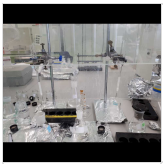

Jan 28, 2021

fatty acid analysis sponges

PLOS One

Anna de Kluijver[1]

[1]Utrecht University, Earth sciences

Anna de Kluijver: Klaas Nierop [Utrecht University, Earth sciences]

1 Works for me dx.doi.org/10.17504/protocols.io.bhnpj5dn

Anna de Kluijver

ABSTRACT

This protocol has been used to analyse fatty acid composition of sponges (Porifera). The original protocol has been developed at NIOZ Yerseke, former NIOO-CEME (Netherlands) (Boschker and Middelburg) and has been used to quantify PLFA in combination with stable isotopes for sediments, plankton, organisms (including sponges). This protocol has been adopted to the procedures of the organic geochemistry lab at Utrecht University.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0241095

DOI

dx.doi.org/10.17504/protocols.io.bhnpj5dn

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0241095

PROTOCOL CITATION

Anna de Kluijver 2021. fatty acid analysis sponges . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bhnpj5dn

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0241095

KEYWORDS

PLFA, BDE, lipids, GC, PLFA identification, derivatization, sponges

CREATED

Jun 18, 2020

LAST MODIFIED

Jan 28, 2021

PROTOCOL INTEGER ID

38319

GUIDELINES

Contamination: work clean and avoid organic contamination, especially plastics throughout the procedure
glassware: use very clean glassware that has been rinsed in decon, dishwasher washed and oven-dried
storage: The samples and extracts should be stored in a freezer at -20°C or colder.

SAFETY WARNINGS

This procedure uses hazardous chemicals

1. Read the MSDS forms for each chemical in the procedure.
2. Use gloves throughout the procedure.
3. Wear a lab coat and safety glasses throughout the procedure.

Dispose of all chemical waste in appropriately labeled containers

BEFORE STARTING

All sponge material should be (freeze)dried, grinded and homogenized

solution preparation

1 Prepare P-buffer: dissolve **8.7 g** K2HPO4 in **1 L** mQ in a 1 L glass bottle, adust to pH 7-9 with **1 Molarity (M)** HCl, check with pH paper

2 Prepare Bligh and Dyer extraction (BDE) mix: methanol (MeOH)/ dichloromethane (DCM) / P-buffer in ratio of 2/1/0.8 (v/v/v), e.g. **250 mL** MeOH, **125 mL** DCM and **100 mL** P-buffer in a 500 mL Erlenmeyer with glass stopper

3 Prepare standards:
20 mg $C_{19:0}$ FAME in **2 mL** hexane (100 x stock)
20 µl $C_{12:0}$ FAME in **2 mL** hexane (100 x stock)

4 Prepare **0.2 Molarity (M)** methanolic NaOH by dissolving **1.08 g** CH_3NaO in **100 mL** MeOH

5 Activate silicic acid gel by heating it at **120 °C** , for at least 2 hours and allow it to cool. Once activated, it can be kept in a closed bottle for 2 or 3 days.

6 Prepare **1 Molarity (M)** acetic acid: add **5.742 mL** glacial acetic acid (**17.416 Molarity (M)**) into **25 mL** miliQ and fill to **100 mL** in a 100 mL vollumetric flask.

Total lipid extraction

7 Weight 30-50 mg dried sponge sample in extraction tubes (Kimax®centrifuge tube).

This amount is enough to do either lipid-class or total lipid analysis, if you want to do both, weight a double amount.

8 Add **28.5 mL** Blight and Dyer mix, close with lid and shake/vortex

9 Extract the sample in an ultrasonic bath for **00:10:00**

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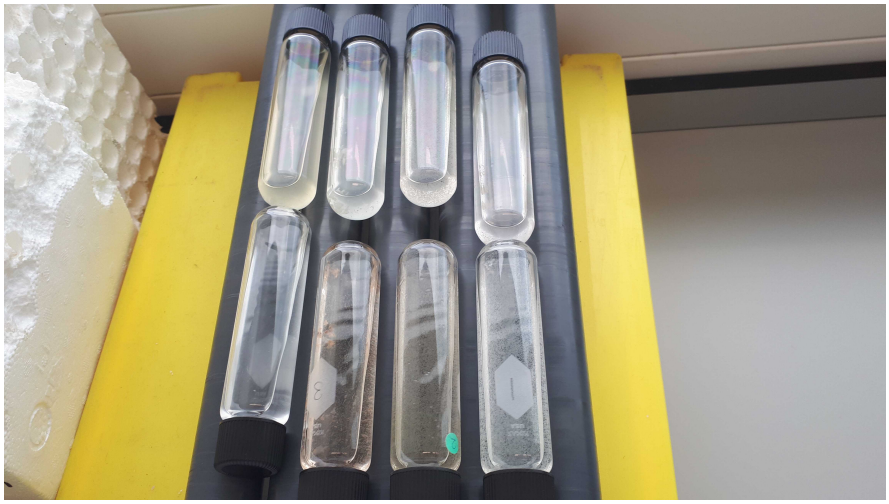
10 Extract for minimal **03:00:00** on a roller table - horizontal shaker. Check that lids are tightly closed!



