43854.1	KEDPAVKAERDKRRQEFLERYKAK-----
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233	2521963319	LLNDAIDAQVASR-----

234
235
236 **Figure S6.** Alignment of the amino acid sequences of the octaheme cytochromes encoded in the
237 genomes of *Ca. Electronema* sp. GS (Ga0183576_10612), *Ca. Electrothrix aarhusiensis* MCF
238 (H206_03164), *Ca. Maribeggiatoa* sp. (BOGUAY_0691), *Ca. Thiomargarita nelsonii*
239 (KHD08390.1), *Ca. Thioglobus autotrophicus* (WP_053951924.1), *Ca. Thioglobus* sp. EF1
240 (2647705483), endosymbiont of *Riftia pachyptila* (2601634560), Nitrospirae bacterium jdFR-81
241 (2730372430), *Ca. Magnetoovum chiemensis* (KJR43854.1), *Calditrichaeota* bacterium
242 (RMH60699.1), the ϵ HaO encoded in the genomes of *Caminibacter mediatlanticus*
243 (WP_007473950.1), *Nautilia profundicola* (WP_015902282.1), *Campylobacter curvus*
244 (WP_011992073.1), *Campylobacter fetus* (WP_002849252.1) and *Thioalkalivibrio* (2521963319).
245 The eight conserved heme binding motives (CxxCH) (yellow), a conserved tryptophan thought to be
246 important for reductive type HaO (green) and conserved residues located at the putative active sites
247 (blue) are highlighted.

248 **S7. Extended discussion**

249 **LINKING SULFIDE OXIDATION TO NITRATE REDUCTION VIA MENAQUINONE**
250 **CYCLING THROUGH LONG-DISTANCE ELECTRON TRANSFER IN CABLE**
251 **BACTERIA**

252 The metabolic model of nitrate reduction in the cathodic nitrate-reducing cells in cable bacteria
253 includes a menaquinone cycle in which a reduced menaquinone donates two electrons to the
254 menaquinol dehydrogenase NapGH, membrane component NapH. This results in the transfer of two
255 protons from the cytoplasm to the periplasm. The menaquinone is reduced back via sulfide oxidation,
256 which however takes place in a distant cathodic cell, most likely via reverse sulfate reduction (1).
257 Assuming the presence of an identical membrane-bound enzymatic apparatus for catalyzing
258 menaquinone oxidation-reduction reactions in the cathodic nitrate-reducing cell and in the anodic
259 sulfide-oxidizing cell, and that these apparatus are electrically connected with a conductor the
260 metabolic system linking sulfide oxidation to nitrate reduction via a menaquinone cycle can be
261 considered as a concentration cell (Fig. S7.1). This is a galvanic cell that has two equivalent half-cells
262 with the same reactants differing only in concentration. Such a concentration cell produces a voltage
263 as it attempts to reach chemical equilibrium, which occurs when the concentration of the reactant in
264 both half-cells are equal. This is achieved by transferring electrons from the half-cell with the highest
265 concentration of reduced compounds to the half-cell with a lower concentration of these. In the
266 following we will investigate, the thermodynamic constraints on the concentration cell model of
267 nitrate reduction in cable bacteria in order to elucidate if the flow of electrons between the
268 menaquinone in pool anodic (half) cells and the menaquinone pool in cathodic (half) cells via the
269 conducting fiber in cable bacteria is thermodynamic possible and can account for current observed
270 running in these organisms.



271

272 **Figure S7.1.** Conceptual scheme of a concentration cell.

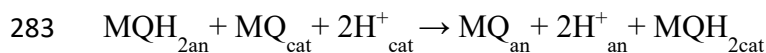
273

274 **The voltage yield of the concentration cell**

275 The voltage produced by the concentration cell can be estimated from the Nernst equation:

$$276 \quad E = E_o - \frac{0.0592}{n} \text{Log}Q \quad (\text{Eq. S1})$$

277 Where E_o is the standard cell potential, which is defined as the sum of the reduction potential and the
 278 oxidation potential of the half reactions. n is the number of moles of electrons transferred, and Q is
 279 the reaction coefficient. The reduction potential (E_{red}) for the reaction $\text{MQ} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{MQH}_2$ is -
 280 67 ± 10 mV (2) and the oxidation potential is consequently $+67 \pm 10$ mV. Hence, E_o equals zero. The
 281 full reduction of 1 mole of menaquinones implies the transfer of two moles of electrons and n
 282 consequently equals 2. The reaction coefficient Q is defined from the overall reaction:



