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Supplementary Information for

NanoSIMS imaging reveals metabolic stratification within current-producing biofilms

Grayson L Chadwick*, Fernanda Jiménez Otero*, Jeffrey A Gralnick, Daniel R Bond, and Victoria J Orphan

*These authors contributed equally to this work.

Victoria J. Orphan
Email: vorphan@gps.caltech.edu

Daniel R. Bond
dbond@umn.edu

This PDF file includes:

Figures S1 to S4

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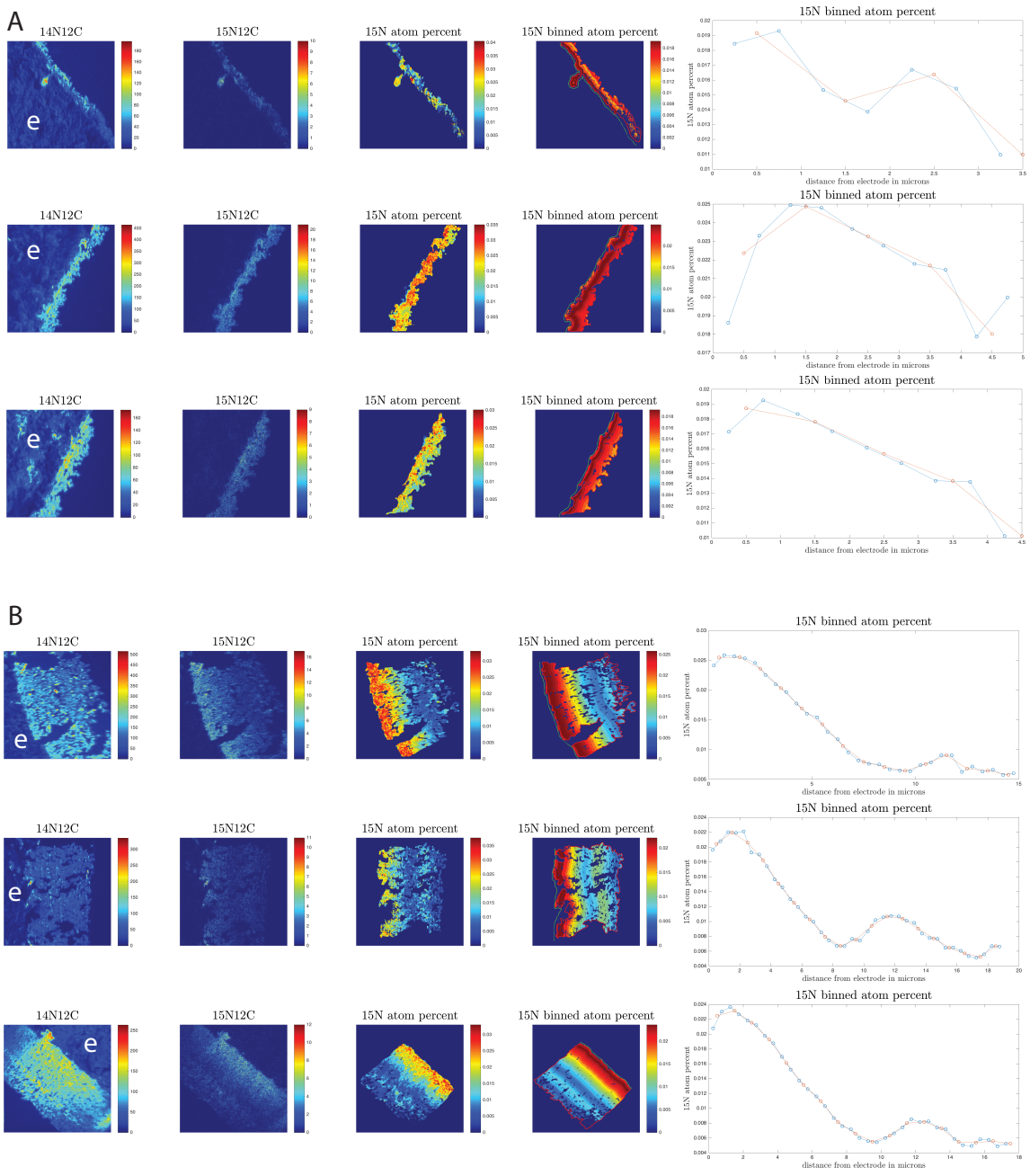

Fig. S1. Repeatability of nanoSIMS analysis of *G. sulfurreducens* biofilms grown using a graphite electrode poised at +240 mV vs. SHE during (A) exponential and (B) current plateau phases. Panels from left to right correspond to $^{14}N^{12}C^-$ ion image demonstrating biofilm morphology and spatial resolution. The location of the graphite anode is labeled on each $^{14}N^{12}C$ ion panel with the letter "e". Second panels correspond to heavy nitrogen isotope image $^{15}N^{12}C^-$, and third panels to ^{15}N fractional abundance image, all revealing an enrichment at the anode surface. Plots to the right of nanoSIMS images correspond to binned anabolic activity patterns in each additional representative *G. sulfurreducens* biofilm. These data all contributed to the average activity plots in Figure 2D and Figure 4D.

