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Deuterium Labels to Study Biodegradation of Plastics with Raman Spectroscopy

Kara Müller, Martin Elsner, and Natalia P. Ivleva
Technical University of Munich, Institute of Hydrochemistry, School of Natural Sciences, Germany (kara.mueller@tum.de)

Biodegradable polymers are considered one of the solutions to the plastic accumulation problem in terrestrial and aquatic systems. It is important to ensure complete degradation since residual micro- and nanoplastics influence soil health and its biota. During biodegradation, microorganisms first colonize the plastic surface, where they then excrete enzymes responsible for the depolymerization. Finally, the mono- and oligomers are utilized by the microorganisms as energy sources (mineralization into CO_2) or for biomass formation. Only by studying the last step the final fate of the anthropogenic pollutant is revealed. Conventionally CO_2 concentrations are measured to monitor microbial activity in samples exposed to plastics in comparison to plastic-free controls. However, this is in no direct relation to the polymer and priming effects or unknown processes due to bacteria adaptation might blur the analysis. Stable isotope labels can be traced from the polymer into $^{13}CO_2$, D_2O and microbial biomass to overcome those obstacles. While many publications covered $^{13}CO_2$ monitoring, only Zumstein et al. additionally traced the carbon label into fungal biomass with nanoscale secondary ion mass spectrometry.[1] In our approach, we use deuterium instead of carbon labels due to reduced costs and enhanced availability of labeled compounds. Although we lose the ability to contribute to a closed mass balance, we use non-destructive Raman microspectroscopy to gain additional chemical information on a single cell level. Heavier isotopes lead to a red shift of the according Raman band due to their larger mass. Deuteration of microbial lipids, proteins, DNA, and carbohydrates leads to an extensive shift of C-H vibrations into the Raman-silent region. C-D vibrations can therefore be quickly detected with a facilitated data analysis.

We incubated the environmental bacterium *Sphingomonas koreensis* with deuterated polylactic acid (dPLA) in an aqueous medium at room temperature under aerobic conditions. After 3 weeks, we observed an additional biomass spectrum for about 50 % of the measured cells besides undeuterated biomass and dPLA particles. After 13 weeks, this spectrum was already recorded for all cells. While the biomass and C□H str. vibrations clearly indicate microbial biomass, the C-D vibrations of the additional spectra differ from reference deuterated biomass spectra obtained with glucose-d_{12} and D_2O labeling. After comparing these untypical C-D vibrations to self-obtained and literature reference spectra, they were interpreted to originate from deuterated biomass with strongly deuterated lipids and inhibited labeling of proteins. Now that we can trace deuterium from labeled plastics into microbial biomass, we want to extend the approach to terrestrial environments. Therefore, cell isolation from the soil matrix was successfully adapted from the

literature[2] to gain adequate Raman spectra. In ongoing experiments, environmental samples will first be exposed to unlabeled PLA for bacteria adaptation and then used for incubation with dPLA in soil microcosms.

References:

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