Metabolic potential for heterotrophic utilization of a large array of organics by coccolithophores determined through experiments at Bigelow Laboratory for Ocean Sciences using BioLog Eco-plates

Website: https://www.bco-dmo.org/dataset/858513
Data Type: experimental
Version: 2
Version Date: 2021-10-05

Project
» Coccolithophore Mixotrophy (Cocco-Mix)

Contributors	Affiliation	Role
Balch, William M.	Bigelow Laboratory for Ocean Sciences	Principal Investigator
Godrijan, Jelena		Contact
Drapeau, David T.	Bigelow Laboratory for Ocean Sciences	Analyst
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset includes results from an experiment determining the metabolic potential for heterotrophic utilization of a large array of organics by coccolithophores. Experiments used the BioLog Eco-plates (BioLog, Haywood, CA, U.S.A.) and were conducted at Bigelow Laboratory for Ocean Sciences, East Boothbay, ME.

Table of Contents

Coverage

Spatial Extent: **N**:59.5 **E**:47.8333 **S**:-13.5833 **W**:-113.56
Temporal Extent: 1951-01-01 - 2013-11-20

Methods & Sampling

BioLog Eco-plates contained 96-wells, prefilled with: (1) a colorless tetrazolium dye that reduces to a violet formazin if the substrate is oxidized, (2) triplicates of 31 different organic compounds in equimolar quantities, and (3) triplicate water blanks as a control for airborne bacterial contamination. We used a single coccolithophore strain in each plate. We started each experiment by inoculating a 96-well plate with 100 µL of log-phase cell suspension from each log phase culture. We carried out all inoculations under subdued light and all incubations in complete darkness. At time-zero (T0), 24 h, 48 h, and 72 h, we measured the reduced violet tetrazolium dye absorption on a FilterMax F5 multimode plate reader (Molecular Devices, LLC, San Jose, CA, U.S.A.) for absorption at 595 nm. We interpreted the increasing optical density at 595 nm as heterotrophic metabolism of the organic compound by the coccolithophore cultures. (Note: For two strains, CCMP298 and CCMP 3337, T72 was not performed, but the measurement was instead taken at T120.)

Several limitations to the BioLog Eco-plates should be noted (Stefanowicz 2006). One limitation is that the technique assumes that the uptake of each substrate is independent from any other (i.e., two substrates are not used in a synergistic fashion). The technique also assumes the concentrations of the substrates in each well of the microtiter plate are optimal for the organism in question, rather than too high (i.e., inhibitory) or low (limiting) based on the organism's specific uptake kinetic parameters. Therefore, we could not make any inferences on the uptake kinetics of DOC utilization using the microtiter plates. Furthermore, ions in seawater (specifically Ca++) can cause false positives in BioLog Eco-plates (Pierce et al. 2014). It is critical to lower the Ca++ concentration from 10 mmol L^{-1} (normal concentration in seawater) to ~ 2.5 mmol L^{-1} in order to eliminate false positives (Pierce et al. 2014). The BioLog Company suggests doing this with chelators. An alternative method was suggested by Tuchman et al. (2006) in which the cultures were centrifuged and pelleted to separate them from the nutrient-rich media, which might contain other growth-inducing substrates. Then the pellet should be resuspended in nutrient-free saline solution. We followed these latter recommendations and resuspended the coccolithophores in ASW with 2.5 mmol L^{-1} calcium.

Data Processing Description

Data Processing:
The potential utilization of each organic compound was expressed as the average compound color development (ACCD). We averaged the absorption observed at five points across the center of each well at each time point and subtracted the average water control well. We then calculated the mean absorbance of replicate wells for each compound. The ACCD was computed as the percentage of the absorbance of each compound over the sum of absorbance of all compounds on a plate.

