Geobacter sulfurreducens biofilms developed under different growth conditions on glassy carbon electrodes: insights using cyclic voltammetry

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Supplementary Information

Experimental

Preparation of Geobacter Sulfurreducens biofilms. Custom built glassy carbon rod (Goodfellow, UK) electrodes were used to form *G. Sulfurreducens* biofilms. These were made by shrouding glassy carbon rods, of various dimensions, exposed in glass tubes using heat-shrink plastic tubing (Alphawire, UK) and establishing an electrical connection at the rear with a 0.3 cm dia copper rod (Farnell electronics, Ireland) and silver epoxy adhesive (Radionics, Ireland). Prior to use, these electrodes were sterilized by placement in boiling 0.1 M H_2SO_4 for 15 min, and washed several times with distilled water, followed by placing in absolute ethanol overnight. Verification of electrical connectivity was established by cyclic voltammetry in buffer electrolyte.

G. sulfurreducens (ATCC 51573, from German Collection of Microorganisms and Cell Culture centre) was used as a source of electro-active bacteria. The strain was sub-cultured in 100 mL air tight, rubber septa-sealed, anaerobic syringe bottles containing 70 mL of growth medium. The growth medium was prepared according to the protocol supplied by the culture centre (http://www.dsmz.de, medium No. 826). The bacteria, prior to inoculation in the electrochemical cell, were cultured in fumarate-containing *Geobacter* growth medium for ~2 weeks (3 sub-cultures) and subsequently grown in the presence of growth medium and 10 mM acetate initial concentration under applied potential, with replenishment of acetate periodically when the amperometric current decreased to baseline levels. CVs were recorded after one hour following removal of the electrodes from the growth medium and replacement in new medium containing 10 mM acetate. All inoculations were carried out in a sterile anaerobic glove box (Coy Laboratory, USA) and incubations were performed at 30 °C in a sterilized controlled-temperature hot room.

Biofilms were formed in duplicate under constant applied potentials (-0.2 V, 0 V, 0.2 V and 0.4 V *vs.* Ag/AgCl) using a multi-channel potentiostat (CHI-1030a, CH Instruments, USA) in a three electrode electrochemical cell. Biofilms were grown simultaneously on graphite rod working electrodes, poised at various applied potentials, in the same electrochemical cell, using a platinum gauze (5 cm x 5 cm) as counter electrode and an Ag/AgCl electrode (BioAnalytical Systems, USA) as reference. Biofilm formation was continuously monitored by cyclic voltammetry at regular intervals under the above conditions.

Scanning electron microscopy analysis (SEM) Electrodes were removed from electrochemical cell and fixation undertaken by placing in the following solutions: a) 1% glutaraldehyde, 2% paraformaldehyde, 0.2% picric acid, 10 mM HEPES (pH 7.4) for 1 h, b) 50mM NaN_3 for 1 h, c) 2% tannic acid for 1 h, d) 1% osmium tetroxide for 2 h, e) 1% thiocarbohydrazide for 30 min, f) 1% osmium tetroxide over night, and g) 2% uranyl acetate for 2 h, while washing with 10 mM HEPES buffer (pH 7.4) between steps . Then samples were dehydrated in a graded series of aqueous ethanol solutions (10–100%) and then oven dried (2 hrs at 40 ^{0}C) to remove residual moisture. The dried samples were mounted over SEM stubs with double-sided conductivity tape and a thin layer of gold metal applied using an automated sputter coater (Emitech, K550) for 1 min. The biofilm samples were then examined using a SEM (Model 4700, Hitachi, Japan).

Epi-fluorescent imaging of Geobacter biofilms. Electrodes were transferred to sterile containers with 50 mL of sterile saline phosphate buffer (3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4). Then the entire biofilm from the electrodes was suspended in the buffer by shaking followed by 30 seconds sonication. The entire sample was then stained with SYBR gold (20× diluted) for 20 min at room temperature. Entire samples were filtered individually onto 0.2 μm pore-size polycarbonate filters (25 mm diameter; Costar Scientific Coproration, Bucks., UK) by applying a slight vaccum. After filtration, filters were mounted in oil and observed immediately by epi-fluorescence microscopy using an Olympus BX40F4 microscope (Olympus, Japan). Slides were viewed using 100× oil immersion (UPlanFl 100× lens), under blue light filter (WB) in the dark.

