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SEM imaging of biofilms supported on natural zeolite

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SEM imaging of biological samples such as biofilms supported on natural inorganic material is not an easy task [1-3]. The main issues that should be taken into account are: Non-conducting surface: when bombarded with electrons, artefacts known as charging are produced. It manifests as image distortion, specimen damage, and dark areas within an image due to repulsion of electrons. In order to prevent radiation and thermal damage to structures, specimen can be coated with conductive material (sputter coating with Au, Pd or carbon). Alternatively, specimen can be observed in ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate, etc). Low atomic weight: biological samples are composed of low atomic number elements thus the electron beam penetration is deep giving rise to a large interaction volume. This is manifested in signal weakening and low resolution. As a solution, metal sputter coating and/or additional staining step of biofilm can be used. Instability of microorganisms in high-vacuum: due to internal pressure biological samples are unstable in high vacuum. Therefore, specimen dehydration is a crucially important step in SEM imaging of microorganisms. Common approaches include critical point drying, freeze drying and chemical drying (ethanol/HDMS). In order to reduce deformation of the specimen during drying, biological samples are usually fixed using glutaraldehyde and/or osmium tetroxide. Alternatively, fixed specimens can be observed in ionic liquid that exclude need in drying. Some of the mentioned approaches are evaluated and compared on biofilm formed on natural zeolite. This contribution is devoted to the discussion of advantages and disadvantages of the tested approaches.

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