BCO-DMO Processing:
- concatenated data from separate Excel sheets into one dataset;
- joined the strain information from original file "CCMP_cocco_strains.xlsx" to the rest of the dataset, matching on the Strain_code field;
- renamed fields to conform with BCO-DMO naming conventions;
- replaced 'n.d.' with 'nd' (no data);
- converted dates to YYYY-MM-DD format;
- removed directionals from latitude and longitude column to allow for column to be typed as numeric;
- removed comma in D,L-α-Glycerol-Phosphate in Substrate column to allow for download as a .csv;
- replaced spaces in Species column and Collection_Site_Sea column with underscores to allow for sorting;
- rounded data as requested by data provider.

[table of contents | back to top]

Data Files

File
metabolic_potential.csv (Comma Separated Values (.csv), 113.02 KB) MD5:ec3f1a70b587e331958f5d99e6e6d47c Primary data file for dataset ID 858513

[table of contents | back to top]

Related Publications

Godrijan, J., Drapeau, D., & Balch, W. M. (2020). Mixotrophic uptake of organic compounds by coccolithophores. Limnology and Oceanography, 65(6), 1410–1421. doi:10.1002/lno.11396
Results

Pierce, M. L., Ward, J. E., & Dobbs, F. C. (2014). False positives in Biolog EcoPlatesTM and MT2 MicroPlatesTM caused by calcium. Journal of Microbiological Methods, 97, 20–24. doi:10.1016/j.mimet.2013.12.002
Methods

Stefanowicz, A. 2006. The Biolog plates technique as a tool in ecological studies of microbial communities. Pol. J. Environ. Stud. 15: 669–676. https://isbnsearch.org/isbn/1230-1485

Methods

Tuchman, N. C., Schollett, M. A., Rier, S. T., & Geddes, P. (2006). Differential Heterotrophic Utilization of Organic Compounds by Diatoms and Bacteria under Light and Dark Conditions. Hydrobiologia, 561(1), 167–177. doi:10.1007/s10750-005-1612-4
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Strain_code	Strain code (CCMP) from the National Center for Marine Algae and Microbiota (NCMA)	unitless
Species	Species name	unitless
Isolated_Date	Strain isolation date; format: YYYY-MM-DD	unitless
Deposit_Date	Strain deposit date; format: YYYY-MM-DD	unitless
Collection_Site_Lat	Latitude of strain collection site; positive values = North	decimal degrees North
Collection_Site_Long	Longitude of strain collection site; positive values = East	decimal degrees East
Collection_Site_Sea	Body of water where strain was collected	unitless
Substrate	Substrate	unitless
T0_AVG	Average absorption at time-zero (T0)	unitless
T0_SD	Standard deviation of absorption at time-zero (T0)	unitless
T24_AVG	Average absorption at 24 hours	unitless
T24_SD	Standard deviation of absorption at 24 hours	unitless
T48_AVG	Average absorption at 48 hours	unitless
T48_SD	Standard deviation of absorption at 48 hours	unitless
T72_AVG	Average absorption at 72 hours	unitless
T72_SD	Standard deviation of absorption at 72 hours	unitless
T120_AVG	Average absorption at 120 hours	unitless
T120_SD	Standard deviation of absorption at 120 hours	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	FilterMax F5 multimode plate reader
Generic Instrument Name	plate reader
Dataset-specific Description	FilterMax F5 multimode plate reader (Molecular Devices, LLC, San Jose, CA, U.S.A.).
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

[table of contents | back to top]

Project Information

Coccolithophore Mixotrophy (Cocco-Mix)

Coverage: Partially lab-based, with field sites in Gulf of Maine and NW Atlantic between the Gulf of Maine and Bermuda

