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Session 5b) Novel Methodology 2: Technical Development

Stable Isotope Raman Microspectroscopy as Novel Approach for Analysis of Microbial Degradation of Microplastics

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Stable isotope-based analytical methods gain increasing relevance in different scientific fields. Here, a combination of Raman microspectroscopy with the stable isotope approach – stable isotope Raman microspectroscopy (SIRM) can offer a non-destructive, quantitative spatially-resolved in situ analysis without interference of water. SIRM provides fingerprint spectra with the spatial resolution of an optical microscope, containing information on stable isotope-labeled substances (based on red-shift of bands) and the amount of a label. It delivers information on the carbon metabolism / flow and the cell activity [1], and hence can be suitable for the analysis of the microbial degradation of a most prominent emerging pollutant in the environment and food – microplastics (synthetic polymer particles & fibers in the size range of 1 µm–1 mm).

Here, we bring forward SIRM at the single-cell level to demonstrate and broaden the mechanistic understanding of microbial degradation of (micro)plastics in natural systems. The use of deuterated, instead of ^{13}C-labeled polymer – perdeuterated D polylactic acid (dPLA) as model for (micro)plastics turned out to be a very suitable (i.e., availability, price, pronounced red-shift in Raman spectra). We traced D-label during incubation experiments into microbial biomass of the environmental bacterium Sphingomonas koreensis using both carotenoid bands (resonance Raman) and C-D vibrations (appear in the Raman-silent region of undeuterated biomass, 2050–2300 cm-1). Single-cell analysis was the key to detect phenotypic heterogeneity and to classify S. koreensis cells in two clusters: one showed a significantly stronger D-level than the negative Escherichia coli control, whereas the other was non-labeled. Thus, the application of SIRM for the analysis of the biodegradation of D-labeled (micro)plastics can provide unambiguous direct information on D-assimilation by microorganisms at the single-cell level [2].

References

[1] Weng, J., Müller, K., Morgaienko, O., Elsner, M., and Ivleva, N.P. (2023) Multi-element stable isotope Raman microspectroscopy of bacterial carotenoids unravels rare signal shift patterns and single-cell phenotypic heterogeneity, Analyst 148, 128-136.

[2] Müller, K., Leung, A.E., Wacklin-Knecht, H., Allgaier, J., Elsner, M., and Ivleva, N.P. Raman microspectroscopy to trace the incorporation of Deuterium from labeled (micro)plastics into microbial cells, submitted.