

Biofilm Structure of *Geobacter sulfurreducens* by Helium Ion Microscopy

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Microbial communities form biofilms on material surfaces in a multitude of ecosystems, from the root hairs of a plant to the human gut. The hallmarks of an established biofilm include (1) the attachment of microbial cells to a surface, (2) production of extracellular polymeric substance, (EPS) (3) a complex structure or “architecture,” and (4) the ability to exchange genetic information between cells¹. *Geobacter sulfurreducens* forms unique, electrically conductive biofilms, a property that can be exploited in production and design of microbial fuel cells. In this work, we examine biofilm formation, and biofilm properties of *Geobacter sulfurreducens* using a Scanning Electron Microscope (SEM) as well as a Helium Ion Microscope (HIM).

SEM is a high-resolution imaging technique used for characterization of a broad variety of materials. However, in order to image highly insulating, soft biological materials, the samples must be coated to inhibit charging. These (typically) metallic coatings create a homogenous surface and may cloak true biological structure and material contrast in the micrograph. In the case of *Geobacter sulfurreducens*, metal coating precludes detailed investigation of microbial attachment, presence of EPS, and fine surface details that may elucidate the mechanisms behind architecture formation and genetic material exchange.

Recently introduced HIM, offers more flexibility in investigating biological samples, as highly insulating samples can be imaged *sui generis*, without the use of a conductive coating². This opens new pathways to capturing high resolution spatial details of biofilm formation and biofilm properties. Furthermore, high-resolution HIM imaging reveals true surface details of *Geobacter sulfurreducens*, such as flagella or pilin typically inaccessible by SEM.

References:

¹ Donlan, R. M. (2002). Biofilms: Microbial Life on Surfaces. *Emerging Infectious Diseases*, 8(9), 881–890. doi.org/10.3201/eid0809.020063

² Joens, M. S., Huynh, C., Kasuboski, J. M., Ferranti, D., Sigal, Y. J., Zeitvogel, F., Obst, M., Burkhardt, C. J., Curran, K. P., Chalasani, S. H, Stern, L. A., Goetze, B., and Fitzpatrick, J. A. (2013). Helium Ion Microscopy (HIM) for the imaging of biological samples at sub-nanometer resolution. *Scientific Reports*, 3(3514). doi:10.1038/srep03514

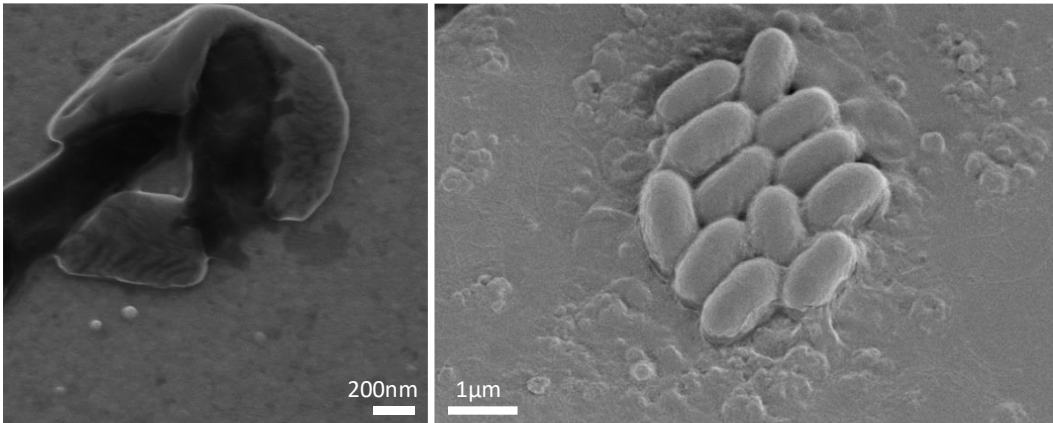


Figure 1. A) Helium Electron Microscope micrograph of Geobacter sulfurreducens on glass. Sample preparation: 1 mL of 4% formaldehyde was pipetted onto glass coverslip with Geobacter sulfurreducens attached, and left for 5 minutes. Sample was tilted, washed twice with 0.5 mL of phosphate buffer saline solution, and then rinsed with 95% ethanol. B) Scanning Electron Microscope micrograph Geobacter sulfurreducens on glass, 10 nm of chromium (no chemical of physical fixation) coating prior to imaging.