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Quantifying Biogenic Silica (bSi) Deposition Rates Adapted Method

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We use this protocol and it's working

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Abstract

This method can be used to assess and quantify the rate of silica deposition (bSi) over time in diatoms to determine their rate of frustule synthesis. This protocol has been adapted for the processing of both cultures as well as environmental samples when inoculated with PDMPO [2-(4-pyridyl)-5-((4-(2dimethylaminoethylaminocarbamoyl)methoxy)phenyl)oxazole], which is a fluorescent dye that is co-deposited with silica during frustule biosynthesis in a 3230:1 Si:PDMPO (mol:mol) ratio.

Guidelines

- PDMPO can be obtained from **Fisher Scientific (Yellow/Blue Lysosensor)** at a 1mM concentration, with 50mL pre-aliquoted volumes.
- PDMPO is to be stored at -20°C in the dark prior to use.
- Thaw in dark at room temp when needed.
- Briefly centrifuge to mix thoroughly and ensure maximum volume is easily accessible via pipette.
- PDMPO is to be added to diatom culture/sample prior to experiment/incubation to a final concentration of 0.125mM.
- Depending on the volume of your culture/sample, this can be a very small volume to be added, in which case one can decide to make the additions of the dye individually or make one large addition to the media prior to inoculation.
- -Mix thoroughly and begin time-series experiment upon additions.

Materials

MATERIALS

LysoSensor™ Yellow/Blue DND-160 - Special Packaging **Fisher Scientific Catalog #**L7545

Safety warnings

See SDS (Safety Data Sheet) for hazards and safety warnings.

Before start

See Guidelines for PDMPO

1. Collection and Preservation of Samples + PDMPO

1 Select polycarbonate filter size based on your diatom/community.

Note

(ex. 0.22mm to collect all potential diatoms from an environmental sample, or 2.0 mm to collect diatoms from axenic cultures).

2 Either process samples immediately or preserve to process later. Proceed for processing.

Note

Samples can be processed immediately (described in the following text) or preserved to be processed later. In the event of one collecting samples over the course of a time series for later comparison, preserving the samples is highly recommended for accurate comparison. It is important to process all samples at the same time in the same conditions when you are hoping to compare them (ex. Process all treatments at the same time, or if this is not feasible prices all replicates per treatment at the same time).

3 the vacuum **Filter** designated volume onto the filter.

Note

It is advised to filter all replicates in a corresponding treatment simultaneously to minimize the chances of error amongst your replicates. Once filtered, turn off the vacuum, release the vacuum pressure.

Volume is based on the initial volume of your cultures and biomass (i.e. Whether you are using 50 mL culture tubes vs. 250 mL culture flasks, or based on diatom concentration or biomass concentration).
Previous experiments have been performed filtering 10 mL of culture to 100 mL of culture.

The later-described fluorometer calibration procedure and formation of a standard curve will adjust the sensitivity of the equipment, allowing for accurate measurements of incorporated PDMPO dye, regardless of the sample volume filtered.

4 Add 20 mL volume onto the filters using sterile growth media if they are cultures.

OR 0.22 mm filtered sample water for environmental samples.

Note

This step is crucial as it washes away any leftover external PDMPO from the cells which will interfere with your later calculations. It is best to turn the vacuum off before adding the volume to ensure the cells on the filter receive equal volumes of media/rinsing.

5 After adding volume to all replicates, turn the vacuum **on** and filter volume through for **rinse 1.**

Note

Ensure no liquid remains around the internal lid of the filter tulip, as this will dilute the next step.

10% HCl incubation

6 Turn the vacuum off and release any built-up pressure from the filter rig.

7 Add 10 mL of 10% HCl onto the filters and incubate for 00:02:00 .

8 Turn vacuum **on** and filter through.

9 Leave vacuum on and rinse the internal lip of the filter tulip with Milli-Q water to ensure the remaining acid is removed from the tulip prior to the next step.

Note

This is of critical importance as even the smallest amount of 10% HCl remaining has the potential to prevent the osmotic lysing of the cells via the Milli-Q incubation.

10 When acid is **completely removed** with the Milli-Q wash, turn vacuum off and release pressure.

Milli-Q incubation

11 Add 10 mL Milli-Q (resistivity of greater than or equal to 18 Ω-cm).

Note

These two incubation steps osmotically lyse the diatom cells and effectively release PDMPO taken up by the diatoms and stored in the silica deposition vesicle, but not yet incorporated into the frustule. Failure to lyse the cells will result in potential large standards of error amongst replicates and inaccurate deposition rate calculations.

12 Incubate for 00:02:00 .

13 Turn vacuum **on** until all Milli-Q has been pulled through.

14 Turn vacuum **off.**

15 Perform one **final rinse** of 20 mL sterile media or filtered environmental water, ensuring the filters are equally covered by liquid.

16 Turn vacuum **on** until media or environmental water has been pulled through.

17 Turn vacuum **off**.

18 Using sterile tweezers, fold filters into quarters and place in labeled 2 mL cryovial.

19 Flash freeze in liquid nitrogen immediately after sampling, then store at -80 °C until processing.

3. Frustule Digestion (Hot NaOH digestion)

20 Frustule digestion is necessary for accurate results and if the frustule digestion is not properly performed, there is a high likelihood of significant error among replicates.

CITATION

Leblanc, K., & Hutchins, D. A. (2005) (2005). New applications of a biogenic silica deposition fluorophore in the study of oceanic diatoms. Limnology and Oceanography. : Methods, 3(10), 462-476..

