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## Using dynamic headspace collections for bacterial volatile sampling

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Barbora Novakova<sup>1,2</sup>, John C. Caulfield<sup>1</sup>, David M. Withall<sup>1</sup>, Michael A. Birkett<sup>1</sup>, Jozsef Vuts<sup>1</sup>, Gareth Thomas<sup>1</sup>

<sup>1</sup>Protecting Crops and the Environment, Rothamsted Research; <sup>2</sup>Department of Biology, University of York

Gareth Thomas: Corresponding author: [gareth.thomas@rothamsted.ac.uk](mailto:gareth.thomas@rothamsted.ac.uk);



**Barbora Novakova**

Rothamsted Research

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**We use this protocol and it's working**

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## Abstract

This protocol describes a step-by-step guide for sampling Volatile Organic Compounds (VOCs) from the headspace of bacterial cultures, using dynamic headspace collections.

## Guidelines

Sterile conditions are required throughout.

## Materials

Lysogeny Broth (LB) (Sigma-Aldrich)

Lysogeny Agar (Sigma-Aldrich)

Ringer's Solution (Sigma-Aldrich)

Falcon tubes (50 mL)

Inoculating loops

Petri dishes (9 cm diam.)

Glass pipettes (10 mL)

Pipette controller

Gilson's micropipette

Glass Pasteur pipette (150 mm)(Thermo Fisher Scientific)

Pipette Bulb Dropper Teat Red Latex 1 mL Capacity (Thermo Fisher Scientific)

Glass chambers (10 cm height × 12 cm diam.)

Metal plates (12cm diam.)

Filter paper (12.5 cm) (Whatman)

Porapak tubes (50 mg Porapak)

Brass fittings

Ferrules

Air entrainment kit

Macherey Nagel Screw Caps (8 mm)(Esslab)

Rubber septa (8 mm, Silicone/Red PTFE) (Kinesis Scientific Experts)

Pointed glass vials (1.1 mL Thermo Fisher Scientific)

Glass syringe

Solvents:

Acetone (99%) (Thermo Fisher Scientific)

Redistilled diethyl ether (99.5%) (Thermo Fisher Scientific)

## Before start

Ensure appropriate risk assessments have been completed and follow any local guidelines that apply.



## Materials to autoclave before experiment

- 1 Prepare 400 mL of LB broth (25 g per litre).
- 2 Prepare 500 mL of Ringer's solution, by placing one tablet of Ringer's solution into 500 mL distilled water.
- 3 Prepare 400 mL of LB agar (37 g per litre).
- 4 100  $\mu$ L Pipette tips.
- 5 Autoclave items.

## Preparing overnight cultures of bacteria

- 6 Transfer 10 mL of autoclaved LB into two 50 mL falcon tubes. 
- 6.1 While we use LB as the primary medium, other media can also be utilized as needed for the protocol.
- 7 Using a sterile inoculating loop, pick a single colony from a bacterial culture inoculated onto a Petri dish.
- 8 Inoculate one falcon tube with the single colony, and leave the other falcon tube containing LB broth alone, as an uninoculated control.
- 9 Place both falcon tubes in a shaking incubator (23°C/170 rpm) for ~16 hours.

## Culturing bacteria prior to headspace collections

- 10 Pour the autoclaved LB agar onto the required number of Petri dishes and leave to dry, until the agar has solidified.



