



Method Article

Lab-scale biodegradation assay using passive samplers to determine microorganisms' ability to reduce polychlorinated biphenyl (PCB) volatilization from contaminated sediment ☆☆☆★



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Lab-scale biodegradation assay using passive samplers to screen microorganisms for the ability to prevent PCB volatilization from contaminated sediment slurry to air

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ABSTRACT

Many PCB-degrading aerobes have been identified which may serve as bioaugmentation strains for aerobic, in situ bioremediation or in combination with dredging operations. The present work describes a lab-scale PCB biodegradation assay which can be used to screen potential bioaugmentation strains or consortia for their ability to decrease PCB mass flux from contaminated sediment to air through biodegradation of freely dissolved PCBs that have desorbed from sediment particles. The assay uses two types of passive samplers to simultaneously measure PCB mass that is freely dissolved in aqueous solution and PCB mass that has volatilized to the headspace of the bioreactor. Using this approach, relative comparisons of PCB mass accumulated in passive samplers between bioaugmented treatments and controls allow for practical assessment of a microbial strain's ability to reduce both freely dissolved and vapor phase PCB concentrations. The method is designed to be conducted using aliquots of homogenized, well-characterized, PCB-contaminated sediment gathered from a field site. This work details the experimental design methodology, required materials, bioreactor set-up, passive sampling, PCB-extraction, sample cleanup, and quantification protocols such that the biodegradation assay can be conducted or replicated. A step-by-step protocol is also included and annotated with photos, tips, and tricks from experienced analysts.

- Relative comparisons of PCB mass accumulated in passive samplers between experimental treatments and controls allow for practical assessment of bioaugmentation strain's ability to reduce both freely dissolved and vapor phase PCB concentrations
- Passive sampler preparation, deployment, PCB-extraction, cleanup procedures, and quantification are detailed step-by-step and annotated by experienced analysts

Abbreviations: PCBs, Polychlorinated Biphenyls.

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* Bako, C. M.; Martinez, A.; Ewald, J. M.; Hua, J. B. X.; Ramotowski, D. J.; Dong, Q.; Schnoor, J. L.; Mattes, T. E. Aerobic Bioaugmentation to Decrease Polychlorinated Biphenyl (PCB) Emissions from Contaminated Sediments to Air. *Environ. Sci. Technol.* 2022, 56 (20), 14338–14349. <https://doi.org/10.1021/acs.est.2c01043>. Bako, C. M.; Martinez, A.; Ewald, J. M.; Hua, J. B. X.; Schnoor, J. L.; Mattes, T. E. Dataset Describing Polychlorinated Biphenyl (PCB) Congener Accumulation on Passive Samplers and Mass Transport in Sediment Slurry Bioreactors Bioaugmented with *Paraburkholderia xenovorans* LB400. *Iowa Res. Online* 2021, V1. <https://doi.org/10.25820/data.006160>. Bako, C. M.; Martinez, A. R Code for Polychlorinated Biphenyl (PCB) Reactive Transport Model. *Iowa Res. Online* 2022. <https://doi.org/10.25820/code.006163>.