Further detail on frustule digestion can be found in LeBlanc and Hutchins 2005.

21 Thaw samples on ice and prepare 1L stocks of [M] 0.2 Molarity (M) NaOH and [M] 1 Molarity (M) HCl .

22 Once samples are thawed, carefully remove filters from cryovials and transfer to 15 mL labeled Falcon tubes.

23 ***Critical Step for Reading Sample protocol***

Protocol

NAME

Reading Sample Fluorescence (PDMPO) via Fluorometer

CREATED BY

Ashley A Humphrey

PREVIEW

Establishing a Standard Curve: Prior to centrifuging samples for reading, establish a standard curve of 5 different concentrations. Standard curve replicates are to be:

- 4 mL NaOH that has been incubated at 80 °C in the hot water bath along with the other samples.

- Chilled on ice and neutralized with + 1 mL [M] 1 Molarity (M) HCl with selected volume of PDMPO then added prior to reading.

- Ensure there is a blank, consisting only of the NaOH-HCl matrix with no PDMPO addition.

23.1 From the home screen of the device, perform the following:

23.1.1 Select the 1. Button for “set-up”

23.1.2 Select the 1. Button again for “mode”

23.2 Confirm machine reads “simple multi-operational” (which is the mode)

press ENTER.

23.3 Select the 2. Button for “calibration procedure”

23.3.1 Use < > arrow buttons to select “Direct concentration” (as the type of measurement to be taken/read)

press ENTER.

23.4 Select the 3. Button for “units”

Then select mg/L, mg/mL, ng/mL etc.

Note

Depending on your own concentrations selected for your standards. We utilized mg/mL or ng/mL for our standards, depending on whether they are cultures or environmental samples.

23.5 **Press ESCAPE** and return to the main home screen.

23.6 Select “max range”

Note

Which is the maximum approximate concentration range you expect, you will have to perform a little trial and error depending on your samples and their concentration.

*This will vary on your cell concentration, incubation conditions, and the volume filtered.

23.7 You will then be prompted to enter the number of standards you are using (5) and **press ENTER**.

23.8 The machine will ask you to insert your highest standard first:

Pipet 4 mL of the standard into a disposable glass 10 mL tube and wiping the outside prior to insertion.

23.9 Enter the concentration (in your prior selected units) from your standard. (This is your highest standard).

Select 1. “OK”. Then **press “*”** to read the standard.

23.10 Machine will prompt you to repeat the same steps for your other standards (Order does not matter).

23.11 Follow prompt to insert a blank, press **ENTER**.

Note

4 mL of NaOH and 1 mL 1M HCl

23.12 Once blank stabilizes, **press "0".**

23.13 Screen will revert to home screen when reading is complete.

23.14 Once calibration is complete and displayed on the computer screen that is hooked up to your fluorometer,

You will use this equation to convert your sample readings (FSU) to actual concentrations of PDMPO and then silica.

Note

It is recommended to record your fluorescence readings (FSU) of your standards as you go through the calibration process and plot them manually to validate and compare the program's results (actual concentration, x-axis) vs. (fluorescence, y-axis).

23.15 The linear regression equation must have an R^2 value higher than 0.95 ($R^2 > 0.99$ preferred).

23.16 Carefully pipette the top 4 mL of matrix from your recently centrifuged samples into a clean 10 mL disposable glass tube.

Note

Do not to disturb the bottom of the 15 mL Falcon tube where filter fragments and unlysed/digested cells may be, as it will interfere with fluorescence readings.

23.17 **Press the "*"** button and begin sample reading which will be visible on computer screen.

Note

The end number is an average of a variety of fluorescence measurements.

23.18 After reading all samples, enter the fluorescence readings into your linear regression equation (y) and solve for the actual concentration of PDMPO (x).

Note

The actual concentration must be converted from **mL/nL** to **mol.** Then converted to silica using the conversion factor 3230:1 for Si: PDMPO (mol:mol).

23.19 Normalize to either **cells/mL** or **chlorophyll-A** data, for each sample, to obtain Si (mol)/cell.

23.20 Information can be graphed to show silica deposition rates over time.

24 Add 4 mL volume of [M] 0.2 Molarity (M) NaOH to all tubes.

Ensure filters are fully submerged in the solution.

25 Screw Falcon tube lids on to prevent evaporation during incubation.

26 Mix gently by hand to remove any air bubbles around filter.

27 Place tubes in a rack in an 80 °C water bath for 01:00:00 incubation in the dark.

28 Remove tubes from water bath and cool in ice bath for 00:15:00 .

29 Once cooled, add 1 mL of [M] 1 Molarity (M) HCl to each tube, resulting in a net volume of 5 mL .

Note

At this point, the frustules of the diatoms should be digested and the only source of PDMPO fluorescence will stem from the PDMPO that has been deposited into the frustule itself via deposition.

30 Invert tubes and hand mix thoroughly.

Note

It is normal for filter to degrade during the hot NaOH digestion.

31 Centrifuge tubes at 13000 rpm for 00:05:00 prior to reading fluorescence of the matrix.

Note

Centrifuge to ensure filter fragments/remaining cells are not interfering with fluorescence measurements.

Citations

Step 20

Leblanc, K., & Hutchins, D. A. (2005). New applications of a biogenic silica deposition fluorophore in the study of oceanic diatoms. Limnology and Oceanography