- 11 After ~16 hours, remove the falcon tubes used in step 9 from the shaking incubator and assess the bacterial growth by visually comparing the bacterial culture to the LB control tube. The bacterial culture should be more cloudy in appearance compared to the LB control tube.
- 12 Centrifuge the bacterial culture into a pellet at 3000 rpm for 10 minutes. 
- 13 Discard the supernatant after centrifugation.
- 14 Transfer 10 mL of Ringer's solution into the falcon tube containing the bacterial pellet. 
- 15 Mix the solution using a plastic pipette (10 mL) attached to a pipette controller.
- 16 Repeat steps 12-15 twice more, to wash bacterial cells.
- 17 Measure the Optical Density (OD) of the bacterial culture in the Ringer's solution using a spectrophotometer, using Ringer's solution alone as a reference blank.
- 18 Dilute the bacterial culture with Ringer's solution, to reach the required optical density (OD=0.1).
- 18.1 Equation for working out dilution volume:  
**Concentration**<sub>(start)</sub> × **Volume**<sub>(start)</sub> = **Concentration**<sub>(final)</sub> × **Volume**<sub>(final)</sub>
- 19 Apply 70 µL (Garbeva, 2014) of the diluted bacterial culture, at the desired Optical Density, to a fresh agar plate. 
- 20 Spread the diluted bacterial culture across the entire plate using a sterile inoculating loop.
- 21 Repeat steps 19-20 for the required number of bacterial isolates/replicates, leaving one agar plate without any inoculum as an uninoculated control.



22 Incubate the Petri dishes in total darkness (48 h at 28°C).



## Conditioning glass chambers and metal plates prior to headspace collections

23 Wash the glass chamber (10 cm height × 12 cm diam.) and glass fittings using Teepol detergent and distilled water.



24 Rinse the detergent off the glass chamber using distilled water.

25 Wash the glass chamber using 100% acetone.

26 Rinse the glass chamber using distilled water.

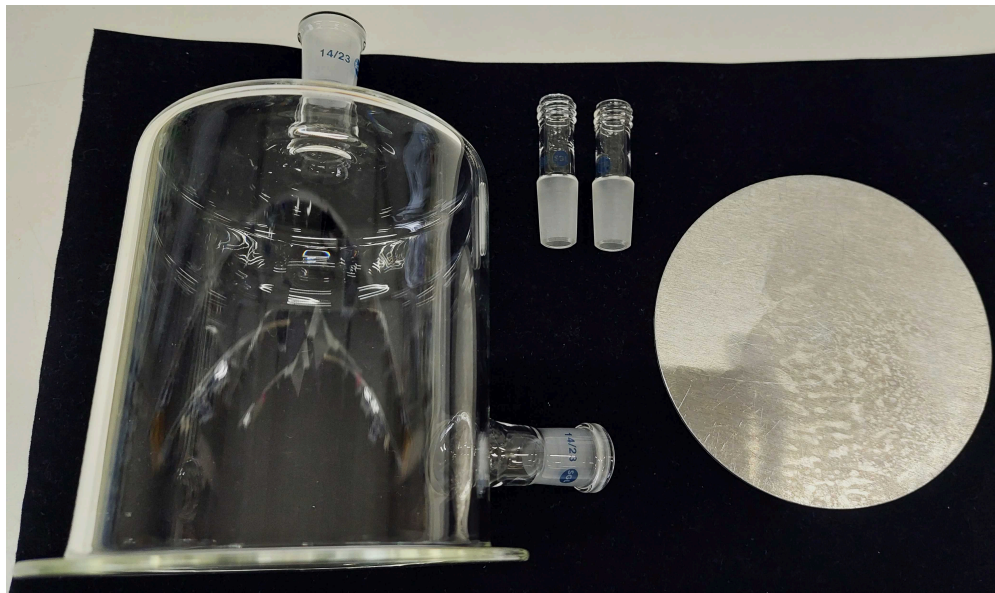
27 Repeat steps 23-26 for the metal plates.

28 Leave the glass chambers and metal plates in a modified heating oven at 150°C for a minimum of 2 hours.

1h 30m



29 Remove the glass chamber and metal plates from the oven using heat resistant gloves and allow to cool, prior to the headspace collections.



Components needed for headspace collection of bacterial volatiles: glass chamber, glass fittings, metal plate.

## Conditioning activated charcoal prior to headspace collections

- 30 Attach the charcoal filter tube to a supply of constant flow of purified nitrogen, using brass fittings, and turn nitrogen supply on. Ensure the nitrogen flow is in the opposite direction to the arrow on the charcoal filter.



Charcoal filter attached to nitrogen supply.

