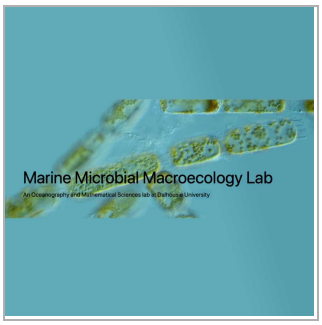

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IN DEVELOPMENT

Biogenic silica measurement from diatom

COMMENTS 0

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Ying-Yu Hu[1], Nuwanthi Samarasinghe[1], Zoe V. Finkel[1]

[1]Dalhousie University

Marine Microbial Macroecology Lab
Tech. support email: **ruby.hu@dal.ca**

Ying-Yu Hu
Dalhousie University

ABSTRACT

Here we describe the measurement of biogenic silica from diatoms. Biogenic silica is digested by wet-alkaline method, using 2 M sodium carbonate to hydrate and depolymerize amorphous silica and yield monosilicic acid. Diatomaceous earth is used as a check standard for the recovery of biogenic silica. The molybdate measurement is adopted from Mortlock et al. (1989) and JGOFS protocols (UNESCO 1994).

Digestion:

CITATION

Shemesh, Aldo; Mortlock, Richard A; Smith, R J; Froelich, Philip N (1970). Determination of Ge/Si in marine siliceous microfossils: separation, cleaning and dissolution of diatoms and radiolaria. Marine Chemistry.

LINK

https://doi.org/10.1016/0304-4203(88)90113-2

Molybdate measurement:

CITATION

David A. Mucciarone. Stanford University Standard Operating Procedures .

LINK

10.17605/OSF.IO/UVHT3

PROTOCOL CITATION

Ying-Yu Hu, Nuwanthi Samarasinghe, Zoe V. Finkel 2022. Biogenic silica measurement from diatom. **protocols.io** https://protocols.io/view/biogenic-silica-measurement-from-diatom-cimmuc46

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biogenic silica, dissolution, diatom, molybdate

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Sample collection

1 Estimation:
Low limit of detection of this assay is ~3 uM silicate per sample in the molybdate assay, equivalent to about 1 ug PON per filter.

2 Filter micro algae in liquid media onto polycarbonate filters, using gentle vacuum pressure (130 mmHg)

Equipment

Filter forceps	NAME
blunt end, stainless steel	TYPE
Millipore	BRAND
XX6200006P	SKU

3 Rinse filter funnel with filtered artificial seawater without macronutrients to avoid sample loss

4 Transfer filter with sample into 2 mL cryogenic vial

5 Filter blank media (without cells) through polycarbonate filter as blank

6 Flash freeze and store at -20 °C

Dissolution

7 [M] 2 M Na_2CO_3

7.1 Each sample requires 10 mL 2 M Na_2CO_3

7.2 In a 1 L polypropylene volumetric flask, add 500 mL Milli-Q

7.3 Add and dissolve 212 g Na_2CO_3 (CAS: 497-19-8, FW 105.99)

7.4 Top final volume to 1 L

8 Turn on airforce oven to 85 °C

Equipment

Forced air oven NAME

VWR BRAND

89511-410 SKU

9 Transfer sample to 50 mL falcon tube with clean filter forceps (rinsed by 95% ethanol and air-dried)

Equipment

Falcon® Centrifuge Tubes NAME

Polypropylene, Sterile, 50 mL TYPE

Corning® BRAND

352070 SKU

10 Diatomaceous

Note

10.1 Weigh ~1000 ug diatomaceous into 50 mL falcon tube, in triplicate.

Celite S diatomaceous earth **Sigma-aldrich Catalog #06858**

10.2 Prepare one empty 50 mL falcon tube as the reagent blank for diatomaceous.

11 Add 10 mL 2 M Na_2CO_3 into each tube with sample or diatomaceous and vortex.

12 Loose the caps and place all samples into the airforce oven overnight (for example, from 5 pm to 9 am). 16h

13 Remove tubes from the oven and cool to room temperature

Note

Prepare standard primary solution and reagents

14 Molybdate reagent stock solution

Note

14.1 Ammonium molybdate **Sigma Aldrich Catalog #09878-100G**

Ammonium paramolybdate:
[(NH4)6Mo7O24.4H2O]
CAS: 12058-85-2

14.2 In the fume hood, add 0.8 g ammonium paramolybdate and 2.4 mL 12 N HCl into a 100 mL plastic volumetric flask.

14.3 Top to 100 mL with MilliQ water.

14.4 Store in a brown polyethylene bottle out of direct sunlight. Discard if white precipitation forms.

15 HCl stock solution

In a fume hood, add 4.8 mL 12 N HCl into a 100 mL plastic volumetric flask and top to 100 mL.

Note

16 Metal-sulfite solution

Note

16.1 4-(methylamino)phenol hemisulfate salt **Sigma Aldrich Catalog #320013**

Sodium sulfite **Fisher Scientific Catalog #5430-500**

16.2 Require:
(1) No.1 Whatman filter paper
(2) Swinnex filter holder
(3) 50 mL syringe

16.3 In the fume hood, add 60 mL MilliQ into a 100 mL plastic volumetric flask.

16.4 Add 1.2 g sodium sulphite and mix to dissolve.

16.5 Add 2 g 4-(methyl amino)phenol hemisulfate, mix to dissolve.

16.6 Top to 100 mL with MilliQ water.

16.7 Cut No. 1 Whatman filter paper into 25 mm disk and load it into Swinnex filter holder.

16.8 Fill syringe with Metal-sulfite solution, filter through the filter paper, collect filtrate into an amber or dark plastic bottle, keep at room temperature.

