Determination of Phosphite (HPO_3^{-2}) by a New IC/MS/MS Method Using an ^{18}O-labeled HPO_3^{-2} Internal Standard

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Project Goals: This project investigates the role of microbial dissimilatory phosphite oxidation (DPO) in the global phosphorus and carbon biogeochemical cycles. As part of this we are investigating the natural occurrence of DPO and phosphite in a broad range of geochemical environments, and examining the fundamental physiological and biochemical aspects of DPO. We are combining systems biology and -omics approaches with physiological and geochemical studies to elucidate the geochemical impact, environmental prevalence and metabolic machinery underlying DPO. To achieve our goals, we are testing three specific hypotheses:

1. DPO is an environmentally prevalent metabolism that co-occurs with global phosphite reserves

2. DPO metabolism is universally conferred by the conserved *ptx-ptd* operon

3. DPO is universally associated with CO_2 fixation

The work described here advances hypothesis 1.

A new method has been developed to determine trace amounts of phosphite (HPO_3^{-2}) in environmental samples using ion chromatography with electrospray tandem mass spectrometry (IC-ESI/MS/MS). The method includes the production and use of an ^{18}O-labeled HPO_3^{-2} internal standard (IS). This isotopically labeled IS significantly improved sensitivity and could account

for matrix suppression. The method detection limit (MDL) was determined as 0.017 and 0.034 µg L^{-1} of HPO_3^{-2} (6.5 and 13 ng P L^{-1}) using a 500 and 25 µL injection loop, respectively. Precision (1–10%) and accuracy (recoveries = 96–106%) were established for a range of environmental samples using known (spiked) addition. The impact of ionic interferences was investigated by evaluating the response of the internal standard in the presence of common anions with respect to distilled deionized water. The most significant interference was due to nitrate (100 mg-NO_3^- L^{-1}) with a 99.99% reduction in IS intensity. The method was successfully applied to wastewater effluent, anaerobic digester influent, centrifugation supernatant (centrate), biosolid, surface water, tap water, and soil samples. Relatively low concentrations < 0.25 µg HPO_3^{-2} L^{-1} were measured in tap water, surface water and wastewater effluent, 1.6 µg kg^{-1} HPO_3^{-2} in soil samples, 2 µg HPO_3^{-2} L^{-1} in centrate, 2.8 µg kg^{-1} HPO_3^{-2} in biosolid, and ~ 4 µg kg^{-1} HPO_3^{-2} in anaerobic digester influent. Limited suppression was observed for all matrices. The largest IS peak area suppression (~98%) was observed in WW effluent with 500 µL injection loop; however, this method was able to quantify HPO_3^{-2} with good recoveries and precision despite the mentioned suppression, supporting the ability of the proposed method to quantify HPO_3^{-2} in different environmental matrices.

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