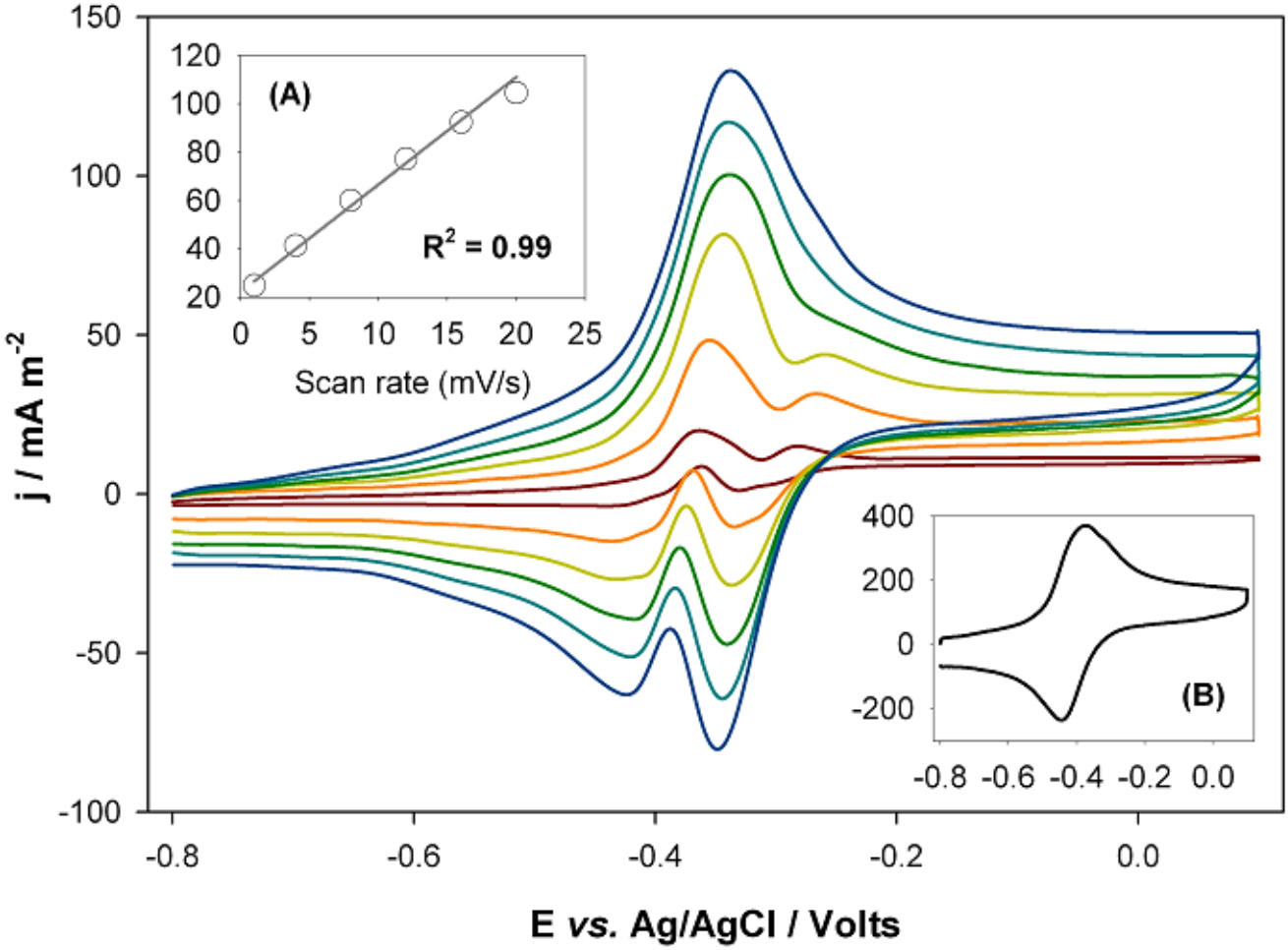

Fig. S1 Scan rate dependent study of a ~300 hrs aged biofilm at substrate limiting condition (~ 180 µM acetate concentration) grown at 0 V *vs.* Ag/AgCl applied potential. Inset figures show the effect of scan rate on peak currents (A) and a CV at 100 mV/sec scan rate of the biofilm (B).