- 31 Check for a flow of nitrogen by attaching a Teflon tube to the end of the filter, and placing the end of the tubing into a vial of diethyl ether, and assess for bubbles.



- 32 Place the charcoal filter into a modified heating oven at 170°C for a minimum of 2 hours.

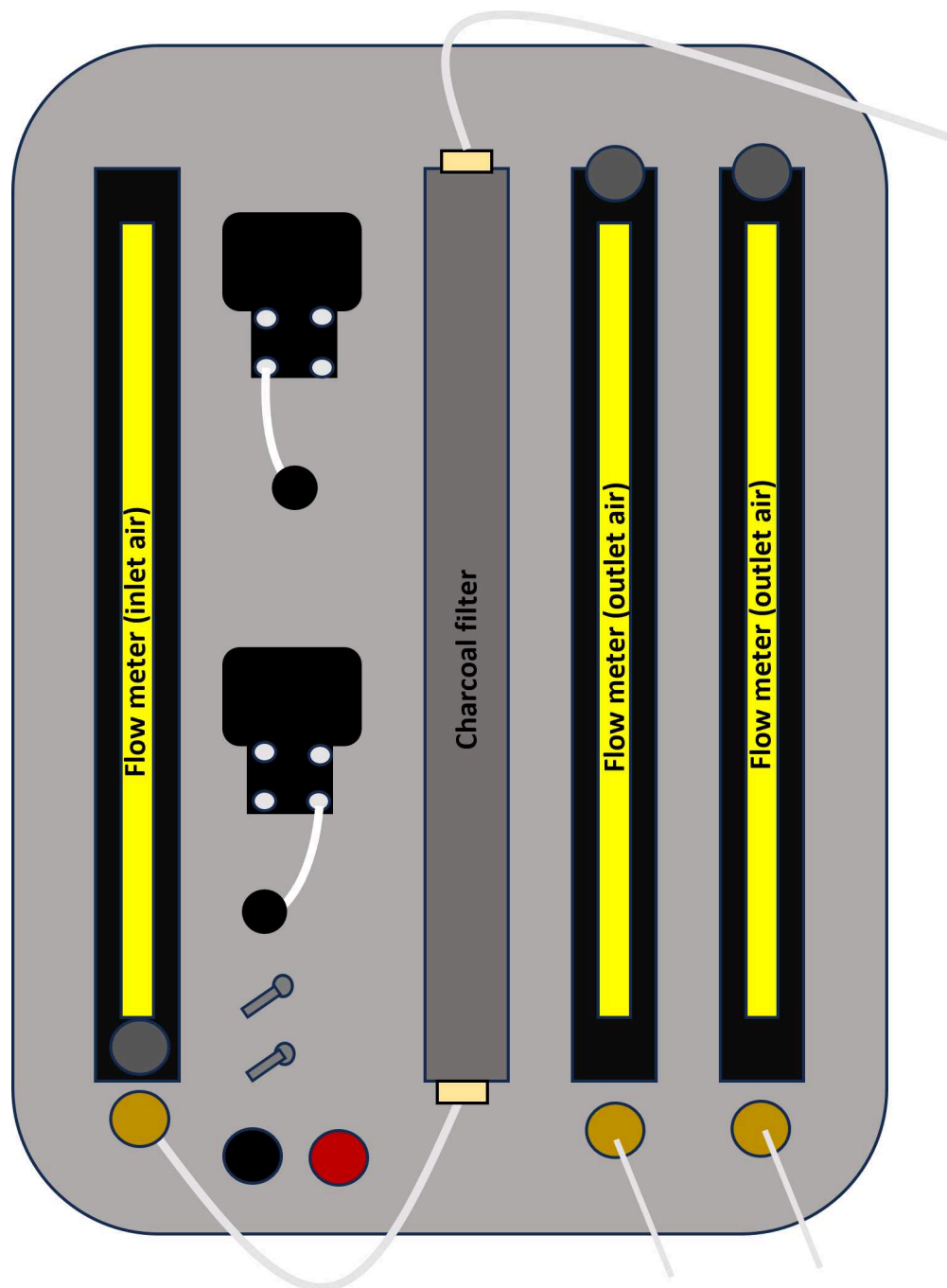
1h 30m



Modified heating oven containing charcoal filter tube, under a constant stream of nitrogen.

