57Fe Wall Loss Experiment data collected as part of a method development study investigating the precipitation and wall loss of labeled 57Fe when added to M9 Minimal Media

Website: https://www.bco-dmo.org/dataset/732864
Data Type: experimental
Version: 1
Version Date: 2018-04-05

Project
» EAGER: Iron-Virus Interactions in the Ocean (Fe-Virus)

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Abstract
This data was collected as part of a method development study investigating the precipitation and wall loss of labeled ^{57}Fe when added to M9 Minimal Media, which is used to grow the model bacterial species Escherichia coli. The bulk media was prepared with the same components and a portion was treated with Chelex-100 to remove metals, while the other portion remained un-chelexed. Half of each treatment was spiked with labeled ^{57}Fe and either 0.2 µm or 0.02 µm filtered for comparison of the dissolved and soluble fractions. The ^{57}Fe content was monitored over four time points for one week in a shaking incubator, under the same conditions used to culture E. coli for labeling experiments.

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Coverage

Temporal Extent: 2017-11-13

Dataset Description

This data was collected as part of a method development study investigating the precipitation and wall loss of labeled ^{57}Fe when added to M9 Minimal Media, which is used to grow the model bacterial species *Escherichia coli.* The bulk media was prepared with the same components and a portion was treated with Chelex-100 to remove metals, while the other portion remained un-chelexed. Half of each treatment was spiked with labeled ^{57}Fe and either 0.2 µm or 0.02 µm filtered for comparison of the dissolved and soluble fractions. The ^{57}Fe content was monitored over four time points for one week in a shaking incubator, under the same conditions used to culture *E. coli* for labeling experiments.

Methods & Sampling

Refer to Supplemental File, "57Fe Wall Loss Experiment diagram" for the steps indicated in **bold**.

All materials were soaked overnight with heating in 1.5% Citrad Citric Acid liquid cleaner in deionized water, rinsed in RO water, and soaked in 10% HCl in Milli-Q ultrapure water for one week (due to time constraints), then rinsed with MilliQ ultrapure water, let dry in an AirClean 400 work station overnight, and double-bagged in polyethylene bags (Mellett et al., 2017). M9 Minimal Media for bacterial cultures was made using Milli-Q ultrapure water, containing final concentrations of 33.7 mM $Na_2HPO_4 \cdot 2H_2O$, 22 mM KH_2PO_4, 8.56 mM NaCl, 18.7 mM NH_4Cl, 0.1 M magnesium chloride, 0.1 M calcium chloride, 2 mg/ml Thiamine HCl in 70% EtOH, and 20% Glucose (Kutter & Sulakvelidze, 2004). Half of the media was chelexed using Chelex-100 resin (Pai et al., 1988) that was not acid-cleaned (**C**), and half remained un-chelexed (**B**). Half of each treatment was spiked with 10 µM labeled $^{57}FeSO_4$(**2**) while half remained un-spiked (**1**). A volume of 25 ml was then filtered through either a 0.2 µm Sterivex PVDF syringe filter for the dissolved fraction (**D**), or a 0.02 µm Whatman Anatop syringe filter for the soluble fraction (**S**) (Gledhill & Buck, 2012). Samples were directly filtered into trace metal clean polycarbonate Erlenmeyer flasks, placed in a clean bag with a small opening to vent, and left shaking at 37 °C for one week. Samples of each treatment were taken initially, then after the 1st, 3rd, and 7th days (t= 00, 01, 03, 07). The samples were diluted, acidified, and analyzed using an ELEMENT XR High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS). The limit of detection (LOD) for ^{56}Fe and ^{57}Fe were 0.3 nM and 0.012 nM, respectively (Shrivastava & Gupta, 2011).

Problem Report:
The original measurements of the M9 minimal media ^{56}Fe contamination was high, so in an attempt to lower background contamination the media was chelexed. However, the Chelex-100 resin was not rinsed with acid, so was less active for removal of metals. This resulted in higher than expected background concentrations of metals.

Three samples were contaminated with bacterial growth (as indicated by visual turbidity and confirmed using SYBR nucleic acid stain and epifluorescence microscopy) over the course of the week. On t=3, sample B2 was contaminated. By t=7, sample B1D and B1S were contaminated.

Data Processing Description

BCO-DMO Processing:
- modified parameter names to conform with BCO-DMO naming conventions (replaced spaces with underscores, removed puncutation, placed letters before numbers);
- formatted year to yyyymmdd;
- replaced blanks with "nd";
- replaced mu symbol with u in DESCRIPTION column.

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Data Files

File	
Fe57_wall_loss_expt.csv	(Comma Separated Values (.csv), 4.77 KB) MD5:adc07831e5bc5bb014195f7e44a83301
Primary data file for dataset ID 732864	

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Supplemental Files

File
57Fe Wall Loss Experiment diagram filename: experiment_diagram.png (Portable Network Graphics (.png), 43.12 KB) MD5:70275bb41c21026a3e2ecf2a222da7c1 Diagram depicting experimental design used in data 732864 (submitted by Kristen Buck).