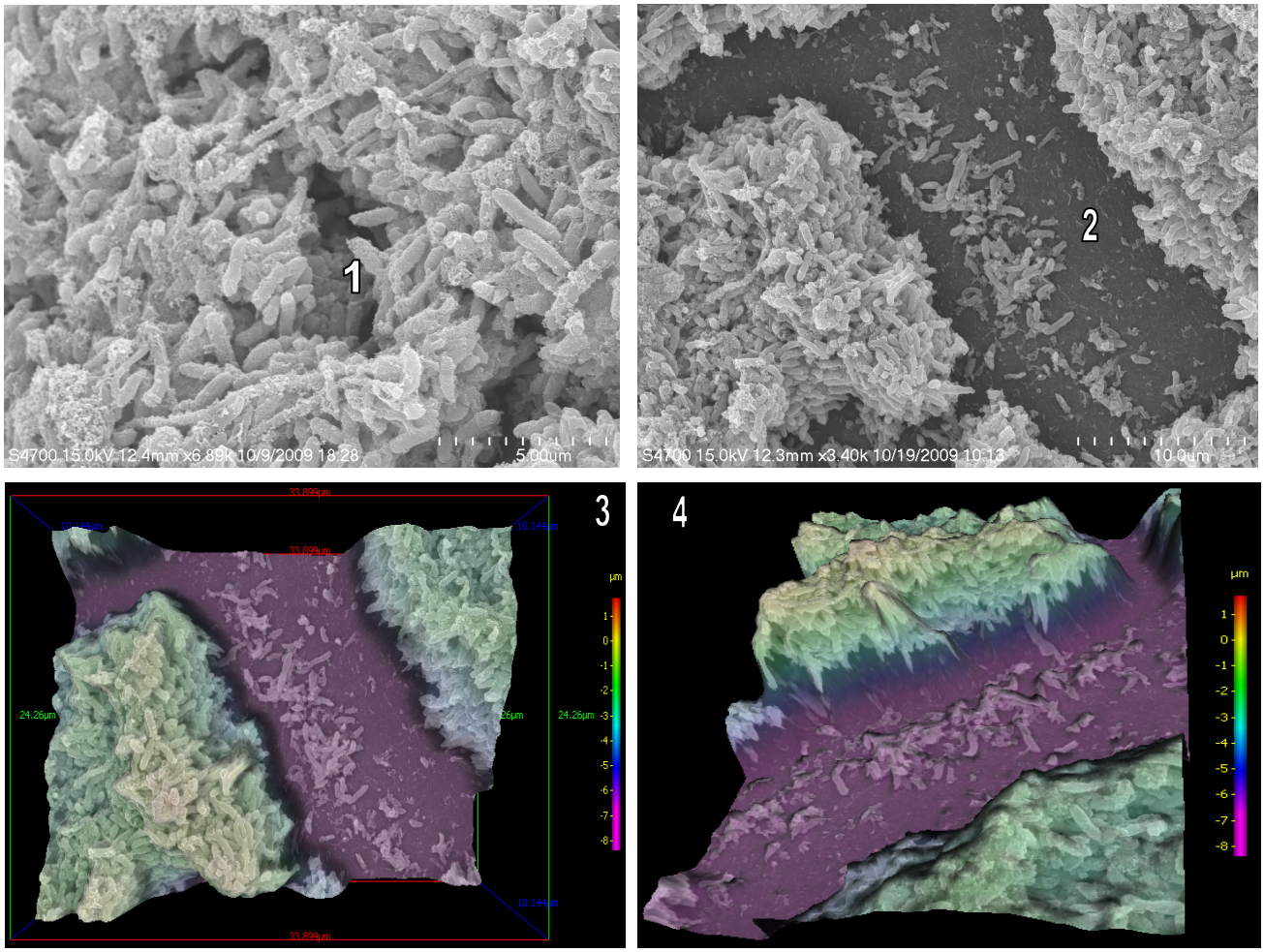

Fig. S2 Scanning electron microscopy (SEM) images of *Geobacter* biofilms on glassy carbon rods grown under constant potential (0 V *vs.* Ag/AgCl). 1 & 2 images are captured at different locations on electrode and 3 & 4 are 3 D - SEM images of biofilm 2.