284 Thus:

$$285 \quad Q = \frac{[\text{MQH}_{2\text{cat}}][\text{MQ}_{\text{an}}][\text{H}^+_{\text{an}}]^2}{[\text{MQH}_{2\text{an}}][\text{MQ}_{\text{cat}}][\text{H}^+_{\text{cat}}]^2} \quad (\text{Eq. S2})$$

286 The proton source for the menaquinone cycle is the cytoplasm. As an equivalent pH of the cytoplasm
 287 of cathodic and anodic half-cells can be assumed, $[\text{H}^+_{\text{an}}]^2/[\text{H}^+_{\text{cat}}]^2$ approximates 1. Hence:

288
$$Q \approx \frac{[MQH_{2cat}][MQ_{an}]}{[MQH_{2an}][MQ_{cat}]} \quad (\text{Eq. S3})$$

289 With a and b denoting the fraction of oxidized menaquinones (MQ) to the total pool of menaquinones
 290 (MQ_{Tot}) in the anodic cell and cathodic cell, respectively we have:

291
$$MQ_{an} = a MQ_{Total\ anode} \quad (\text{Eq. S4})$$

292
$$MQH_{2an} = (1-a) MQ_{Total\ anode} \quad (\text{Eq. S5})$$

293 for the anodic cell, and:

294
$$MQ_{cat} = b MQ_{Total\ cathode} \quad (\text{Eq. S6})$$

295
$$MQH_{2cat} = (1-b) MQ_{Total\ cathode} \quad (\text{Eq. S7})$$

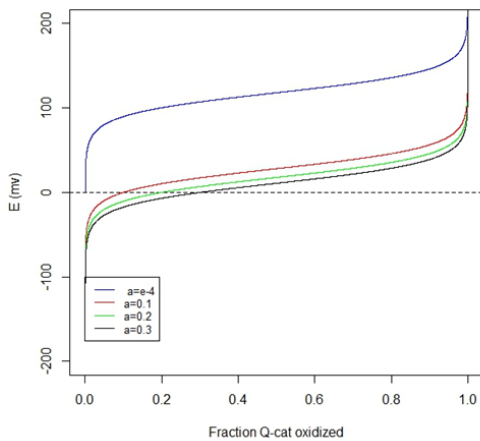
296 for the cathodic cell. Thus:

297
$$Q = \left(\frac{[(1-b) MQ_{Total\ cathode}][a MQ_{Total\ anode}]}{[(1-a) MQ_{Total\ anode}][b MQ_{Total\ cathode}]} \right)$$

$$= \left(\frac{(1-b) a}{(1-a) b} \right) \quad (\text{Eq. S8})$$

298 With this expression for Q the voltage produced by the concentration cell is given by:

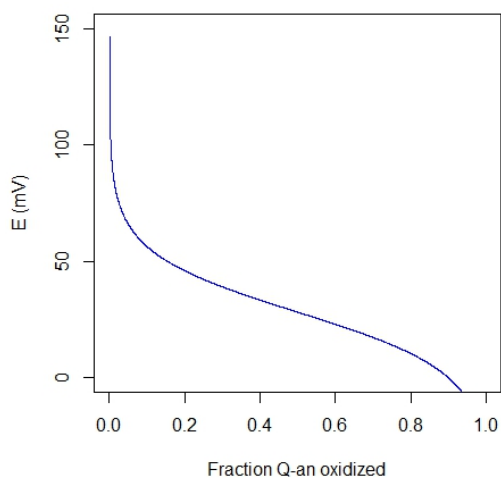
299
$$E = -\frac{0.0592}{2} \text{Log} \left(\frac{(1-b) a}{(1-a) b} \right) \quad (\text{Eq. S9})$$



300 **Figure S7.2.** *The voltage yield (E) of the concentration cell for different values of a (the fraction of*
301 *oxidized menaquinones in the anodic half-cell) as function of the fraction of menaquinones oxidized*
302 *in the cathodic half-cell (b).*