NSF Award Abstract
Coccolithophores are single-cell algae that are covered with limestone (calcite) plates called coccoliths. They may make up most of the phytoplankton biomass in the oceans. Coccolithophores are generally considered to be autotrophs, meaning that they use photosynthesis to fix carbon into both soft plant tissue and hard minerogenic calcite, using sunlight as an energy source ("autotrophic"). However, there is an increasing body of evidence that coccolithophores are "mixotrophic", meaning that they can fix carbon from photosynthesis as well as grow in darkness by engulfing small organic particles plus taking up other simple carbon molecules from seawater. The extent to which Coccolithophores engage in mixotrophy can influence the transfer of carbon into the deep sea. This work is fundamentally directed at quantifying coccolithophore mixotrophy -- the ability to use dissolved and reduce carbon compounds for energy -- using lab and field experiments plus clarifying its relevance to ocean biology and chemistry. This work will generate broader impacts in three areas: 1) Undergraduate training: Two REU undergraduates will be trained during the project. The student in the second year will participate in the research cruise. 2) Café Scientifique program: This work will be presented in Bigelow Laboratory's Café Scientifique program. These are free public gatherings where the public is invited to join in a conversation about the latest ideas and issues in ocean science and technology. 3) Digital E-Book: We propose to make a digital E-book to specifically highlight and explain mixotrophy within coccolithophores. Images of mixotrophic coccolithophores would be the primary visual elements of the book. The E-book will be publicly available and distributed to our educational affiliate, Colby College. The goal of the book is to further communicate the intricacies of the microbial world, food web dynamics, plus their relationship to the global carbon cycle, to inspire interest, education, and curiosity about these amazing life forms.

Coccolithophores can significantly affect the draw-down of atmospheric CO2 and they can transfer CO2 from the surface ocean and sequester it in the deep sea via two carbon pump mechanisms: (1) The "alkalinity pump" (also known as the calcium carbonate pump), where coccolithophores in the surface ocean take up dissolved inorganic carbon (DIC; primarily a form called bicarbonate, a major constituent of ocean alkalinity). They convert half to CO2, which is either fixed as plant biomass or released as the gas, and half is synthesized into their mineral coccoliths. Thus, coccolithophore calcification can actually increase surface CO2 on short time scales (i.e. weeks). However, over months to years, coccoliths sink below thousands of meters, where they dissolve and release bicarbonate back into deep water. Thus, sinking coccoliths essentially "pump" bicarbonate alkalinity from surface to deep waters, where that carbon remains isolated in the abyssal depths for thousands of years. (2) The "biological pump", where the ballasting effect of the dense limestone coccoliths speeds the sinking of organic, soft-tissue debris (particulate organic carbon or POC), essentially "pumping" this soft carbon tissue to depth. The biological pump ultimately decreases surface CO_2. The soft-tissue and alkalinity pumps reinforce each other in maintaining a vertical gradient in DIC (more down deep than at the surface) but they oppose each other in terms of the air-sea exchange of CO_2. Thus, the net effect of coccolithophores on atmospheric CO2 depends on the balance of their CO_2-raising effect associated with the alkalinity pump and their CO2-lowering effect associated with the soft-tissue biological pump. It is virtually always assumed that coccolith particulate inorganic carbon (PIC) originates exclusively from dissolved inorganic carbon (DIC, as bicarbonate), not dissolved organic carbon (DOC). The goal of this proposal is to describe a) the potential uptake and assimilation of an array of DOC compounds by coccolithophores, b) the rates of uptake, and potential incorporation of DOC by coccolithophores into PIC coccoliths, which, if true, would represent a major shift in the alkalinity pump paradigm. This work is fundamentally directed at quantifying coccolithophore mixotrophy using lab and field experiments plus clarifying its relevance to ocean biology and chemistry. There have been a number of technological advances to address this issue, all of which will be applied in this work. The investigators will: (a) screen coccolithophore cultures for the uptake and assimilation of a large array of DOC molecules, (b) perform tracer experiments with specific DOC molecules in order to examine uptake at environmentally-realistic concentrations, (c) measure fixation of DOC into organic tissue, separately from that fixed into PIC coccoliths, (d) separate coccolithophores from other phytoplankton and bacteria using flow cytometry and e) distinguish the modes of nutrition in these sorted coccolithophore cells. This work will fundamentally advance the state of knowledge of coccolithophore mixotrophy in the sea and address the balance of carbon that coccolithophores derived from autotrophic versus heterotrophic sources.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635748

[table of contents | back to top]