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Investigation of a bioflotation interface with infrared spectroscopy

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Abstract. In this paper we report an approach for the structural analysis of mineral-collector interfaces of (bio)flotation systems by means of attenuated total reflection Fourier-transform infrared spectroscopy (ATR FT-IR). The extraction of rare earth metals from electronic waste materials is an important challenge for the recycling industry. In a current project bacteriophage are used as biocollectors to develop a bioflotation model system for the separation of lanthanum phosphate doped with cerium and terbium ($LaPO_4:Ce^{3+},Tb^{3+}$) from mixed fluorescent phosphors. As an initial analytical concept fluorescence microscopy was successfully applied to investigate particles of spent fluorescent lamp powders and to visualize the bacteriophage on the surface of the waste material. However, due to the restrictions of this technique we are not able to identify the molecular interactions of the bacteriophage with the recycled material. ATR FT-IR was found to be an effective tool to detect the major coat protein of the bacteriophage biocollectors on the surface of the $LaPO_4:Ce^{3+},Tb^{3+}$ and sense their specific bonding interaction opening the gates for the high level chemical characterization of the interface.

Introduction

Flotation is one of the most traditional methodologies in mineral processing. The technique is based on changing the hydrophobic behavior of the mineral surfaces using organic flotation collector molecules. Recently, biomolecules and microorganisms as flotation biocollectors have garnered great attention, because they potentially allow smoother reaction conditions during alternative bioflotation processes, when classical chemical flotation reagents are not applicable.

Describing the mineral-collector interactions in any flotation system is very important to characterize the nature of the reaction and the specified behavior of the reactants. Several studies have already been published about the flotation interface and the adsorption conditions of flotation collectors on mineral surfaces with several techniques including electrochemical methods [1,2], atomic force microscopy [3] or FT-IR spectroscopy [4]. However there is little known about the interactions of biocollector microorganisms with minerals or any inorganic target materials. In our previous work [5] the identification of highly effective $LaPO_4:Ce^{3+},Tb^{3+}$ (LAP) binding peptides bound to phage particles (as biocollectors) was successful. LAP is one component of the fluorescent phosphors of compact fluorescent light bulbs. The recycling of the rare earth components that are part of the fluorescent powder is not yet possible due to the very similar chemical and physical characteristics of rare earth minerals. Peptides providing highly specific binding sites for individual lamp phosphor components will close the gap. In our previous study, fluorescent microscopy was applied to visually probe the phage biocollectors connected to the LAP targets, which is generally unable to provide any chemical or structural information about the system. The goal of this work is the identification of the interactions between the phage biocollectors and the LAP matter and to demonstrate the chemical detectability of the phage on the LAP surface by means of ATR FT-IR.

Materials and methods

LAP was provided by Leuchtstoffwerk Breitungen GmbH, Breitungen, Germany. For the sample preparation we used the phage clone that expresses the peptide sequence RCQYPLCS which was previously identified using the phage peptide library f88.4 LX4 [5]. TRIS-buffered-saline (TBS) contained 50 mM TRIS-HCl, 150 mM NaCl at pH = 7.5. Phosphate-buffered-saline (PBS) was the mixture of 137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4 at pH 7.4 and it was used together with 10 mg/ml bovine serum albumin (BSA). TBST (0.5 %) and TBST (0.05%) were made from TBS and 0.5 % and 0.05 % Tween-20. PBST (0.05 %) was similarly the mixture of PBS and 0.05 % Tween 20. Anti-fd phage antibody (1:1000) was purchased from Sigma Aldrich. The secondary antibody aniti-rabbit IgG conjugated with Alexa 594 (1:1,000) was bought from Molecular Probes.

Fluorescence microscopic characterization of the samples were carried out using a Nikon Eclipse Ti microscope equipped with epi fluorescence and images capture using NIS Elements imaging software. A specific TRITC filter was applied to make bound phage visible (red) and an FITC filter was employed to visualize the LAP with a different color (green).

The ATR FT-IR measurements were carried out on a Bruker Vertex 80/v spectrometer equipped with a mercury cadmium telluride detector and an attenuated total reflection (ATR) accessory that is a horizontal diamond crystal with nine internal reflections on the upper surface and an angle of incidence of 45° (DURA SamplIR II, Smiths Inc.). Transmission spectra were recorded in the range from 650-1,800 cm^{-1} with a spectral resolution of 4 cm^{-1}. For each spectrum 256 individual spectra were averaged.