- 33 After 2 hours, remove the charcoal filter from the oven using heat resistant gloves and allow to cool. Keep the nitrogen flow on whilst the charcoal filter is cooling.
- 34 Once the charcoal filters have cooled, turn off the nitrogen supply and attach the charcoal filter back onto the air entrainment kit.

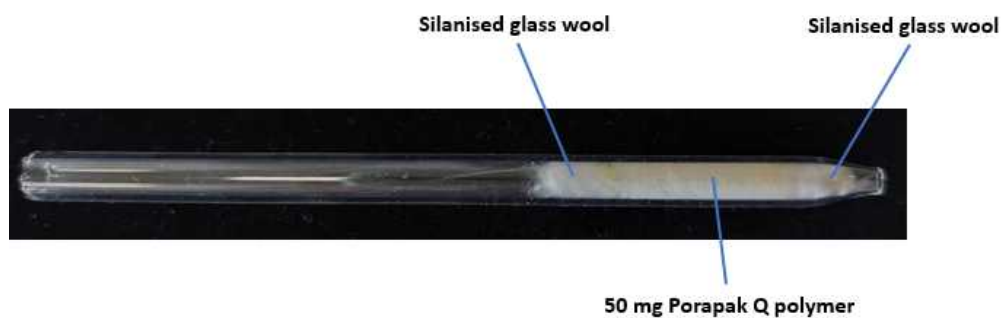




Schematic overview an air entrainment kit.

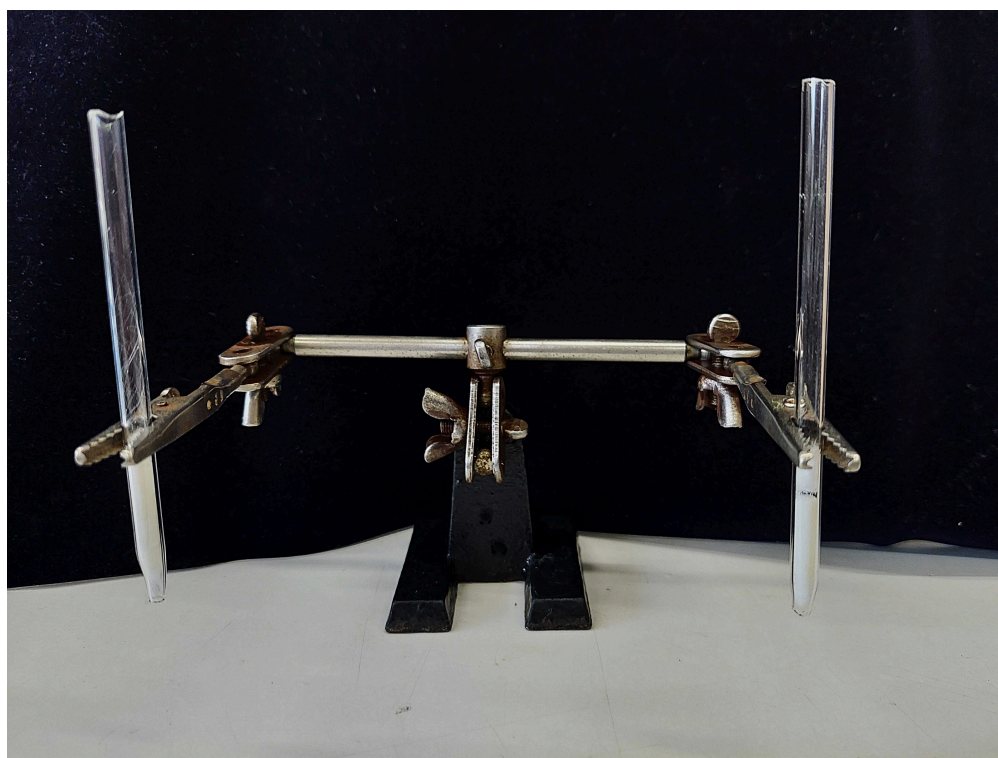
## Conditioning Porapak tubes prior to headspace collections

- 35 Porapak tubes contain 50 mg of Porapak Q polymer (mesh size 50/80) within a glass tube, held between two plugs of silanised wool.