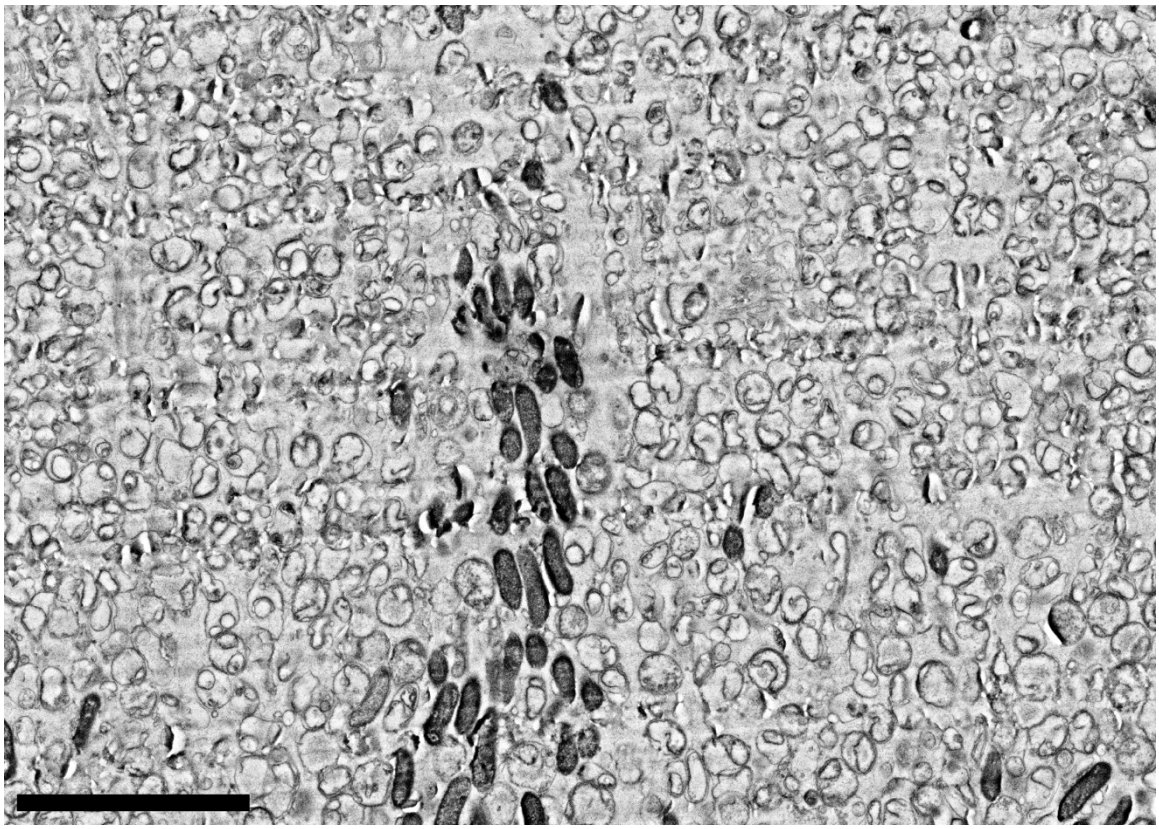

Fig. S2. SEM image of a region of lysing cells in thin section of weeks old *G. sulfurreducens* biofilms (detail of biofilm from Fig. 6a in the main text). Cells with more electron density retained the characteristic size and shape of healthy *Geobacter* rods, while low density material appeared to be made up of lysed cells with irregular broken membranes. Intact cells yielded much greater $^{14}N^{12}C^-$ ion counts in nanoSIMS images, indicating dense biological material, and are oriented perpendicular to the surface of the anode (~20μm below the region displayed here). Scale bar is 5 μm.