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Related Publications

Gledhill, M. (2012). The organic complexation of iron in the marine environment: a review. Frontiers in Microbiology, 3. doi:10.3389/fmicb.2012.00069
Methods

Kutter, E., & Sulakvelidze, A. (2004). Bacteriophages: biology and applications. CRC Press. https://isbnsearch.org/isbn/9780203491751
Methods

Mellett, T., Brown, M.T., Chappell, P.D., Duckham, C., Fitzsimmons, J.N., Till, C.P., Sherrell, R.M., Maldonado, M.T., and Buck, K.N. (2017). The biogeochemical cycling of iron, copper, nickel, cadmium, manganese, cobalt, lead, and scandium in a California Current experimental study. Limnology and Oceanography, 63(S1), S425–S447. doi:10.1002/lno.10751
Methods

Pai, S.-C., Whung, P.-Y., & Lai, R.-L. (1988). Pre-concentration efficiency of chelex-100 resin for heavy metals in seawater. Analytica Chimica Acta, 211, 257–270. doi:10.1016/s0003-2670(00)83685-5 https://doi.org/10.1016/S0003-2670(00)83685-5
Methods

Shrivastava, A., & Gupta, V. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles of Young Scientists, 2(1), 21. doi:10.4103/2229-5186.79345
Methods

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Parameters

Parameter	Description	Units
DATE	Date (UTC) when media was filtered and experiment initiated (t=0), in format YYYYMMDD	unitless
SAMPLE_ID	Sample identifier designated B for un-chelexed, or C for chelexed; 1 for un-spiked with 57FeSO4, 2 for spiked with 10 uM 57FeSO4; D for dissolved (0.2 um filtered) or S for soluble (0.02 um filtered)	unitless
DESCRIPTION	Description of treatment type	unitless
CHELEX	Solution was treated with Chelex-100 resin (Yes) or not (No), units in Yes/No	unitless
Fe57_SPIKE	57FeSO4 spike concentration as added to the media	micromolar (uM)
FILTER_SIZE	Pore size used to filter media, using 0.2 um Sterivex PVDF syringe filter or 0.02 um Anatop syringe filter	micrometers (um)
DAY	Time when treatment was sampled, t=n, units in days	days
Fe56	Concentration of 56Fe as determined by HR-ICP-MS	nanomolar (nM)
Fe57	Concentration of 57Fe as determined by HR-ICP-MS	nanomolar (nM)
Fe57_to_Fe56	Ratio of 57Fe concentration to 56Fe concentration as measured by the HR-ICP-MS, multiplied by 100 (in percentage)	unitless (percentage)
NOTES	Some samples contaminated by bacterial growth were designated as such	unitless

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Instruments

Dataset-specific Instrument Name	ELEMENT XR High Resolution Inductively Coupled Plasma Mass Spectrometer
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
Dataset-specific Description	ELEMENT XR High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS)
Generic Instrument Description	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

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Project Information

EAGER: Iron-Virus Interactions in the Ocean (Fe-Virus)

Iron is an essential micronutrient for phytoplankton that is required for photosynthesis and respiration. Insufficient iron has been shown to limit phytoplankton growth in large regions of the surface ocean, and correspondingly, iron cycling is directly linked to carbon cycling in much of the marine environment. Nearly all iron in seawater (>99%) exists as complexes with organic molecules called ligands, which govern the concentration of iron dissolved in the water and the bioavailability of that iron to phytoplankton. However, despite the importance of iron-binding organic ligands, their sources and identities are largely unknown. Viruses, the majority of which are phages (viruses that infect bacteria), are extremely abundant in seawater and are in the same size fraction as dissolved iron. Recent evidence that non-marine phages contain iron as part of their structures has led to the proposal that marine phages may represent a previously overlooked class of organic iron-binding ligands. This project is determining the contribution of marine phages to dissolved iron pools and culture phage-host systems in the laboratory to determine if phages utilize bacterial iron-uptake receptors for infection in the manner of a Trojan horse. As the first study to examine the biogeochemical impact of trace elements contained within the structure of highly abundant marine phage particles, successful completion of the proposed research will be transformative for biological and chemical oceanography and have far-reaching implications for other fields, including human health where iron availability plays an important role in microbial pathogenesis. This project contributes to the multidisciplinary training of a graduate student and postdoctoral researcher. Research results will be disseminated through scientific publications and presentations, and the public will be educated about linkages between viruses and ocean chemistry via a hands-on exhibit for the annual St. Petersburg Science Festival.

Building upon evidence from non-marine model systems demonstrating the presence of iron ions in phage tail proteins and phage utilization of cell surface receptors for siderophore-bound iron, this project combines field and laboratory-based experiments to test the following three hypotheses regarding iron-virus interactions in the oceans: (1) Iron incorporated into phage tails originates from bacterial cell reserves, reducing the amount of iron available for remineralization upon lysis; (2) Phages constitute important iron-binding ligands in the oceans, accounting for a substantial portion of organically complexed colloidal dissolved iron; (3) Marine phages compete with siderophore-bound iron for uptake receptors on the bacterial cell surface and use iron in their tails as a Trojan horse for infection. Initial calculations predict that phages could account for up to 70% of the colloidal fraction of organically complexed dissolved iron in the surface ocean; therefore, this project is critical for advancing knowledge of trace-metal cycling as well as phage-host interactions. Additionally, if a portion of the cellular iron thought to be released from bacterial cells for remineralization following lysis is already incorporated into phage tails, then these findings will have significant implications for oceanic biogeochemical models. Through a combination of laboratory-based culture experiments and field sample measurements, this project could reveal the identity of a ubiquitous component of colloidal organic iron-binding ligands, modify the estimates of iron concentrations and species released through viral lysis, and potentially identify a novel receptor type for marine phage that may compete with the acquisition of siderophore-bound iron by host bacteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1722761

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