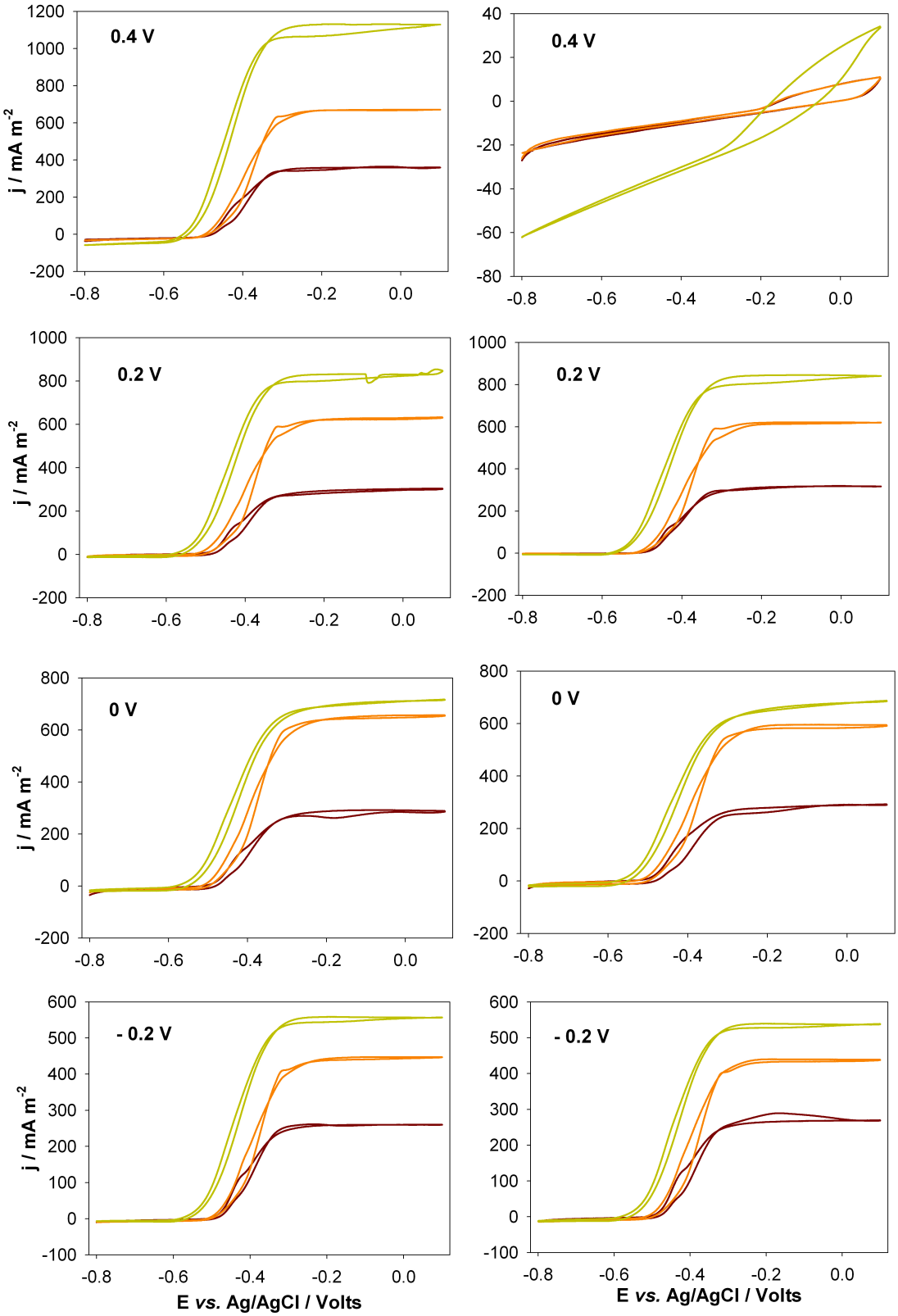

Fig. S3 CVs of *G. sulferreducens* biofilms developed under different applied potentials. Maroon, orange and pale green lanes represent 50, 100 and 250 hrs aged biofilms. The steady-state currents increase with increase in biofilm age and the magnitude in steady-state current densities increase with increase in