Stable-isotope Raman Microspectroscopic Studies on Accumulation of Pollutants by Biofilms in Aquatic Systems

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Most microbial cells live in the form of biofilms in natural environment. These communities of microorganisms (bacteria, protozoa, algae and fungi) embedded in a hydrogel matrix of extracellular polymeric substances (EPS, biopolymers such as polysaccharides, proteins, nucleic acids, lipids) are called biofilms [1]. They play an important role in the degradation of pollutants, but they are very sensitive to varying boundary conditions. Therefore a rapid and noninvasive analytical tool for chemical characterization with high spatial resolution and sensitivity is required.

With Raman microspectroscopy (RM) an *in situ* nondestructive chemical analysis of biofilm matrix in the µm-range can be performed [2]. This vibrational spectroscopy allows a noninvasive acquisition of Raman spectra without the interference of water. However, the quantum efficiency of $10^{-6} – 10^{-8}$ for the Raman effect and hence the sensitivity is rather limited. Therefore surface-enhanced Raman scattering (SERS) can be used to enhance the intensity of the Raman bands significantly. SERS occurs if a molecule is attached to, or in immediate proximity of a nanometer-roughened metal (e.g. Ag, Au) surface. With this technique enhancement factors in the range of $10^3 – 10^6$ (up to 10^6 at "hot spots") due to the electromagnetic (surface plasmon resonance) and chemical enhancements (charge transfer complex) can be reached [3]. With the improvement of the sensitivity of RM with SERS a reproducible and rapid analysis of the biofilm matrix is possible [4].

In this project we analyze the accumulation/degradation of water quality-related substances in cooperation with Helmholtz Zentrum München, Institute of Groundwater Ecology. Stable-isotopes (i.e. ^{13}C-tracer) are used to achieve a better understanding of degradation pathways of pollutants. It is already known that the Raman bands of proteins or nucleic acids in ^{13}C-labeled microorganisms show a characteristic red-shift in the Raman spectrum [5]. The analysis of sulfate reducing bacteria cultivated with ^{13}C-naphthalene show a clear red-shift of the bands in the Raman spectra of the microbiologic cells. Additional Raman and SERS measurements of ^{12}C/^{13}C-glucose and phenylalanine mixtures were successfully performed, and calibration curves were created. The results should help to understand the influence of biofilms on the flux, turnover and fate of natural and anthropogenic pollutants in regional water cycles.

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