Study of Chromium biosorption by *Citrobacter freundii* using scanning transmission x-ray microscopy

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The elucidation of the mechanism of bio-reduction of Cr(VI) to Cr(III) assumes significance in the bio-remediation process. Previous works from our group have employed FTIR, EDS, and XPS to explain the mechanism. Scanning transmission X-ray microscopy (STXM) is a more direct analytical tool, which provides high resolution chemical speciation information down to ~30 nm. In this study, for the first time, STXM technique at SOLEIL Synchrotron facility was utilised to analyse the spatial distribution and chemical speciation of biosorbed chromium present on the *Citrobacter freundii* bacterial cells by image stacks and line scans at Cr L-edge. Ratio of intensities at 577.4 eV [Cr(III)] and 580.6 eV [Cr(VI)] indicate that Cr(III) is predominantly present owing to bio-reduction. The ratio of Cr $2p_{3/2}$(L3) to Cr $2p_{1/2}$(L2) suggest that biosorbed Cr^{3+} is predominantly present in the form of oxides, hydroxides or oxyhydroxides (Cr_2O_3, $Cr(OH)_3$). Analysis across O K-edge showed two peaks at 531 eV and 535 eV with a sharp split around 533 eV, which is a characteristic feature of Cr_2O_3. A significant shift in 535 eV peak is observed for different regions of the sample indicating the effect of interaction of the bacterial cell with biosorbed chromium. Furthermore, it was observed that, varying concentrations of chromium does not alter the Cr L-edge signals as opposed by significant variation in the O K-edge signal. This supports the hypothesis that cell-bound oxygen atoms of hydroxyl and carboxyl groups are involved in chromium binding, reinforcing the FTIR spectral data. In addition, the elucidation of the molecular structure of the chromium binding unit will facilitate the design of selective sensors for chromium detection.