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Specifications table

Subject area	Engineering
More specific subject area	Environmental Engineering; Bioremediation
Name of your method	Lab-scale biodegradation assay using passive samplers to screen microorganisms for the ability to prevent PCB volatilization from contaminated sediment slurry to air
Name and reference of original method	[1] - Martinez, A.; Wang, K.; Hornbuckle, K. C. Fate of PCB Congeners in an Industrial Harbor of Lake Michigan. <i>Environ. Sci. Technol.</i> 2010 , <i>44</i> (8), 2803–2808. https://doi.org/10.1021/es902911a . [2] - Martinez, A.; O'Sullivan, C.; Reible, D.; Hornbuckle, K. C. Sediment Pore Water Distribution Coefficients of PCB Congeners in Enriched Black Carbon Sediment. <i>Environ. Pollut.</i> 2013 , <i>182</i> , 357–363. https://doi.org/10.1016/j.envpol.2013.07.015 . [3] - Herkert, N. J.; Martinez, A.; Hornbuckle, K. C. A Model Using Local Weather Data to Determine the Effective Sampling Volume for PCB Congeners Collected on Passive Air Samplers. <i>Environ. Sci. Technol.</i> 2016 , <i>50</i> (13), 6690–6697. https://doi.org/10.1021/acs.est.6b00319 . [4] - Marek, R. F.; Thorne, P. S.; Herkert, N. J.; Awad, A. M.; Hornbuckle, K. C. Airborne PCBs and OH-PCBs Inside and Outside Urban and Rural U.S. Schools - Supporting Information. <i>Environ. Sci. Technol.</i> 2017 , <i>51</i> (14), 7853–7860. https://doi.org/10.1021/acs.est.7b01910 . [5] - Herkert, N. J.; Jahnke, J. C.; Hornbuckle, K. C. Emissions of Tetrachlorobiphenyls (PCBs 47, 51, and 68) from Polymer Resin on Kitchen Cabinets as a Non-Aroclor Source to Residential Air. <i>Environ. Sci. Technol.</i> 2018 , <i>52</i> (9), 5154–5160. https://doi.org/10.1021/acs.est.8b00966 . [6] - Martinez, A.; Awad, A. M.; Herkert, N. J.; Hornbuckle, K. C. Determination of PCB Fluxes from Indiana Harbor and Ship Canal Using Dual-Deployed Air and Water Passive Samplers. <i>Environ. Pollut.</i> 2019 , <i>244</i> , 469–476. https://doi.org/10.1016/j.envpol.2018.10.048 . [7] - Jahnke, J. C.; Hornbuckle, K. C. PCB Emissions from Paint Colorants. <i>Environ. Sci. Technol.</i> 2019 , <i>53</i> (9), 5187–5194. https://doi.org/10.1021/acs.est.9b01087 . [8] - Bako, C. M.; Mattes, T. E.; Marek, R. F.; Hornbuckle, K. C.; Schnoor, J. L. Biodegradation of PCB Congeners by <i>Paraburkholderia xenovorans</i> LB400 in Presence and Absence of Sediment during Lab Bioreactor Experiments. <i>Environ. Pollut.</i> 2021 , <i>271</i> , 116364. https://doi.org/10.1016/j.envpol.2020.116364 . [9] - Bannavti, M. K.; Jahnke, J. C.; Marek, R. F.; Just, C. L.; Hornbuckle, K. C. Room-to-Room Variability of Airborne Polychlorinated Biphenyls in Schools and the Application of Air Sampling for Targeted Source Evaluation. <i>Environ. Sci. Technol.</i> 2021 , <i>55</i> (14), 9460–9468. https://doi.org/10.1021/acs.est.0c08149 .
Resource availability	Software: Mass spec screening software, MassHunter Qualitative Analysis Agilent Hardware: Accelerated Solvent Extraction (ASE) Thermo Fisher Scientific - US More resource details provided in 'detailed protocol' section.

Method details

Experimental methodology

The assay described in this work tests whether an aerobic PCB-degrading microbe can significantly biodegrade PCBs, such that the accumulation of PCB mass in passive samplers deployed within lab-scale bioreactors is reduced relative to non-bioaugmented controls. Two types of passive sampling media are used: [1] polyurethane foam (PUF) plugs as flask stoppers and [2] solid phase microextraction fibers coated with polydimethylsiloxane (SPME-PDMS) directly immersed in sediment slurry. This methodological protocol refers to results and procedures and data from a companion study [10–12] where necessary or helpful.

To make accurate pairwise comparisons between controls and treatments, the control group should be identical to the treatment group except that it does not contain the bioaugmentation strain or consortium being evaluated. Each replicate bioreactor represents a sacrificial sample. We recommend that control and treatment groups both be conducted in at least triplicate ($n = 3$), but the number of replicates used depends on the goals of the study, desired statistical power, number of sampling timepoints, material availability, and/or lab space constraints. An example experimental design matrix is shown in Fig. 1.

Sediment homogenization

The sediment used in the companion study [10–12] was gathered from a PCB-contaminated field site in Altavista, VA [8]. We thoroughly homogenized the sediment by mixing for approximately 20 min with a Masterforce® Tornado Power Mixer (paddle mixer accessory) attached to a DeWalt® DW235G 1/2" Heavy Duty Side Handle Drill inside a standard-size (20 gal) solid PCB-waste container provided by University of Iowa Environmental Health and Safety (UI EHS; Fig. 2). We drilled a hole in the center of the bin lid using a $\frac{1}{2}$ " bit. Then, we inserted the paddle mixer accessory through the hole in the lid and attached it to the drill chuck. We reattached the lid and made sure it was securely sealed to the bin before mixing. We also added water gathered from the field site to aid mixing, when necessary. We requested an additional bin lid from UI EHS to use during storage at 4 °C. Alternatively, a smaller bucket with an existing hole in the lid can be used as a mixing container. Texture characterization and physico-chemical analyses were conducted on the homogenized sediment by Minnesota Valley Testing Laboratories (MVTLL; New Ulm, MN).