Porapak tube containing silanised glass wool and 50 mg of Porapak Q polymer.

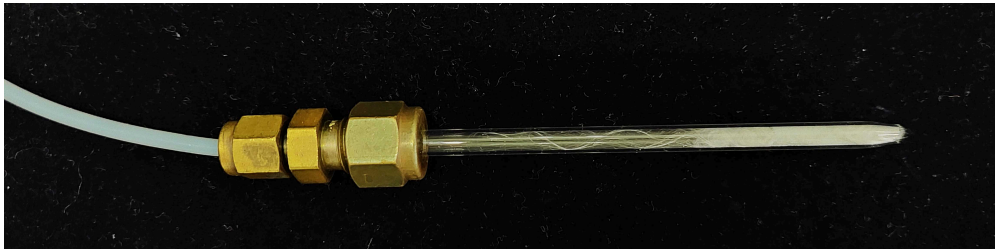
- 36 In a fume hood, clamp the Porapak tubes so they are suspended.



Porapak tubes suspended in a fume hood.

- 37 Using a glass Pasteur pipette, take up 2 mL of redistilled diethyl ether and pass through the Porapak tubes.
- 38 Attach the Porapak tubes to a supply of nitrogen, using brass fittings, and turn the nitrogen supply on.





Porapak tube attached to nitrogen supply.

- 39 Check for a flow of nitrogen by placing the end of the Porapak tube into a vial of diethyl ether and assess for bubbles.
- 40 Place the Porapak tubes into a modified heating block at 132°C for a minimum of 2 hours.

2h



Modified heating block containing a Porapak tube, under a constant stream of nitrogen.



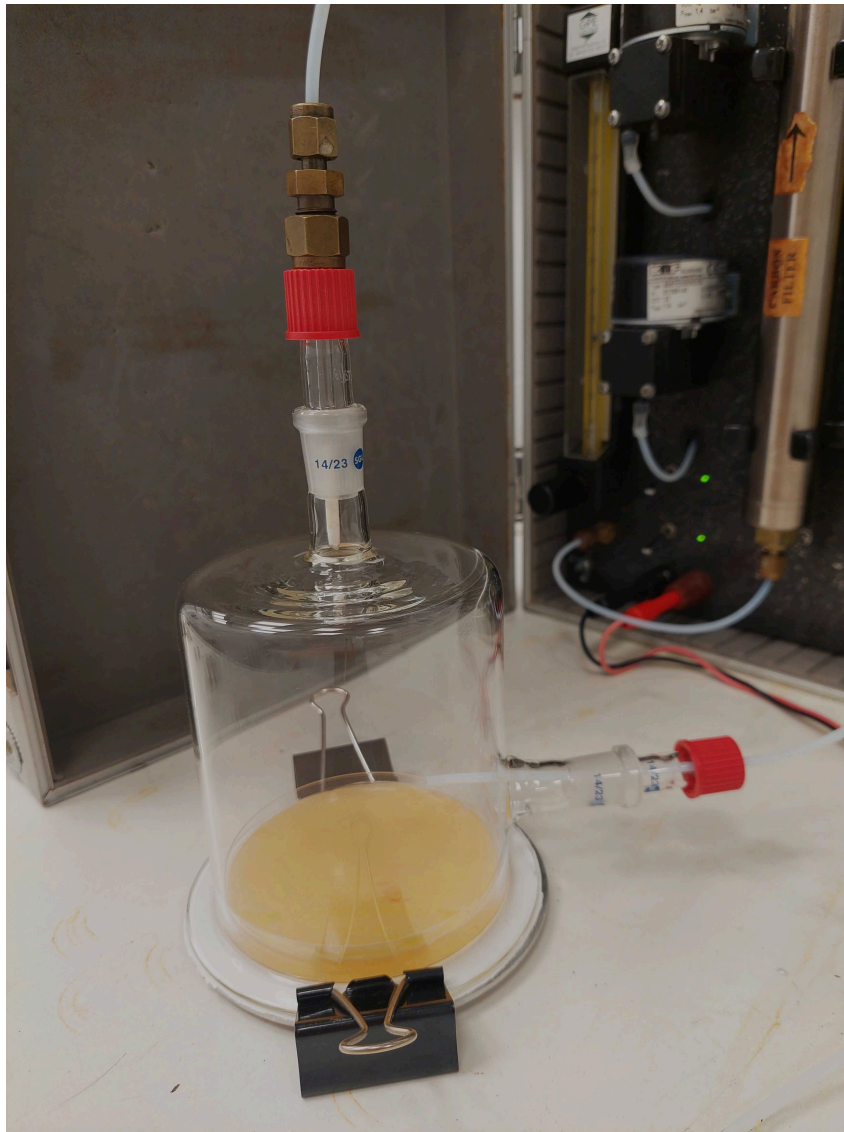
- 41 After 2 hours, remove the Porapak tubes from the heating block using heat resistant gloves and allow the Porapak tubes to cool. Keep nitrogen flow on whilst the tubes are cooling.
- 42 If using newly made Porapak tubes, repeat steps 36-41 twice more (three conditioning rounds in total) to remove any contaminants on the Porapak.

