

## Characterizing Riverine Biofilms Using Scanning Transmission X-ray and Transmission Electron Microscopies

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Biofilms, which can form on many moist surfaces, are a complex mixture of cells and extracellular polymeric substances (EPS). The EPS serve many functions, including: (i) sorption of organic (e.g., antimicrobial agents) and inorganic (e.g., metals) compounds; (ii) trapping of particulates such as organic debris, humic substances, clays and other minerals; (iii) compound transformations. X-ray absorption spectroscopy using synchrotron light sources can identify and quantify chemical species in complex matrices at a spatial resolution of 50 nm [1]. The X-ray absorption spectral signals in scanning transmission X-ray microscopy (STXM) are used to map biomolecular structure and allow the correlation of elements such as Ca, K, Fe, Mn, Ni, etc. with protein, lipid, carbohydrate and nucleic acid components of the biofilm [2]. Since the chemical and structural integrity of the sample after STXM analysis remains intact due to reduced radiation damage [3,4], further analysis using transmission electron microscopy (TEM) is possible. Conventional TEM is useful for studying the microstructure of biofilms at high resolution and can provide analytical information on the elemental composition of a sample. These approaches provide a combination of suitable spatial resolution and chemical information at the microscale and may be used to create a detailed correlative map of biofilm structure and composition (Fig. 1). Our studies are intended to improve understanding of the organization of biofilms and their capacity to sequester trace organic and inorganic compounds from the surrounding environment.

### References

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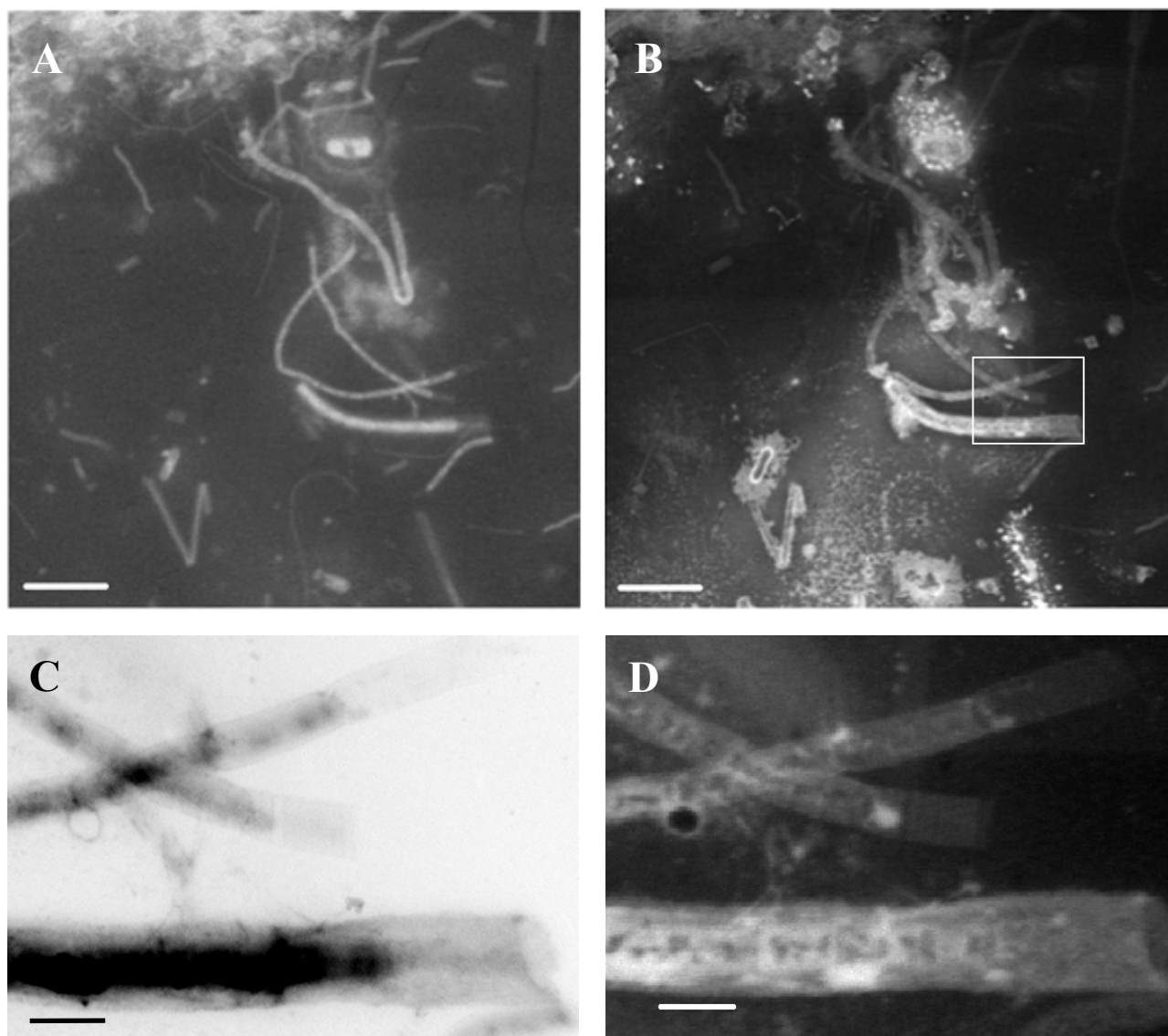


Fig. 1. Six week methanol-fed riverine biofilm that was frozen, cryo-sectioned and mounted on a Formvar-coated grid. A. STXM map of biological species (mostly protein as detected by absorption at 288.2 eV minus that at 282 eV). B. STXM map of calcium (absorption at 352.5 eV minus that at 350.3 eV) in the same area of biofilm. C. and D. Comparative imaging of the biofilm area delineated by a rectangle in B using TEM (C) and STXM Ca mapping (D). Scale bars = 5  $\mu\text{m}$  for A and B. Scale bars = 1  $\mu\text{m}$  for C and D.