Bioreactor setup

Bioreactors are 250 mL Erlenmeyer flasks which contain a PCB-contaminated sediment slurry (10% mass to volume ratio; ~10 g weathered sediment [wet weight] to 100 mL aqueous solution; determined gravimetrically; Fig. 3). Record the exact mass of sediment

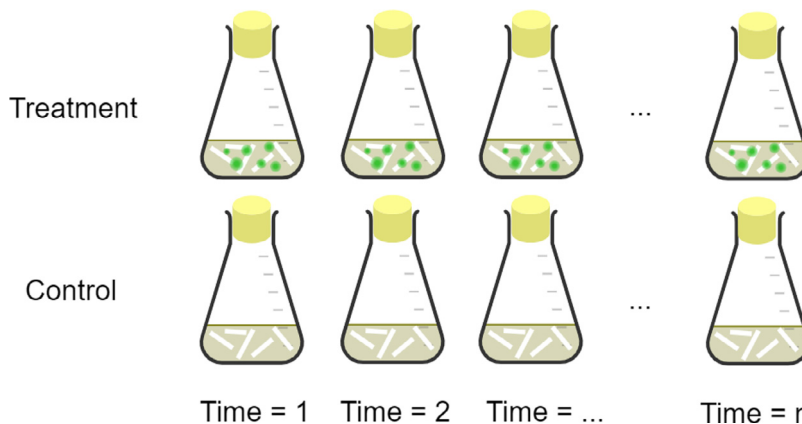


Fig. 1. – Example experimental design matrix with n timepoints. Time-series samples can be used to observe PCB uptake in passive samplers. Alternatively, a single timepoint can be taken when equilibrium has been reached. Determining time to equilibrium requires preliminary sampler calibration studies.

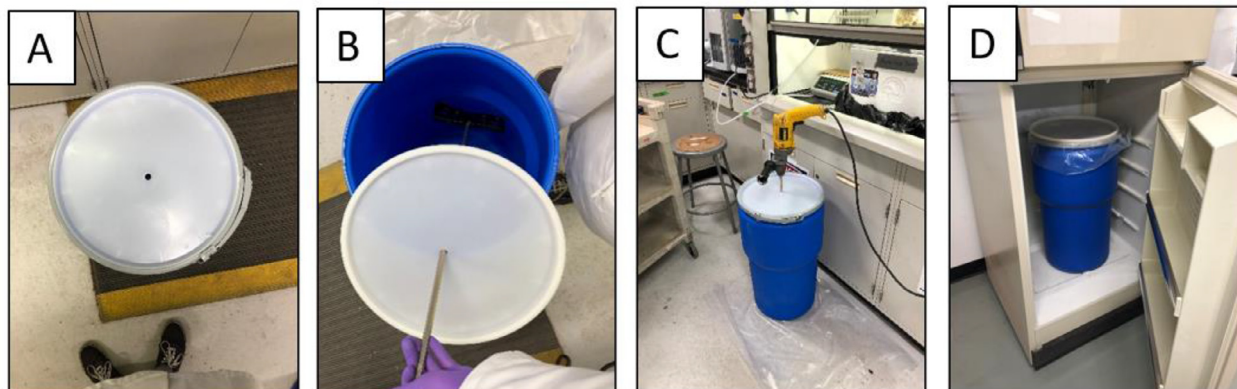


Fig. 2. – (A) $\frac{1}{2}$ " hole drilled in the top of the mixing container (B) paddle mixer passed through the lid of the mixing container (C) paddle mixer attached to power drill (D) mixing container in storage location.



Fig. 3. – Established bioreactor from companion study (aluminum foil cover over PUF not shown).