potential applied to grow biofilms. Experiments conducted in duplicate, with an obvious high-resistance, and therefore poor electrical connection, hampering the analysis of the duplicate for the 0.4 V polarised electrode (top right).

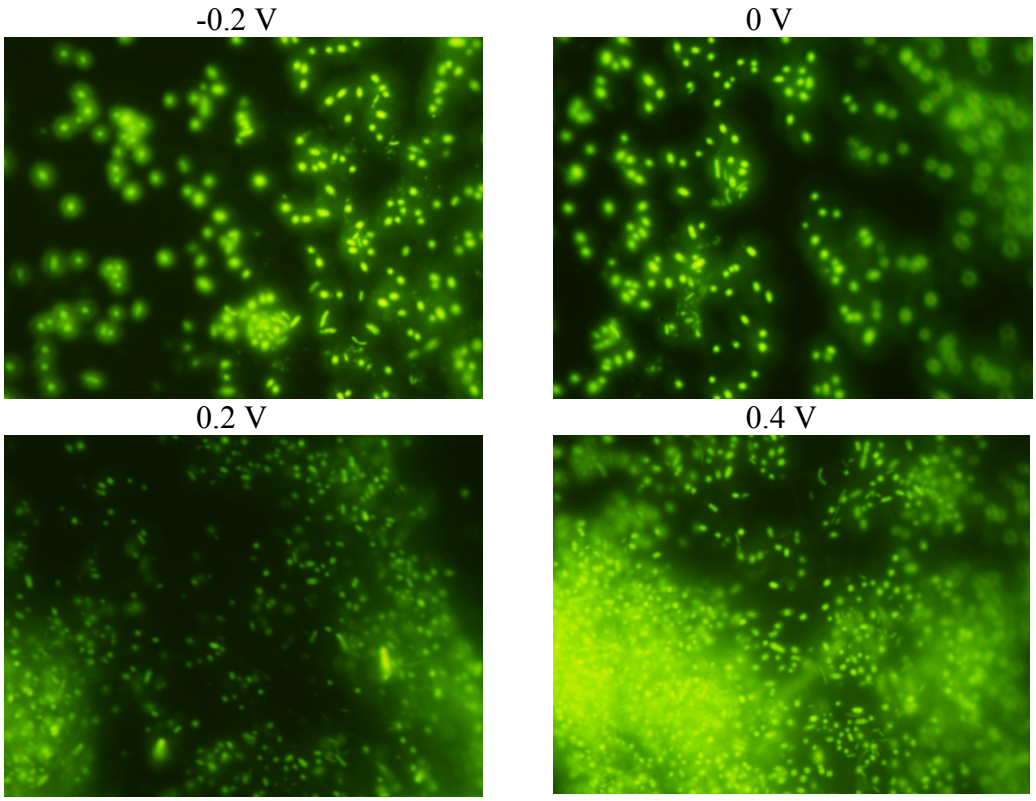

Fig. S4 Epifluorescent images of biofilms grown under different applied potentials.