Removal of toxic heavy metals by biosorption and biofilm formation of indigenous bacteria in soil

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Many studies have pointed out the problem of heavy metals - contaminated soil in Korea, but research work on clean-up technology of the contaminated soil has rarely performed. Recently the application of cost-effective and environment-friendly bioremediation technology to the heavy metals - contaminated sites has been gradually realized in Korea. The objective of this study is to remediate the sites through the reduction of heavy metal mobility by using indigenous bacteria from the soil, and to evaluate the characteristics of biosorption according to various environmental factors which can affect the sorption of toxic heavy metals.

Indigenous bacterium having a tolerance to high Pb and Cd toxicity was isolated from soil concomitantly contaminated with Pb and TPH (total petroleum hydrocarbons). The bacterium was identified as *Bacillus thruingiensis* and named SPb-1. As a result of batch-type biosorption experiments, the highest removal rates of aqueous Pb and Cd were observed with initial concentration of Pb and Cd less than 100 mg/L and 5 mg/L, respectively. The optimal operating conditions of Pb and Cd biosorption were as follows; culture age of over 16 hours to stationary growth phase, biomass amount of more than 2 g/L, pH 5-9, and temperature at 25°C and 35°C for Pb and Cd, respectively. Monolayer sorption to cell surface occurred for Cd biosorption, whereas the mechanisms of Pb biosorption were due to intracellular accumulation by metabolism of bacteria and precipitation with extracellular polymeric substance such as sulfide or phosphate.

Effluent volume ratio of acetate, lactate and glucose through column compared to deionized water showed 98.5 %, 97.3 %, and 94.7 %, respectively, due to the biofilm formation in pore and the flow of injected carbon source solution. The measurement of effluent volume through column could indirectly confirm the biofilm formation. Maximum removal rate of metals by biofilm depends on the carbon source solution, for example, glucose for Cr and Cd and acetate for Cu and Zn.

Copper isotopic fractionation in acid mine drainage

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Acid mine drainage (AMD) is a chronic problem that results from oxidative sulfide mineral dissolution, thereby releasing acidic, metal-rich waters into the environment. We use Cu isotope measurements to better understand the biogeochemical mechanisms contributing to Cu mobility in AMD.

The δ^{65}Cu values (based on ^{65}Cu/^{63}Cu) of primary Cu sulfide minerals near an AMD-impacted stream are lower than those for stream water. In this system, the average isotopic fractionation (quantified as $\Delta_{aq\text{-}min} = \delta^{65}Cu_{aq} - \delta^{65}Cu_{min}$, where Cu_{aq} is leached Cu and Cu_{min} is the mineral) is 1.43 ± 0.14‰ and 1.60 ± 0.14‰ for chalcopyrite and enargite, respectively. In addition to this field survey, we simulated sulfide leaching in batch experiments at pH 2.0 and found that, as in the field, leached Cu_{aq} is isotopically enriched relative to chalcopyrite and enargite-bearing samples when microorganisms are absent (average $\Delta_{aq\text{-}min}$ = 1.18 ± 0.14‰ for chalcopyrite, and 0.94 ± 0.14‰ for enargite). Leaching of sulfides in the presence of *Acidithiobacillus ferrooxidans* results in smaller average fractionation in the opposite direction for chalcopyrite ($\Delta_{aq\text{-}min}$ = -0.61 ± 0.14‰) and no apparent fractionation for enargite ($\Delta_{aq\text{-}min}$ = -0.01 ± 0.14‰).

We hypothesize that the isotope effect during leaching is the same in both abiotic and biotic experiments, but additional fractionation resulting from the association of $^{65}Cu_{aq}$ with *A. ferrooxidans* likely causes the isotopic depletion of remaining Cu_{aq} in the biotic experiments.

To investigate association of Cu_{aq} with cells, we grew *A. ferrooxidans* in medium at pH 2.0 with 0, 0.1, 1, and 10 mM Cu added. We then used Transmission Electron Microscopy (TEM) and Energy Dispersive Spectroscopy (EDS) to image and characterize cells grown under each condition. All cells exhibited electron dense (i.e., metal-rich) areas on cell membranes and/or within the cell. Preliminary EDS data for cells grown in 0.1 and 10 mM Cu medium shows that metal-rich areas on the outer cell membrane and in granules within the cell have slightly higher P, Fe, and Cu contents relative to the background, suggesting that cells are indeed a sink for dissolved Cu.