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Key words: Multispecies natural biofilms, biofilm sampling, sample preparation, cell labelling, Exopolysaccharides labelling, lectins.

Among biofilm's properties, the ability to interact with/catch pollutants can have applications in bioremediation. Here, biofilm interactions with metals (as iron nanoparticles (NanoFer 25S)) was evaluated using various approaches in microscopy. For this, biofilm growth, sampling, labelling and treatment were developed for each type of microscopy to access the surface or inside of the biofilm, biofilm composition, and metal location.
Multispecies biofilms were grown on sand or in PVC tubes inoculated with aquifer water spiked with a nutritive solution to enhance denitrification, and then put in contact with nanoparticles**.** According to the targeted microscopy, biofilms were (i) sampled as flocs or attached biofilm, (ii) submitted to cells (DAPI) and/or lectins (PNA and ConA coupled to FITC or Au nanoparticles) labelling, and (iii) prepared for observation (fixation, cross-section, freezing…). Fluorescent microscopy revealed that nanoparticles were embedded in the biofilm structure as 0.5-5µm size aggregates. SEM observations also showed NP aggregates closed to microorganisms but it was not possible to conclude a potential interaction between nanoparticles and the biological membranes. STEM-in-SEM analysis showed NP aggregates could enter inside the biofilm over a depth of 7-11µm. Moreover, microorganisms were circled by an EPS ring that prevented the direct interaction between NP and membrane. TEM(STEM)/EDS revealed that NP aggregates were co-localized with lectins suggesting a potential role of exopolysaccharides in NP embedding. The combination of several approaches in microscopy is thus a good tool to better understandi and characterize biofilm/pollutant interaction.