303

304 Equation S9 allows to calculate the voltage yield (E) of the concentration cell for different values of
305 a and b . A positive voltage yield ($E > 0$) implies that the concentration cell operates in the forward
306 direction (electron transfer from the anodic half-cell to cathodic half-cell), whereas a negative voltage
307 yield implies electron transfer in the opposite direction. As shown in Figure S7.2, electron transfer
308 from the menaquinone pool in an anodic half-cell to the menaquinone pool in a cathodic half-cell is
309 most favorable if the electrons are delivered from an anodic half-cell with an almost completely
310 reduced (99.99%, $a = 0.0001$) menaquinone pool. For such a system, electron transfer in the forward
311 direction is possible if only 0.01% ($b = 0.0001$) or more of the menaquinone pool is kept oxidized in
312 the cathodic half-cell. The thermodynamic threshold increases when electrons are delivered from
313 anodic half-cells with a less reduced menaquinone pool. If electrons are delivered from anodic half-
314 cells having 90% of the menaquinone pool reduced the recipient half-cell should keep more than 10%
315 of the menaquinone pool oxidized for the reaction to proceed, and for anodic cell having 70% of their
316 menaquinone pool reduced, the recipient cell should keep more than 30% of its menaquinone pool
317 oxidized, etc. The voltage yield is highest if electrons can be delivered from an anodic half-cell having
318 an almost fully reduced menaquinone pool, and for such a system the voltage produced falls in the
319 range 80-150 mV for $5\% < b < 92\%$. The voltage yield drops significantly when the menaquinone
320 pool in the anodic half-cell becomes more oxidized. Figure S7.3 shows the voltage yield for a
321 concentration cell, where 90% of the menaquinone pool in the cathodic half-cell is oxidized ($b = 0.1$).
322 The 80-150 mV range is only obtained for cells where more than 98% of the menaquinone pool in
323 the anodic half-cell are reduced.



324

325 **Figure S7.3.** *The voltage yield of a concentration cell as function of the fraction of menaquinones*
 326 *oxidized in the cathodic half-cell (b) where 90% of the menaquinone pool in the cathodic half-cell*
 327 *are oxidized.*

328

329 **The current in the concentration cell**

330 The above considerations points to the idea that sulfide oxidation coupled to nitrate reduction via
 331 menaquinone cycling, through long-distance electron transfer, is thermodynamically possible and the
 332 next question to be addressed is the if the voltage generated from the concentration cell in a certain
 333 configuration can drive an electric current comparable to the current running in metabolic active cable
 334 bacteria. For simplicity, we will consider a steady-state situation. The drivers of the model are the
 335 concentrations of oxidized and reduced menaquinones in the anodic and cathodic half-cells (eq. S2)
 336 We will assume that the redox state of the menaquinonens in the anodic and cathodic cells of the cable
 337 bacteria are controlled by: 1) the rate of menaquinone reduction via sulfide oxidation in the anodic
 338 cells, 2) the rate of menaquinone oxidation in the anodic cells via the current generated from the
 339 concentration cell, 3) the rate of menaquinone reduction in the cathodic cells via the current generated

340 from the concentration cell, and 3) the rate of menaquinone oxidation via nitrate reduction. At steady-
 341 state, (i.e. a, and b are constant) these rates are equal in magnitude. We will further assume that the
 342 total pool of menaquinones (MQ_{Tot}) in the anodic cell equals the total pool in the total pool of
 343 menaquinones (MQ_{Tot}) in the cathodic cells and that the rate constant for menaquinone reduction in
 344 the anodic cell is equal to the rate constant for menaquinone oxidation in the cathodic cell. This
 345 implies that $a = (1-b)$ in equation S9, which then can be simplified to:

$$346 \quad E = -\frac{0.0592}{2} \text{Log} \left(\frac{b^2 - 2b + 1}{b^2} \right) \quad (\text{Eq. S10})$$

347 With this expression we can estimate the current in the steady-state situation by means of ohms law.

$$348 \quad I = E/R$$

349 Where I is the current, E the voltage yield of the concentraion cell, and R is the resistance of the wire.

350 The resistance of wire can be determined as in (3), *i.e.*,:

$$351 \quad R = \frac{l}{\sigma A} \quad (\text{Eq. S11})$$

352 Here l is the length of the wire, σ the conductivity and A the cross section area of the wire.

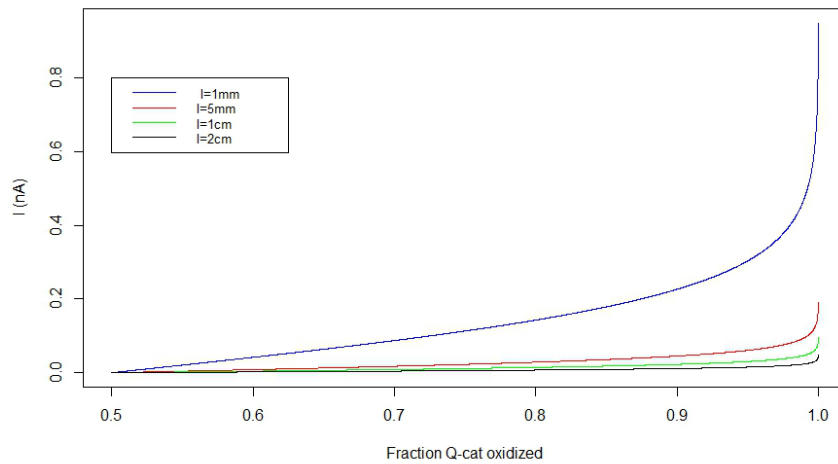
353 The current can therefore be estimated as:

$$354 \quad I = -\frac{0.0592}{2} \text{Log} \left(\frac{b^2 - 2b + 1}{b^2} \right) \frac{\sigma A}{l} \quad (\text{Eq. S12})$$