Experimental

Prior to fluorescence microscopic investigation the following sample preparations were carried out: LAP sample was blocked for 1 hour with PBS containing 10 mg/mL BSA. After rinsing the mineral with TBS, 10^{10} phage expressing the peptide sequence RCQYPLCS in TBST (0.1%) with BSA (0.1%) were added and incubated for 1 hour at room temperature with mixing. The samples were washed 8 times with TBST (0.5%), and then washed twice with TBST (0.05%). The samples were incubated with anti-fd phage antibody (1:1,000, Sigma) over night at 4˚C with mixing. Samples were washed 3 times with PBST (0.05%). The secondary antibody anti-rabbit IgG conjugated with Alexa 594 (1,000, Molecular Probes) was used for the mineral LAP to localize bound phage specific antibody.

The sample preparation process was modified for the ATR FT-IR investigations. In order to clearly demonstrate the interaction between LAP and the major coat protein of the phage BSA protein was completely abandoned. This way we can ensure that every protein-LAP interaction is originated from the major coat protein of the phage. LAP sample was blocked for 1 hour with PBS without any BSA. After rinsing the LAP mineral with TBS, 10^{10} phage expressing the peptide sequence RCQYPLCS in TBST (0.1%) were added and incubated for 1 hour at room temperature with mixing. Finally, the samples were washed 3 times with TBST (0.5 %). Prior to the IR measurements LAP and the LAP-phage bioproduct were dispersed in water with 2.5 g/L and 1.9 g/L as well as the bacteriophages were homogenized in water with 10^{11} pfu/mL concentrations. Then, aliquots of 2.5 µL from each of the dispersions were dried on the surface of the ATR crystal in a gentle nitrogen stream.

Results and discussion

Figure 1 shows a phase contrast image of a LAP-phage system using the optical Nikon Eclipse Ti microscope. The identification of the bound phage particles on the LAP surface is possible above the optical resolution limit of the instrument. In case of LAP, the fluorescence light due to the presence of the doping Ce^{3+} and Tb^{3+} ions can be seen in Figure 2. Using the TRITC filter a

different fluorescence activity is observed, which was originated from the labeling antibodies of the phages (as shown in Figure 3). Figure 4 presents the infrared spectroscopic investigations of a LAP-phage system. The reveal of phage specific bands in the spectrum of the phage-LAP combined system clearly indicates the presence of bacteriophages on the LAP surface and the detectability of an interaction of the biocompounds with the particles.

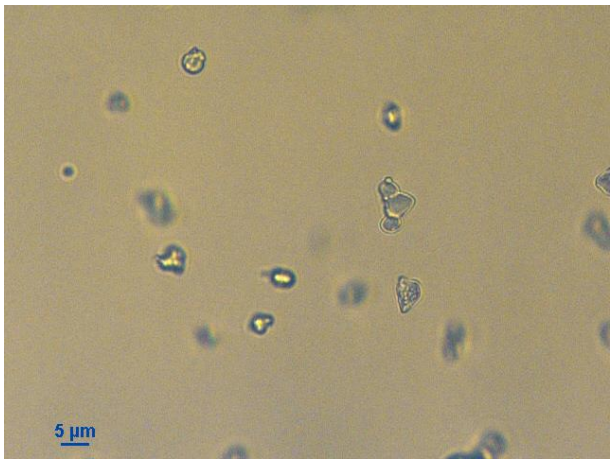

Figure 1. Phase contrast image of phage bound to LAP surface.

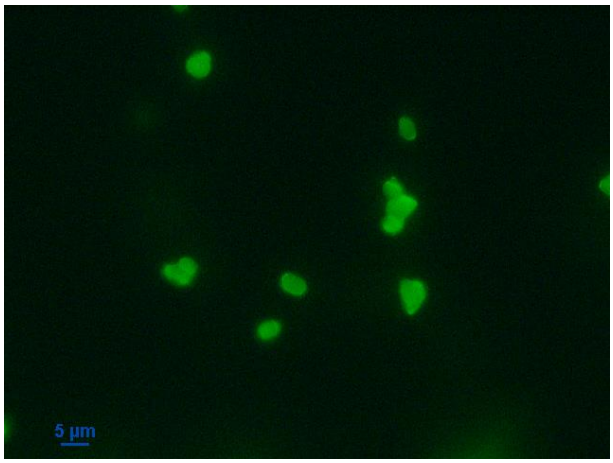

Figure 2. Fluorescence microscopic visualization of LAP particles using the FITC filter

