Stable Isotope (Resonance) Raman Microspectroscopic and SERS Analysis of Single Microbial Cells

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Microorganisms living in diverse natural environments usually form communities, so called biofilms [1], which are embedded in a hydrogel matrix of extracellular polymeric substances. Because of their ubiquitous occurrence information about e.g. metabolic pathways and carbon flows, especially at single cell level, is an important field of microbiological research. Therefore a rapid analytical and nondestructive method for chemical characterization with high spatial resolution and sensitivity is requested.

Stable isotope Raman microspectroscopy (SIRM) is an \textit{in situ} nondestructive chemical analysis in the µm-range, which enables molecular and isotopic characterization of microbial cells. However, the quantum efficiency of $10^{-6}$ – $10^{-8}$ for the Raman effect and therefore the sensitivity limits a broad applicability in environmental analysis. Though, a few methods can be used to enhance the intensity of the Raman bands and shorten analysis times. For example resonance Raman effects can be exploited when the microbial sample possesses resonance Raman active substances such as cytochrome \textit{c} [2]. Alternatively, surface-enhanced Raman scattering (SERS) can be used if a molecule is attached to, or in immediate proximity to a nanostructured metallic surface (e.g. Ag, Au). With this technique enhancement factors up to $10^{11}$ can be reached. Both methods enable a sensitive, reproducible and rapid SIRM analysis of the isotopic labeling and molecular composition of microorganisms at single cell levels.

In our study [3] we explored the possibilities and limitations of SIRM in combination with resonance Raman and SERS analysis of \textit{G. metallireducens} and \textit{E. coli} respectively. First, with an acquisition time of only 6 seconds per single \textit{G. metallireducens} cell, very reproducible spectra with strong resonance Raman bands of cytochrome \textit{c} could be measured. Cultivation of \textit{G. metallireducens} with $^{13}$C-acetate shows a significant red-shift of the cytochrome \textit{c} bands in comparison to $^{12}$C-\textit{G. metallireducens}. Second, the SERS analysis of \textit{E. coli} cultivated with either $^{12}$C or $^{13}$C-glucose showed a very good reproducibility. Most important, we found a very sharp band at 733 cm\textsuperscript{-1} in the spectra of $^{12}$C-\textit{E. coli} cells which can be assigned to adenine or adenine containing compounds. This marker band is clearly red-shifted to 720 cm\textsuperscript{-1} in $^{13}$C-labeled \textit{E. coli} cells and therefore, for the first time, a rapid detection of stable isotope labeled \textit{E. coli} cells and comparison with $^{12}$C-cells by means of SERS becomes possible. These results can help to open new applications for Raman microspectroscopy in understanding of different metabolic processes, which have not been possible so far.

References