16.9 Prepare fresh every month.

17 Oxalic acid solution

Note

17.1 Oxalic acid dihydrate **VWR international Ltd Catalog #BDH4556-500G**

17.2 In a 100 mL plastic volumetric flask, dissolve 6 g oxalic acid in MilliQ and top to 100 mL.

17.3 Let the solution stand at room temperature overnight.

17.4 Decant the solution from the crystals into a plastic bottle.

17.5 Keep at room temperature.

18 Sulphuric acid (0.5 M)
If following Stanford protocol, it shall be 50%. Then prepare as 1 part concentrated sulphuric acid into 1 part of MilliQ
Wait for Nuwanthi's confirmation

18.1

Note

18.2 In a 100 mL plastic volumetric flask, add 50 mL MilliQ

18.3 Slowly add 2.778 mL concentrated sulphuric acid

18.4 Allow the mixture cool to room temperature and then top to 100 mL with MilliQ water.

18.5 Store in a plastic bottle at room temperature.

19 Primary silica standard solution (~ 1 mM Si)

19.1 Sodium hexafluorosilicate **Sigma Aldrich Catalog #250171**

19.2 Transfer 1 g sodium fluorosilicate in a plastic vial

19.3 Keep the vial in a vacuum desiccator overnight to remove excess water (do not heat or fuse)

19.4 In a one litre plastic volumetric flask, dissolve ~ 0.1881 g (log the actual mass) of dry sodium fluorosilicate in MilliQ water and top to 1 L with MilliQ water.

19.5 It takes about 30 min to complete the dissolution. This cannot be rushed.

19.6 Store in a plastic bottle at room temperature.

Acidification

20 [M] 1 Molarity (M) HCl

20.1 12 N HCl : Milli-Q = 1 : 11 (v/v)

20.2 Require 5 mL 1 M HCl per sample

21 Gradually add 5 mL 1 M HCl to each tube (diatomaceous references, blank for diatomaceous reference, diatom samples and diatom sample blanks), mix well before adding the next portion of acid to avoid vigorous reaction.

22 Transfer resulted solution from falcon tube to 25 mL polypropylene volumetric flask

23 Fill empty falcon tube with about 2 mL Milli-Q, cap tightly and vortex. Transfer all liquid to the volumetric flask.

24 Fill empty falcon tube with about 2 mL Milli-Q, cap tightly and vortex. Transfer all liquid to the volumetric flask.

25 Fill empty falcon tube with about 2 mL Milli-Q, cap tightly and vortex. Transfer all liquid to the volumetric flask.

26 Top final volume to 25 mL with Milli-Q.

27 Shake and thoroughly mix the solution.

28

Note

3h

Molybdate reaction

29 Standard working solutions

Standards	Primary (uL)	MilliQ (uL)	Conc. (uM)
S1	0	1000	0
S2	5	995	5

Standards	Primary (uL)	MilliQ (uL)	Conc. (uM)
S3	10	990	10
S4	15	985	15
S5	30	970	30
S6	60	940	60
S7	80	920	80
S8	100	900	100
S9	200	800	200
S10	300	700	300

30 Molybdate working solution

Note

30.1 **1 part** Molybdate stock reagent
1 part HCl stock reagent

31 Reducing solution

Note

31.1 **1 part** Metol-sulfite solution
1 part oxalic acid solution
1 part sulphuric acid solution

32 **Standard working solutions**:

Transfer 50 µL standard working solution into 2 mL microtube, and add 450 µL MilliQ water

33 **Diatomaceous reference and blank**:

33.1 Transfer 50 µL solution from volumetric flask to a 2 mL microtube, add 450 µL MilliQ.

33.2 Transfer 50 µL diluted solution into a new 2 mL microtube, add 450 µL MilliQ.

34 **Diatom samples and blanks**:
Transfer 50 µL solution from volumetric flask into 2 mL microtube, and add 450 µL MilliQ water

35 Add 200 µL Molybdate reagent into each tube.

36 After yellow color is developed in 15 min, add 300 µL reducing solution into each tube.

37 Vortex each tube and then shake at Room temperature for 03:00:00

3h

3h

Colorimetric measurement

38 Load microplate with 250 µL reactant from each tube, duplicate.

Equipment	
96-Well Microplates, Polystyrene, Clear,	NAME
Greiner Bio-One	BRAND
655101	SKU

39 Read plate in microplate reader

A	B
Shake duration	00:00:05
Shaking type	Continuous
Shaking force	High
Shaking speed [rpm]	600
Wavelength [nm]	812
Use transmittance	No
Pathlength correction	No
Measurement Time [ms]	100

Equipment

Varioskan LUX Multimode Microplate Reader NAME

Thermo Fisher BRAND

VL0L00D0 SKU

Waste disposal

3h

40 Collect all solution with paramethylaminophenol sulphate in waste container.

Calculation

3h

41 Subtract the average absorbance at 812 nm of the blank standard replicates from the absorbance at 812 nm of all other standard working solutions.

42 Subtract the average absorbance at 812 nm of the blank sample (i.e. blank filter) replicates from the absorbance at 812 nm of all other individual samples.

43 Prepare a standard curve by plotting the average blank-corrected 812 nm absorbance for each standard working solution versus its concentration in uM.

44 Use the standard curve to determine the silicate concentration of each unknown sample by using its blank-corrected 812 nm absorbance.

45 (Si per sample)_umol = Si_uM X (25)_mL X (0.001)

46 Diatomaceous recovery = 100 X Si_uM X DF X (25)_mL X (0.001) X (60.1_ug/umol)/(M_ug X Purity X 0.01)
Where, DF is the dilution factor (DF=10), M is the mass of diatomaceous, purity of SiO2 in Celite S diatomaceous earth (06858) is 90.2%.

Note