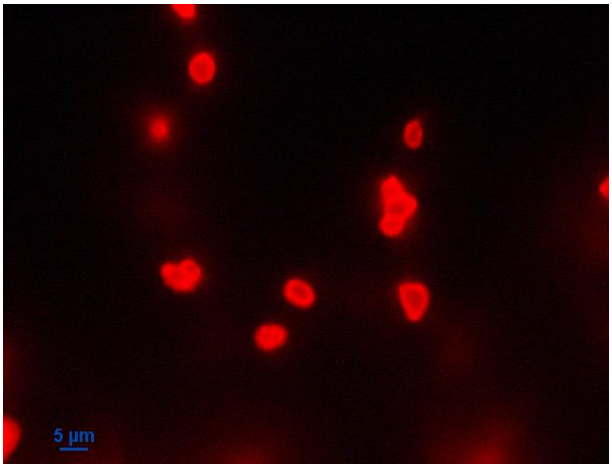

Figure 3. Fluorescence microscopic visualization of phage on LAP surfaces using the TRITC filter.

Conclusions

An optical spectroscopic characterization of a specified bioflotation interface was carried out by means of ATR FT-IR spectroscopy as a possible continuation of the already developed fluorescence microscopic approach. The interaction between the biological and inorganic components of the system was identified and significant protein bands were detected in the interface. Our initial results confirm the potential of infrared spectroscopy to get a more detailed fundamental understanding of the interface processes and open the gates for a high level structural study about the LAP-phage interactions.

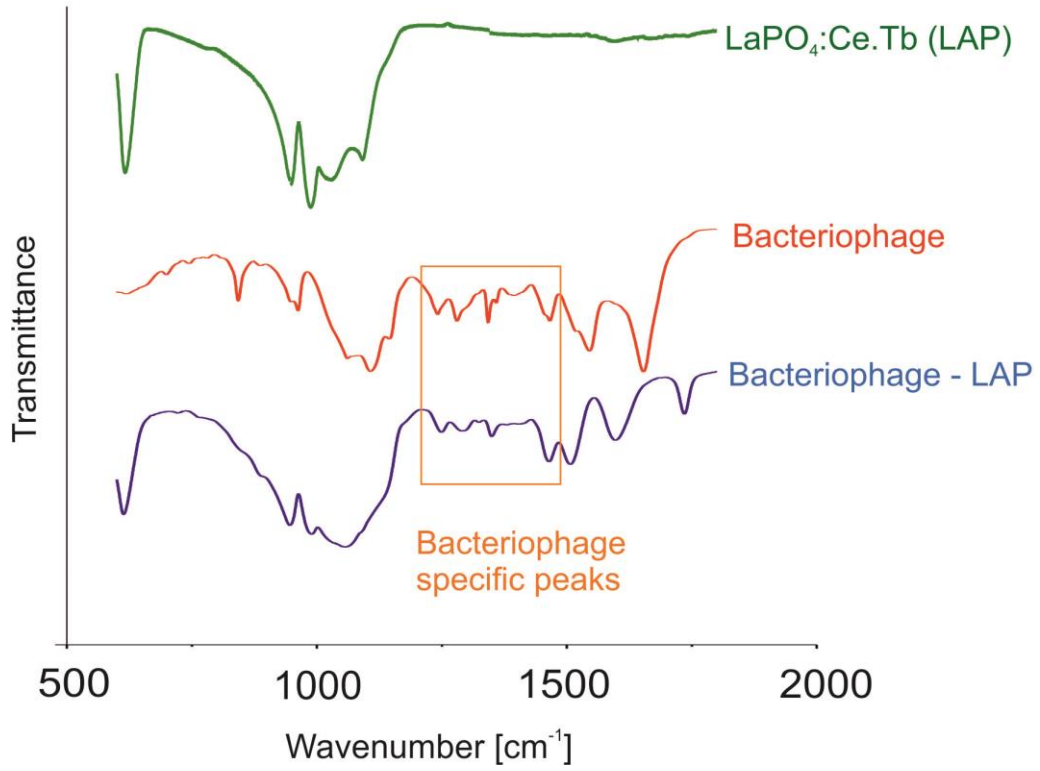

Figure 4. FT-IR spectra of the components of a LAP-phage system.

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