355 The conductive element in cable bacteria is a periplasmatic network of discrete fibres (4). Each of
 356 those with a diameter of ca. 50 nm, corresponding to a cross section area of $2 \times 10^{-15} \text{ m}^2$, and a
 357 counductivity of 20.1 S cm^{-1} , corresponding to $2.01 \times 10^3 \text{ S m}^{-1}$ (4).

358 Figure S7.4, shows the current running between two half cells, connected with a wire having
 359 conductive properties similar to a cable bacteria fibre. The current flows from the anocic half cell to
 360 the cathodic half cell if the fraction of oxidized menaquinones to the total pool of menaquinones in

361 the cathodic half cell (b) is > 0.5 . The current increases for $b \rightarrow 1$. There is an inverse relationship
362 between the length (l) of the wire connecting the anodic and cathodic half-cells and the magnitude of
363 the current running through it. Half cells connected with a short wire in general have a higher current
364 flowing between them than half-cells connected with a long wire.



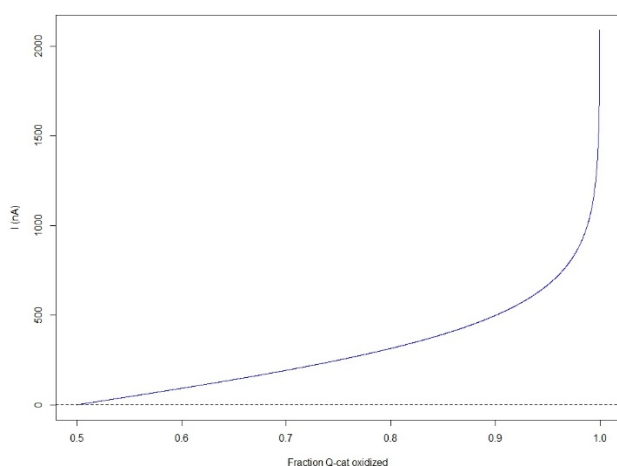
365

366 **Figure S7.4.** Steady state current between half cells in the concentration cell, as function of b : the
367 fraction of oxidized to total menaquinones in the cathodic half cell. The half cells are electrically
368 connected with a wire having conductive properties similar to a conducting fibre in cable bacteria.
369 Colored lines represent the current in wires at different length (l).

370

371 A cable bacterium can be considered as a composite of concentration cells with a series of anodic half
372 cells coupled to a few cathodic half cells via the conducting periplasmic network of fibres. We will
373 for simplicity consider a virtual cable bacterium with 5000 anodic cells, each coupled to a cathodic
374 cell. The cathodic cell can in principle be the same for all anodic cells. 5000 cells spans a distance of
375 2 cm and in our simplified model the wire connecting the most distant anodic cell to the cathodic cell
376 is 2 cm long while the wire connecting the nearest anodic cell to the cathodic cell is 4 μm long. In
377 general, the length of the wire for the cells between the most distant and the nearest cell is equal to
378 their distance to the cathodic cell, which is in a fixed position for all anodic cells. According to

379 equation 12 and Fig S2.4, the implication of such a configuration is that the metabolic activity of the
380 cells in the filament decreases with the distance to the cathodic cell. Figure S7.5 shows the current
381 integrated for all 5000 cells in the filament. As seen, the current generated from this composi-
382 tution concentration cell model of a cable bacterium vastly exceeds the 0.2 - 0.36 nA estimated for natural
383 cable bacteria (5-7) for most redox states of the menaquinone pool in the cathodic cell. Already
384 with 50.11% of the menaquinones in the cathodic cell being oxidized, the current is >1 nA.



385

386 **Figure S7.5.** Current produced from 5000 individual half-cells (the approx. number of cells in a 2 cm
387 long filament). Dashed line indicates the max filament specific current production (0.4 nA) reported
388 in the literature.

389

390 Conclusion

391 Here we have shown using the concentration cell model, that electron transport from a menaquinone
392 pool in an anodic cell to a menaquinone pool in a cathodic cell is thermodynamically possible, and
393 when the concentration cells are brought together in a way that simulates a cable bacterium, that the
394 voltage produced from such cells can be sufficiently high to drive an electric current that exceeds the
395 current reported for cable bacteria.

396
397

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