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Enabling Science and Technology through European Electron Microscopy

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Deliverable 6.2

Report on protocols and standards developed in ESTEEM2

REPORT OF ACTIVITIES BY TU GRAZ

Sample Preparation for Nanoscale Analysis (STEM EELS) of Biochar with Ultramicrotomy

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Biochar, a highly porous material produced from plant waste, is mostly used in agriculture as a soil conditioner, in livestock farming as a feed supplement, and in metalworking as a reducing agent. It is also suitable for cleaning “grey water”, as an absorber in sports clothing, in batteries and many other applications.

Strict guidelines for sample preparation:

- No fixation (for example with glutaraldehyde) and no embedding, this treatment leads to oxidation of the surface.
- No preparation in cryogenic state, because the used sucrose during the cryo-ultramicrotomy sample preparation as supporting material cannot be removed without any residue.
- Target preparation only at the edge of the biochar.
- Special consideration of the carbon chemistry of the sample to allow for analytical TEM investigations.

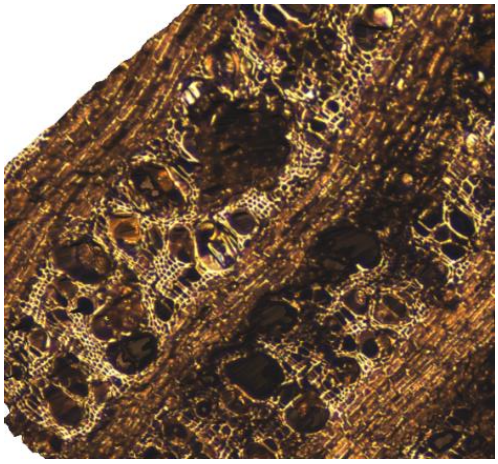
The steps outlined shall suffice preparation needs of similar, organic specimen.

Sample preparation

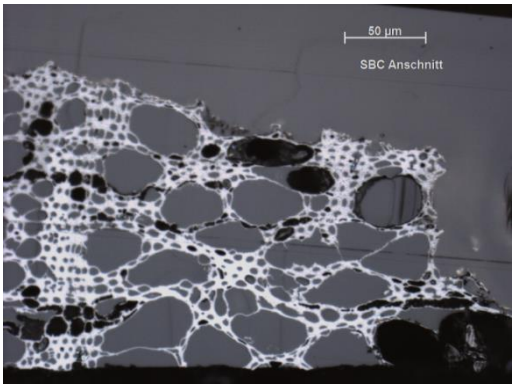
A new specimen preparation idea based on the sample requirements has been applied to produce reliable specimens for STEM and ionization edge fine structure investigations. The aim was to achieve ultrathin, large prestine areas from functionalized areas on the surface of the black carbon particles.

The biochar particles are sputtered with a ~300 nm Au protective layer in a Leica EM ACE600 (Leica Microsystems) using a rotating device to enable 3D coating (45mA, 8,0E -3mbar, working distance 50mm). This guaranteed the protection of the functionalized surfaces during the next preparation steps.
The in this way sealed particle is then embedded in Spezifix40 (Struers) for stabilization of the highly porous sample system.
With an Ultramicrotome UC6 (Leica Microsystems) equipped with an ultrasonic 35° diamant knife (Diatome) ultrathin sections are sliced at a clearance angle of 4°. The nominal feed was generally set in the range from 50 to 60 nm and the cutting speed was chosen between 0.8 and 1.2 mm/s.
The slices were transferred from the water with a perfect loop on a 200 mesh grid.

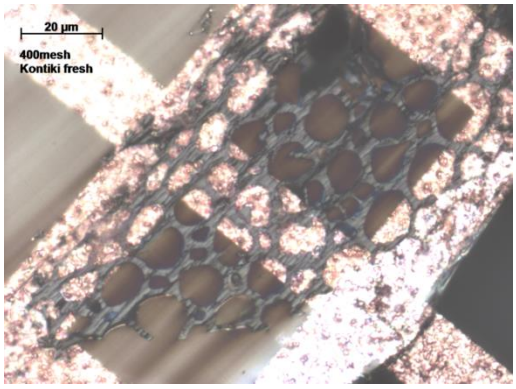
Original biochar sample



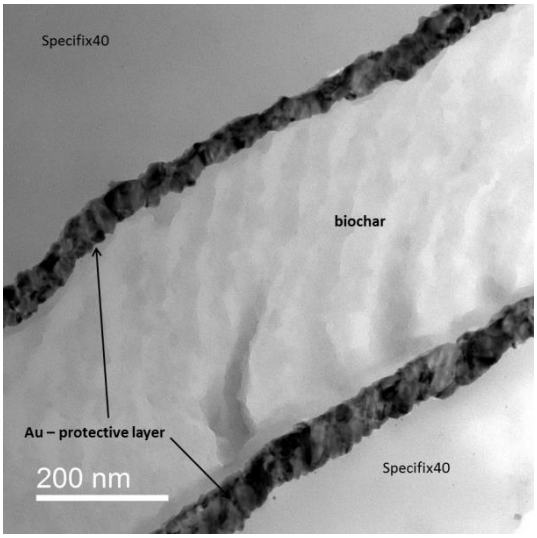
3D infinite focus light microscope image of the Au sputtered sample



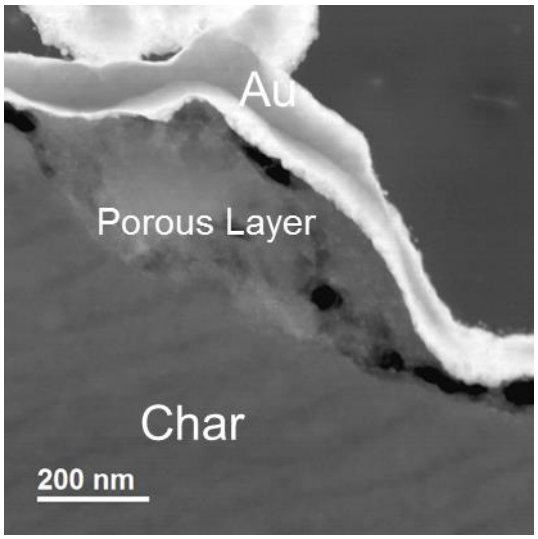
Cutting surface of the Au protected and embedded biochar sample



LM image of a 50nm section on a TEM grid



TEM BF image: Au protected biochar comb wall



STEM HAADF image of the functionalized char surface