11 add **7.5 mL** DCM, close lid and shake/vortex

12 add **7.5 mL** P-buffer, close lid and shake/vortex

13 Let layers separate by either leaving the tubes overnight in the freezer of by gentle centrifugation
170 x g, 00:03:00 (room temperature)

14 Collect the DCM layer (bottom) with a Pasteur pipette or a glass syringe into 50 mL glass vials. The type of vials depend on the evaporator. Take a bit of air into the syringe or pipette. Bring the tip carefully in the bottom layer. Let a few air bubbles out to clean the tip of the syringe/pipette. Collect the DCM and avoid taking the fluffy layer.
When using a glass syring, clean it in DCM: methanol (1:1) between samples.

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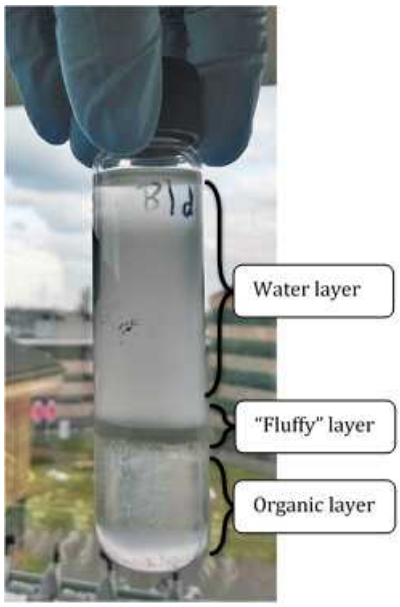

courtesy S. Hoetjes/ D. Lankes

15 Add again **7.5 mL** DCM, let layers separate (centrifugation or freezer) and collect DCM layer into the same vial

16 Dry samples completely under a gentle N_2-stream in a turbovap (**30 °C** , maximum 5 psi pressure, but start with 1-2 psi)

17 Add 1 ml **1 mL** of DCM and evaporate again.

18 Transfer sample in **1 mL** (approximately) DCM:MeOH (1:1) to a pre-weighed 4 ml vial and dry in a Flexivap **26 °C** under a gentle N_2 stream

19 Optional: if your sample contains some residue, it can be cleaned over a pasteur glass pipette with pre-extracted cotton wool in DCM: MeOH (1:1)

20 Weigh your vials to obtain total lipid weight /sponge dry weight. Now you have your Bligh and Dyer extract (BDE)

Separation into polarity classes

21 Prepare columns: add a ball of pre-extracted cotton-wool into the tip of a 10 mLglass pipette. Add 1 spoon (~0.5 g) on top. The whole column is ~3 fingers width, 3 cm. The column is stored into a cylinder filled with DCM. Make sure the silica layer is soaked in DCM. Gently tap the column to get rid of air bubbles.

22 Put the column in a holder, place a vial or beaker glass underneath and wash the column with DCM

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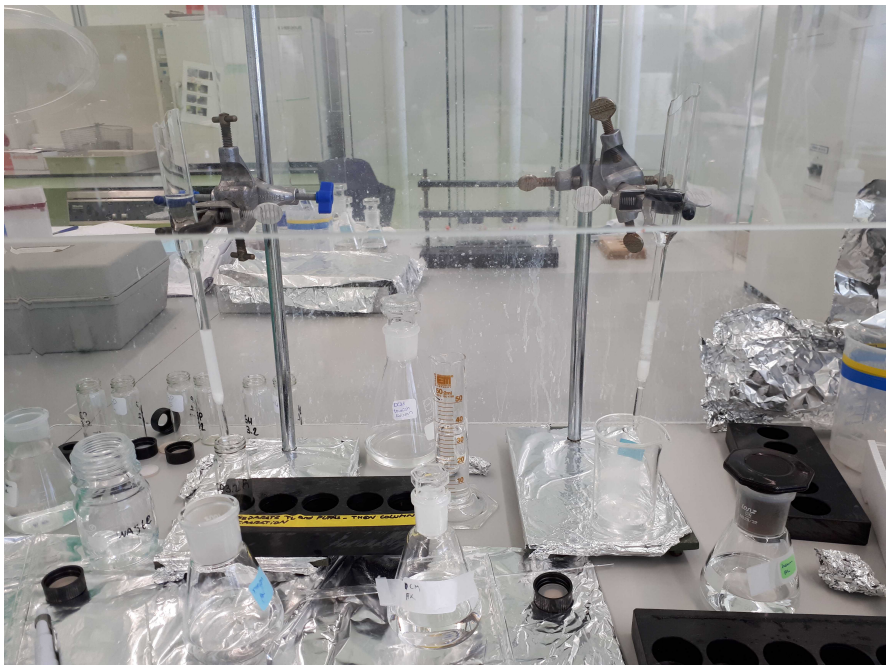