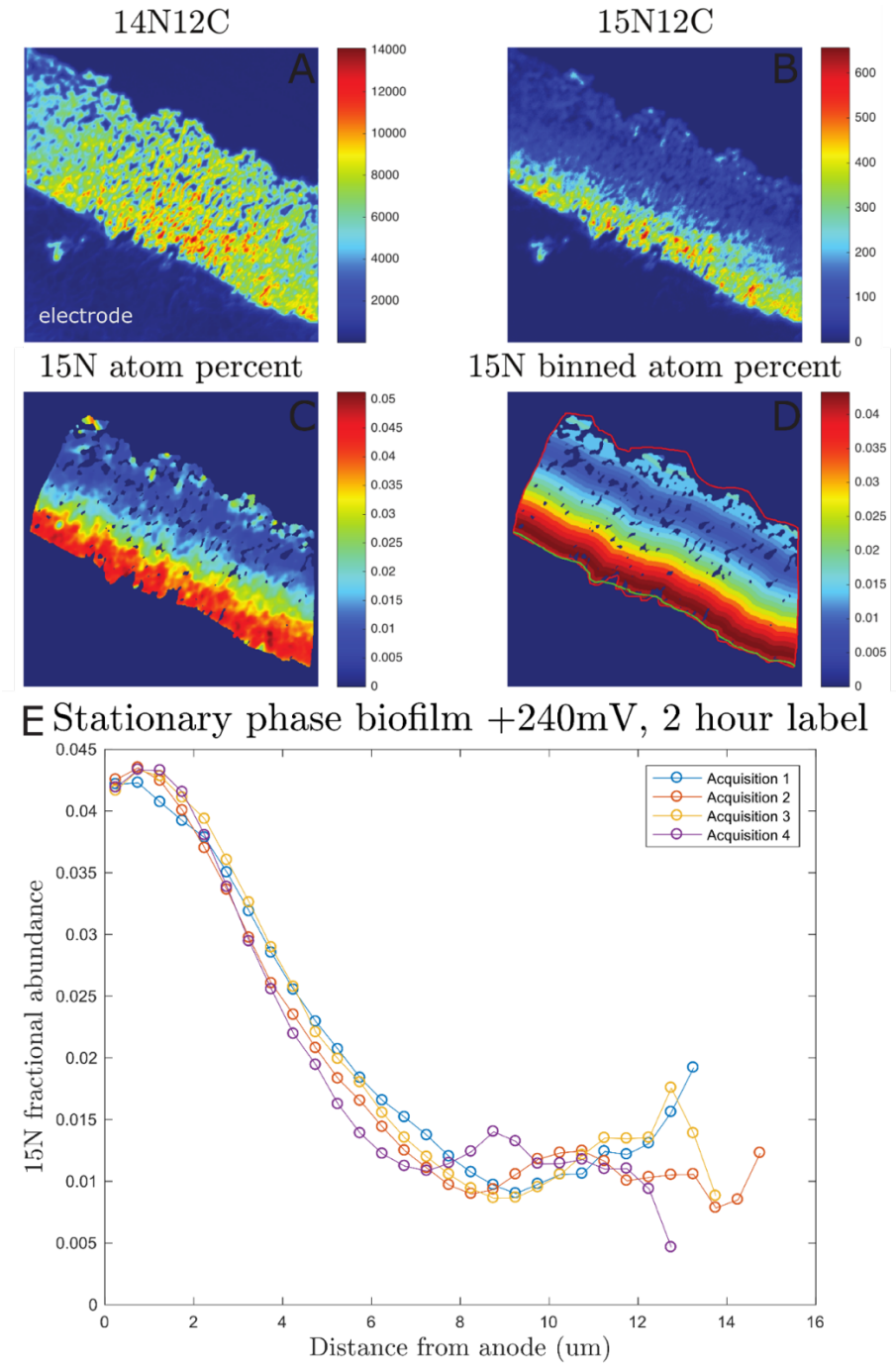

Fig. S3. Short stable isotope probe experiment with higher percent heavy isotope label reveals similar activity patterns. (A-D) Representative nanoSIMS image of the biofilm grown at +240 mV and labeled for ~2 hours with 3x heavy isotope percent medium once current plateau was reached. Acquisition region 30x30µm. In (C) and (D) only regions defined as biofilm by manual tracing and with 14N12C counts above background are shown (as described in the methods). In (D) manual trace of the biofilm is shown in red, with electrode trace in green. (E) Binned fractional abundance of 15N in the biofilm at four different acquisition locations demonstrating the characteristic enriched activity at the anode interface and small peak in activity at the top of the biofilm.

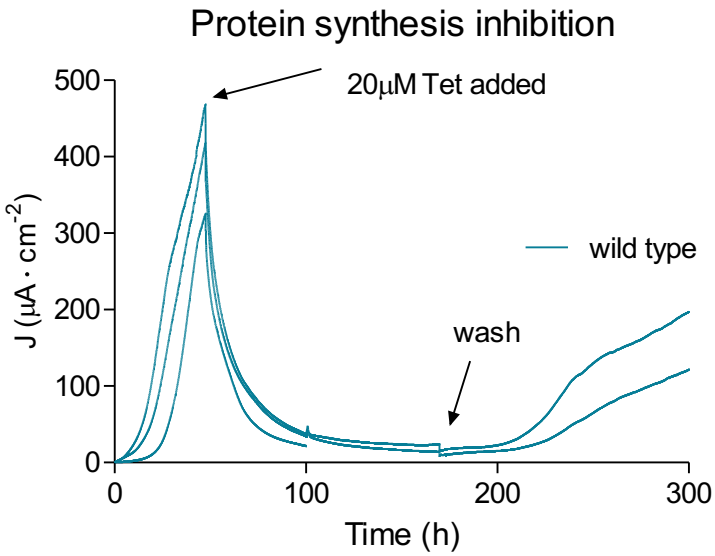

Fig. S4. Current production of wild type *G. sulfurreducens* biofilms grown in triplicate until current reached plateau, then 20 μM tetracycline (Tet) was added. Once current density remained below 30 μA·cm-2 for 24 hours, medium was exchanged for fresh medium with no antibiotic.