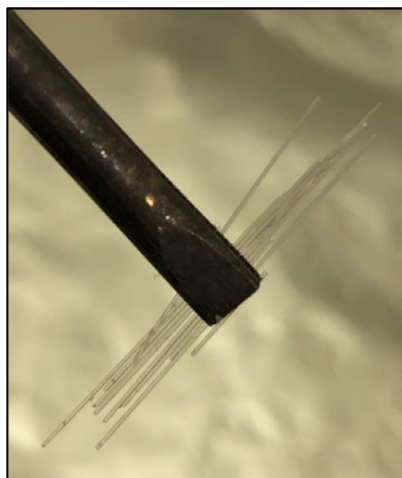


Fig. 4. – Segments of SPME fibers (≤ 2.5 cm) retrieved from bioreactors.

added to the bioreactors so that measured PCB mass can be normalized to that value. To make appropriate pairwise comparisons in the companion study, sterile liquid microbiological medium was used as aqueous solution in the control group (instead of sterile water) because the bioaugmentation strain added to treatment group was also suspended in the same liquid medium [10]. The order in which the various components of the bioreactor were added to the flasks was as follows: [1] liquid microbiological medium [2], ~ 10 g PCB-contaminated sediment [3], SPME passive samplers [4], bioaugmentation solution containing live cells (treatment only), and [5] PUF passive samplers (see **Detailed Protocol** section for step-by-step instructions). Cover the mouths of the established bioreactors with aluminum foil to prevent the lowest molecular weight congeners from volatilizing off PUF stoppers. Mix the sediment slurry by placing bioreactors on a platform shaker at 150 rpm to disperse SPME fibers and accelerate desorption of PCBs such that mass flux is observable over the relatively short experimental timescale. It may also be possible to adapt the bioreactor setup to accommodate anaerobic conditions, but the present method is designed solely to evaluate aerobic microorganisms.

Passive sampling

PCB congener mass can be measured in two separate bioreactor compartments using this protocol: [1] the air phase (headspace) and [2] the freely dissolved aqueous phase. To measure airborne PCBs, cut $3''h \times 1.5''d$ PUF cylinders (TE-1015; Tisch Environmental Inc., Ohio) to desired dimensions ($h \approx 1''$ in the companion study) [10–12] and place them in the neck of the flasks to measure PCB congeners that volatilize to the headspace of the bioreactor, over time. In the companion study [10–12], SPME fibers manufactured by Fiberguide Industries, Inc. (Stirling, NJ) with a $210 \mu\text{m}$ inner diameter and a $10 \mu\text{m}$ -thick layer of PDMS coating were directly immersed in the sediment slurry to measure freely dissolved PCB congeners that readily desorbed from sediment particles [2]. PDMS coating volume on the fibers used in the companion study [10–12] was 6.9×10^{-8} L/cm-fiber, but specifications can vary between manufacturers. Deploy SPME fibers as ~ 2.5 cm segments totaling approximately 30–40 cm per bioreactor with a target recovery length of 20 cm (Fig. 4). SPME fibers can be deployed in permeable secondary containers or casings for easier recovery but this method was not used in the companion study [10–12] and therefore not described in this work [13]. For instructions on how to prepare each type of passive sampler for deployment see the *Sampler Preparation* sections in the **Detailed Protocol**.

Measurements can be taken in time-series to observe the kinetic phase (PCB mass uptake in passive samplers) or at a single timepoint once equilibrium within the system is established. Kinetic phase sampling time points in the companion study [10–12] were $T_1=3\text{d}$, $T_2=11\text{d}$, $T_3=16\text{d}$, and $T_4=35\text{d}$ to observe uptake of PCB congeners in PUF samplers. It is important to note that the time for a passive sampler to reach equilibrium will vary based on temperature, mixing conditions (i.e., shaking speed), sediment characteristics (e.g., texture, organic matter content, pH, etc.), and congener-specific physical-chemical properties. Therefore, identifying the time required to reach equilibrium for each type of sampler will require preliminary calibration experiments with sediment specific to the study being conducted [14–17].

