# *Raman Microspectroscopy and Surface-Enhanced Raman Scattering for Biofilm Analysis: Focus on Stable-Isotope Technique*

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Biofilms are the predominant form of microbial life on our planet. These multicellular aggregates, where different microorganisms are enclosed in a self-produced matrix of extracellular polymeric substances (EPS: polysaccharides, proteins, glycoproteins, nucleic acids, etc.), usually present near interfaces (solid-liquid, liquid-liquid, liquid-air, and solid-air). As biofilms can be prevalent in natural, industrial and hospital settings, detailed and spatially resolved information on composition and structure of biofilms is of high importance in various fields.

Raman microspectroscopy (RM) is a powerful tool for characterization of multispecies biofilm matrices, which provides *in situ* chemical information about microbial constituents/components and EPS [1-4]. Moreover, the sensitivity of biofilm analysis can be significantly increased by applying surface-enhanced Raman scattering (SERS) [5]. We used colloidal silver nanoparticles as media for SERS imaging of biofilms and found than SERS allows for the detection of different components and the determination of their relative abundance in the complex biofilm matrix even at low biomass concentration. Furthermore, the combination of RM with a stable-isotope technique seems to be a promising tool for microbial ecology [6]. It has been shown that bacteria, which were $^{13}$C-labelled through incorporation of the isotope (by cultivating with $^{13}$C-labelled glucose) exhibit key red-shifted peaks of proteins and nucleic acids. The calculated 'red shift ratio' correlates very well with the $^{13}$C-content of the cells [6].

We apply stable-isotope RM to characterize the accumulation and degradation of pollutants by biofilms related to ground water. First, the feasibility of stable-isotope RM technique for quantitative analysis has been proved by examination of reference compounds ($^{12}$C/$^{13}$C-glucose and phenylalanine mixtures). As the next step, we study the accumulation of $^{13}$C-naphthalene by N47 sulfate-reducing bacteria with the resolution at a single-cell level. We found a clear red-shift of the bands in the Raman spectra of the microorganisms. Furthermore, we prove the potential of SERS in combination with stable-isotope probing for sensitive analysis of single microorganisms. This study should help in understanding the role of biofilms in the fate of water quality-related substances and clarifying the degradation pathways of pollutants.

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