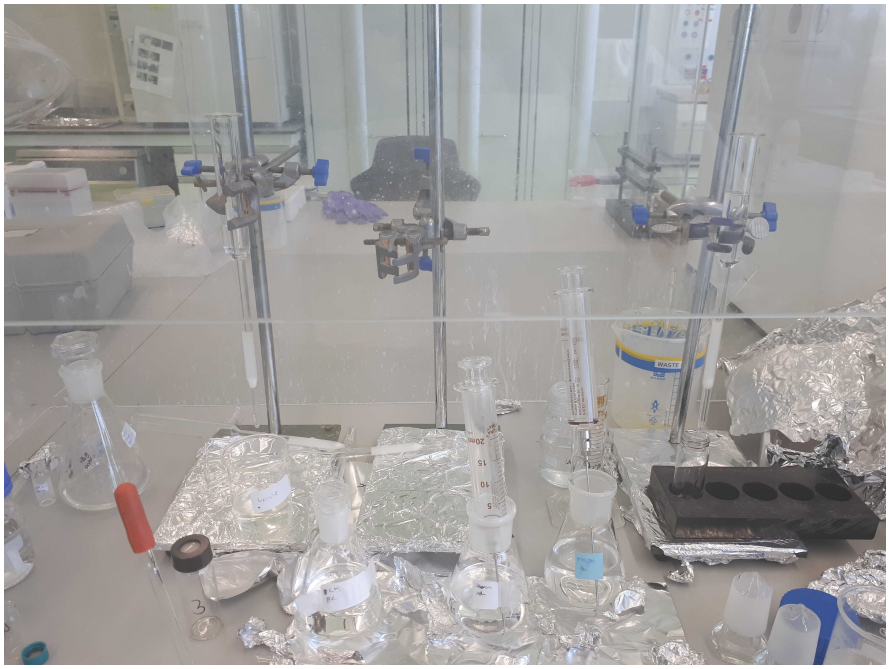

23 Dissolve and add your sample in **0.5 mL** DCM.

24 Don't disturb the coyour neutral lipid fraction. Collect in a 10 mL vial if you want to analyze it or in a waste beaker/vial if it can be discarded.
Don't disturb the column!

25 Elute with **7 mL** acetone (picograde pestinorm), this is your glycolipid fraction. Collect in a 10 mL vial if you want to analyze it or in a waste beaker/vial if it can be discarded.

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26 Elute with **15 mL** MeOH into a 20 m glass vial , this is your phospholipid fraction that will be used for further analysis.

27 Dry the MeOH fraction in the turbovap (**30 °C** max 5 psi, but start with 1-2 psi)

mild alkaline methylation

28 Add to dried sample 1 ml MeOH/toluene (1:1 v/v)
Add 1 ml 0.2 M methanolic NaOH (exactly with glass syringe/pipette)
Add **50 µl** **0.1 mg/ml** $C_{19:0}$ FAME standard

29 Incubate at **37 °C** **00:15:00** in a oven

30 Stop methylation by adding
2 mL hexane
0.3 mL **1 Molarity (M)** acetic acid (exactly, with pipette)
2 mL milliQ

31 Mix very well by shaking or vortexing. Let layers separate. The upper (hexane) layer should be clear

32 Collect the upper layer with a Pasteur pipette into a 10 ml glass vial. Avoid taking the water layer.

33 Add again **2 mL** hexane, repeat 31-32, collect in the same vial

34 Add again **2 mL** hexane, repeat 31-32, collect in the same vial

35 Evaporate hexane until complete dryness
Step 35 includes a Step case.
Acidmethylation

GC analysis

step case

Acidmethylation

- Add 0.5 mL DCM:MeOH (1:1) to dry sample vials
- Transfer the samples into a 4 ml glass vial

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- N_2 dry samples
- Add 0.5 mL BF_3-methanol (Merck Millipore: product code 801663, 20%)
- Incubate samples at 60°C for 10 minutes
- Add 0.5 mL MilliQ water and 40 µl standard $C_{19:0}$ FAME
- Add 1 mL DCM
- Collect the DCM layer (bottom layer) into another 4 mL glass vial
- Repeat the wash procedure 2 more times
- Dry under N_2

36 Add **50 µl** $C_{12:0}$ FAME and ~ **100 µl** hexane or ethyl acetate Transfer samples into GC analysis vials.

Store in **-20 °C** freezer until analysis

Hexane and ethyl acetate give comparable results. Make sure that $C_{12:0}$ FAME is in the same solvent

Step 36 includes a Step case.

DMDS

hydrogenation

Alox separation

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