XANES/EXAFS Study of Zinc Biosorption on Sulfur Ligand-binding Sites in Genetically-engineered Bacteria

Kuen-Song Lin (林錕松)[1], Ling-Yun Jang (張凌雲)[2], Jyh-Fu Lee (李志甫)[2], and Yao-Wen Yang (楊耀文)[2]

[1]Department of Chemical Engineering & Materials Science, Yuan-Ze University, Chungli, Taiwan
[2]National Synchrotron Radiation Research Center, Hsinchu, Taiwan

Many bacteria or fungi have a cell wall or envelope that is capable of passively adsorbing high levels of dissolved metals usually via a charge-mediated attraction. The cell surface of many microorganisms, including cyanobacteria, consists of biopolymer such as polysaccharides, proteins, and lipids, which act as a basic binding site of zinc metals. The functional groups within the cell wall provide the amide, amine, hydroxyl, carboxylic, imidazole, sulfate, sulfydryl, phosphate, and thiol groups that can bind zinc ions.

The valency and fine structure of Zn atom adsorbed or accumulated in fungal or bacterial biomass have not been well studied. Analysis with EXAFS or XANES may provide a novel view into the details of the valency and fine structure of these toxic Zn metals adsorbed in bacterial biomass. The XANES and EXAFS offer the basic knowledge of understanding the oxidation states and fine structures of Zn atom in biomass toward a further study on the biosorption and bioaccumulation mechanism of Zn metals.

The bacterial biosorbent used in this work was genetically-engineered *Escherichia coli* strains capable of overexpressing periplasmic metal-binding proteins (MerP) originated from gram-negative and gram-positive bacteria. The bacterial strains were grown with appropriate medium and the biomass was harvested from cultures in the early-stationary phase. The biosorbents were prepared in the form of freely suspended cells.

The EXAFS and XANES spectra were collected at The EXAFS and XANES spectra were collected at the TXR BL16A1 at the NSRRC of Taiwan. The electron storage ring was operated with an energy of 1.5 GeV and a current of 100-150 mA. A Si(111) DCM was used for providing highly monochromatized photon beams with energies of 0.9 to 9 keV and resolving power (E/ΔE) of up to 7000. Data were collected in fluorescence or transmission mode with a Lytle ionization detector for the Zn (9659 eV)/S (2472 eV) K edge experiments at room temperature. The EXAFS data will be analyzed by using the UWXAFS 3.0 program and FEFF 8.0 codes.

The genetically-engineered *Escherichia coli* bacteria had the enhanced abilities of absorbing Zn ions and might congregate or concentrate the strain structures for stabilizing the cells. The cell surface of genetically-engineered *Escherichia coli* bacteria might consist of biopolymer such as polysaccharides, proteins, and lipids, which act as a basic binding site of zinc ions. The functional groups within the cell wall of genetically-engineered *Escherichia coli* bacteria provided the sulfate (SO_4^{2-}), thiol (mercaptan) or sulfhydryl (mercapto, R-SH) groups that can bind zinc ions. Especially, thiol or sulfate functional groups are more stronger sites bound with zinc ions and may form Zn-SH or $ZnSO_4$ complex. The Zn and S EXAFS spectra indicating the Zn-S species with bond distances of 2.05 Å and 2.13 Å, respectively were found in Figures 1(a) and 1(b). Coordination numbers (CN) of the Zn-S species from Zn and S EXAFS spectra were 2.3 and 2.5, respectively. The uptake of zinc ions can take place by entrapment in the cellular structure and subsequent sorption onto the binding sites present in the cellular structure of genetically-engineered *Escherichia coli* bacteria.

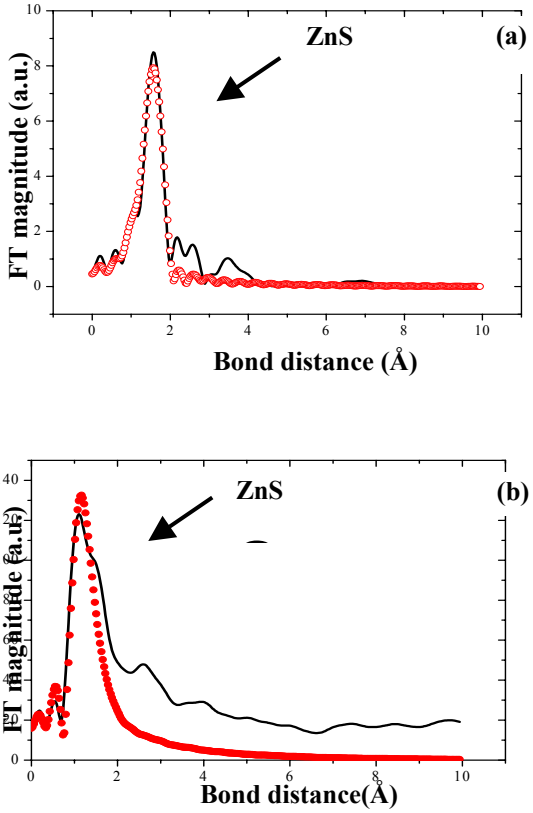

Figure 1. Fourier transform (FT) of the (a) zinc and (b) sulfur K edges of ZnS EXAFS of Zn-absorbed genetically-engineered *Escherichia coli* bacterial biosorbents. The best fitting of the EXAFS spectra are expressed by the circle lines.