At the time of sampling, remove PUF samples from the bioreactors, wrap them in clean aluminum foil, and store at -10°C until PCB extraction and analysis. Before retrieving SPME fibers, establish a working area within a fume hood by laying down clean aluminum foil on which recovered fibers can be set (Fig. 5A). Recover deployed SPME fibers from bioreactors using tweezers by transferring the volume of sediment slurry back and forth from the flask to a separate container inside a fume hood. In the companion study [10–12], a porcelain evaporating dish with a small spout was used. However, in more recent applications of this method a large petri dish was used, and is preferred. A large petri dish traps most of the fibers when bioreactor volume is transferred back to the flask. It can be very difficult to see SPME fibers with the naked eye and may require repeated volume transfers to recover length sufficient for analysis. Twenty cm was the target recovery length in the companion study [10–12] but a length shorter than the target was recovered for some bioreactors and, for others, a length longer. Ensure a higher average recovered length of fiber by increasing the

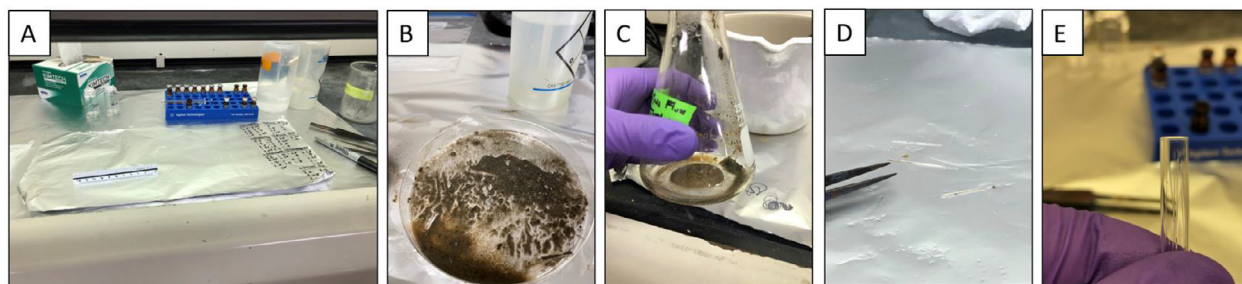


Fig. 5. – (A) Working area within fume hood – length of recovered fibers recorded on aluminum foil inside the fume hood and later transferred to permanent records. (B) Sediment which remained in the petri dish after transferring liquid back to the flask. (C) Sediment not suspended in aqueous solution that has settled at the bottom of the empty bioreactor flask. (D) Lengths of SPME fiber retrieved from the bioreactor that are still covered in sediment debris. (E) Rinsed, dried, and measured fibers placed within glass GC-vial insert.

deployed length. Note that deployed fibers may bunch together, stick to the side of laboratory glassware (either the bioreactor flask or the container receiving sediment slurry volume; Fig. 5B), or become covered by sediment that has settled to the bottom of the flask (Fig. 5C). It can be easier to locate the fibers by looking along the inside wall of the flask through the neck from the top, rather than from the side. Rinse the length of recovered SPME fiber segments with ultrapure water to remove particulate sediment debris (Fig. 5D), gently wipe dry with a Kimwipe™, measure and record the length, and place in a glass GC-vial insert (Fig. 5E). Use the edge or point of the tweezers to push the fibers down into the glass insert so that there is no length overhanging the edge of the glass insert. Carefully fill the insert with hexane to cover the entire length of recovered fibers and facilitate complete desorption of PCBs from the fiber's PDMS coating. Fibers much longer than 2.5 cm will not fit within the glass insert. It is important to leave a small volume available in the insert such that the desired volume of internal standard can be added. No additional extraction or cleanup steps are required after the glass inserts are spiked with internal standard, placed in GC-vials, and capped. SPME samples were not spiked with surrogate standards in the companion study [10–12]. Store SPME samples at $-10\text{ }^{\circ}\text{C}$ until analysis by GC–MS/MS.

PCB extraction from PUF & sample extract cleanup

Extract PCBs from PUF by pressurized liquid extraction (PLE) with equal parts acetone and hexane by volume using an accelerated solvent extractor (ASE; Thermo Scientific™). ASE method settings in the companion study [10–12] are shown in the **Detailed Protocol**: section. If available, smaller volume ASE cells ($\leq 34\text{ mL}$) are preferred to larger volume cells ($\geq 66\text{ mL}$) because water vapor within the unoccupied cell volume (volume that is not occupied by the PUF sample) can become entrained in the sample extract during PLE. Water will become visible as a separate, clear or yellow-ish phase layer at the bottom of the ASE collection vial. If small volume ASE cells are not available, water in sample extracts can be removed with drying agents such as sodium sulfate but may result in lower surrogate standard recoveries. Alternatively, a skilled analyst can conduct a secondary liquid-liquid extraction (LLE) with hexane to separate water and solvent layers in a PLE sample extract. Regardless of cell size used, prior to PLE spike the ASE cell containing the PUF sampler with surrogate standards to measure the efficiency of the extraction procedure. Surrogate standards used in the companion study [10–12] are PCB 14 (50.81 ng; 3,5-dichlorobiphenyl), deuterated PCB 65-d5 (52.5 ng; 2,3,5,6-tetrachlorobiphenyl-d5, deuterated) and PCB 166 (52.56 ng; 2,3,4,4',5,6-hexachlorobiphenyl; Cambridge Isotope Laboratories, Inc.).