## Setting up headspace collections

20h

- 43 Place a filter paper disc (12.5 cm) on top of one of the metal plates.
- 44 Place one of the agar plates containing the bacteria on top of the filter paper.
- 45 Place the glass chamber on top of the agar plate.
- 46 Attach the glass chamber to the metal plate using bulldog clips.
- 47 Attach a smaller ferrule and a red screw cap to the inlet at the side of the glass chamber.
- 48 Insert the tube from air entrainment kit through the inlet into the glass chamber.
- 49 Attach a larger ferrule and red screw cap to the outlet at the top of the glass chamber, and insert a conditioned Porapak tube.
- 50 Attach to the air entrainment kit using brass fittings.





Set-up for the collection of VOCs from the headspace of bacteria.

- 51 Repeat steps 43-50 for the required number of treatments/replicates (minimum of four replicates per treatment, and include an uninoculated growth media control for each round of sampling).
- 52 Turn on the air entrainment kits.
- 53 Set the flow of inlet air to  $600 \text{ mL min}^{-1}$  and outlet air to  $500 \text{ mL min}^{-1}$ , ensuring a positive pressure within the system.
- 54 Leave in darkness for 20 hours.

20h



## Elution of volatiles following headspace collections

- 55 After 20 hours, turn off the air entrainment kits.
- 56 Remove the Porapak tubes from each glass chamber.
- 57 In a fume hood, clamp the Porapak tubes so they are suspended.
- 58 Place a labelled 1.1 mL pointed GC vial beneath each Porapak tube. Ensure that the Porapak tube for a given treatment/replicate is matched with the correctly labelled GC vial.



Porapak tubes suspended over GC vials in a fume hood.

- 59 Take up 750  $\mu$ L of redistilled diethyl ether using a glass syringe and pass through the Porapak tubes.



- 60 Allow the ether to flow through the Porapak tube into the GC vial.
- 61 Place the rubber septa and the screw cap onto the GC vial, ensuring the red side of the septa is pointing downwards, and the white side of the septa is pointing upwards.
- 62 Store the GC vials at -20°C for further analysis.

## Protocol references

Garbeva, P., Hordijk, C., Gerards, S., & de Boer, W. (2014). Volatiles produced by the mycophagous soil bacterium *Collimonas*. *FEMS Microbiology Ecology*. <https://doi.org/10.1111/1574-6941.12252>