Stable isotope resonance Raman microspectroscopic and SERS analysis of microorganisms at single cell level

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Microorganisms, often found in multicellular communities, are usually embedded in a hydrogel matrix of extracellular polymeric substances (EPS), so called biofilms. Due to their ubiquitous occurrence in the environment and especially in aquatic systems they play a major role in the degradation of pollutants. A rapid analytical and nondestructive method for chemical characterization with high spatial resolution and sensitivity is therefore highly requested.

Stable isotope Raman microspectroscopy (SIRM) fills this gap as it enables a nondestructive chemical analysis of the molecular and isotopic composition of microbial cells *in situ*. However, low sensitivity and long acquisition times limit a broad applicability of the method in environmental analysis. Though, a few methods are available to significantly increase sensitivity and decrease measurement times in SIRM. For example resonance Raman microspectroscopy can be used, if the sample possesses resonance Raman active substances like carotenoid[1] or cytochrome *c*. A fast analysis is simply possible by choosing an adequate laser wavelength. Alternatively, surface-enhanced Raman Scattering (SERS) can be used when the sample is in direct contact to nanostructured metallic surfaces (i.e. Ag, Au). Enhancement factors of up to 10^{11} can be reached. With this increased sensitivity of SIRM with either the resonance effect or SERS a reproducible and rapid analysis of the isotopic labeling and molecular composition of microorganisms at single cell levels is possible.

In this study[2], *G. metallireducens*, which is known for its high cytochrome *c* content, was used for resonance Raman measurements. Spectra with very good reproducibility, strong resonance Raman bands of cytochrome *c* and low signal-to-noise ratio could be generated with acquisition times of only 6 seconds per spectrum. Finally, the resonance Raman analysis of *G. metallireducens* cells cultivated with ^{13}C-acetate shows a significant red-shift of the cytochrome *c* bands in comparison to normally cultivated ^{12}C-*G. metallireducens* cells. Second, *E. coli* was used as a model organism to explore the possibilities of SERS in combination with stable isotopes at single cell level. The SERS analysis of *E. coli* cultivated with ^{12}C or fully labeled ^{13}C-glucose showed a very good reproducibility. Notably, a very sharp marker band at 733 cm^{-1} for ^{12}C-*E. coli* cells could be found. This band is significantly red-shifted to 720 cm^{-1} for ^{13}C-labeled *E. coli* cells. This fact enables for the first time a direct comparison of ^{12}C-cells und ^{13}C-cells by means of stable-isotope SERS. These results can open new possibilities in understanding microbiological processes at single cell levels, which have not been possible so far.

[1] M. Li, et al., *ISME J*. 2012, *6*. 875-885.
[2] P. Kubryk, et al., *Anal. Chem.* submitted.