Gypsum (CaSO$_4$ 2H$_2$O) precipitation experiments were carried out in Ca rich media, inoculated with S oxidizing bacteria *Acidithiobacillus thiooxidans* strain GB30-2C and S$^{0}$ as the reduced S source. Microbial oxidation of S$^{0}$ increases SO$_4$ concentration in the media until the solution eventually becomes oversaturated with respect to gypsum. An abiotic and a killed control were also run, wherein the reaction proceeded by the gradual addition of 1M H$_2$SO$_4$. Experiments lasted 51-65 days, and the solutions were sampled every 2-4 days for Ca and SO$_4$ concentrations, pH and cell density. The final precipitates were imaged via SEM to determine average aspect ratios. The Ca isotopic compositions (δ$^{44/40}$Ca SRM915a) of both the solutions and crystals were measured.

As the experiments progressed, SO$_4$ concentrations (0 - 61mM) and cell density (44 - 70%) increased and pH decreased (4.2 - 1.6), consistent with microbial sulfur oxidation. Effective isotopic fractionation factors ($\Delta^{44}$Ca = $\delta^{44}$Ca$_{crystal}$ - $\delta^{44}$Ca$_{solution}$) were -1.34 to -1.04‰ in the biotic experiments, -1.04‰ in the killed control and -0.96 to -0.84‰ in the abiotic controls, which implies the presence of a biological isotope effect of ~0.3‰. Log precipitation rates (µmol/h), determined from the Ca concentration profiles, were similar between the biotic experiments (0.07±0.42 to 0.68±0.25) and the abiotic (0.19±0.25) and killed (-0.09±0.46 to 0.37±0.39) controls, indicating that the isotope effect was independent of rate. Crystals from the biotic experiments and the killed control had smaller aspect ratios (average AR = 8.05±3.99) than the abiotic control, which produced long thin individual needles (average AR = 31.9±8.40). Saturation states just prior to precipitation were ~1.5 to 0.2 units higher in the biotic experiments than in the the killed and abiotic controls.

These observations suggest that the presence of both soluble and insoluble organic material present in both living and dead microbial cultures can fractionate Ca isotopes during gypsum precipitation by altering the morphology of the crystal via growth inhibition on the surface. The results from these experiments can be used to interpret Ca isotopic variations observed in the Frasassi cave system, in which we hypothesize that the isotopically light cave crystals have experienced growth inhibition via microbial interaction. Therefore Ca isotopes may be used in conjunction with other geochemical proxies as a biosignature for microbial life.