Following water removal (if necessary), concentrate the sample extract resulting from PLE to approximately 1 mL under a steady stream of nitrogen gas. In the companion study [10–12], the PLE sample extract was collected directly from the ASE into a TurboVap® collection vial and concentrated using a TurboVap® II Concentration Workstation (Biotage, Uppsala, Sweden). Pass the final, concentrated hexane extract through a Pasteur pipette filled with approximately 0.1 g of combusted silica gel and 1 g of acidified silica gel (2:1 silica gel:sulfuric acid by weight) and elute with approximately 10 mL of hexane. The custom column holder shown in Fig. 6 was made by modifying a ring stand (see *Cleanup of PUF Sample Extract* and *Acidified Silica Gel Column Setup* sections in the **Detailed Protocol** for more details). Eluted samples were again concentrated to approximately 1 mL and transferred to a gas chromatography vial. Immediately before analysis on GC–MS/MS, the final sample volume was spiked with PCB 204 (19.6 ng; 2,2',3,4,4',5,6,6'-octachlorobiphenyl; Cambridge Isotope Laboratories, Inc.) as the sole internal standard in the companion study [10–12].

PCB Quantification with internal standard method

In the companion study [10–12], GC–MS/MS (Agilent 7890A GC system, Agilent 7000 Triple Quad, Agilent 7693 autosampler) was used in multiple reaction monitoring mode (MRM) to identify and quantify all 209 PCBs as 171 individual or co-eluting chromatographic peaks. The GC was equipped with a Supelco SPB-Octyl capillary column (50% n-octyl, 50% methyl siloxane, $30\text{ m} \times 0.25\text{ mm}$ ID, $0.25\text{ }\mu\text{m}$ film thicknesses) with ultra-high purity helium as the carrier gas flowing at 0.75 mL/min and ultra-high purity nitrogen as the collision gas.

Use the internal standard method to quantify PCB congeners by converting individual or co-eluting chromatographic peak areas to corresponding PCB congener mass. Accurate results can be achieved using this method by spiking a precise volume of “internal”



Fig. 6. – Sample extracts being eluted through acidified silica gel columns and collected in TurboVap® tubes.

standard compounds (“IS”) into each sample belonging to a batch or “run” of samples being analyzed. The advantage of using this method is that a precise extract volume is not required. The IS compounds should also be present at certified concentrations within a “calibration” sample (referred to as the “cal” in a run of samples) along with all 209 unlabeled PCB congeners at certified concentrations. Determine the mass of an individual PCB congener (PCB_i ; ng) in a sample extract by calculating the “relative response factor” (RRF; dimensionless) for each congener present in the cal. Eq. (1) relates the instrument peak response of the IS compound(s) to the peak response of all other congeners to which the RRF was applied.

$$RRF_{PCB_i} = \frac{PCB_i^{cal} / PCB_{i \text{ peak area}}^{cal}}{IS^{cal} / IS_{\text{peak area}}^{cal}} \quad (1)$$

Where PCB_i^{cal} is the certified concentration of PCB_i present in the cal, $PCB_{i \text{ peak area}}^{cal}$ is PCB_i 's corresponding integrated peak area (arbitrary units) detected in the cal by GC–MS/MS, IS^{cal} is the certified concentration of the IS compound present in the cal, and $IS_{\text{peak area}}^{cal}$ is the IS compound's corresponding integrated peak area (arbitrary units) detected in the cal by GC–MS/MS. Recalculate RRF after every run to account for slight shifts in column performance resulting from longer than average method run times (70.86 min, total, in the companion study) [10–12] and periodic maintenance that can occur between runs. The mass of PCB_i in a sample extract is then given by Eq. (2).

$$PCB_{i \text{ mass}} = RRF_{PCB_i} * PCB_{i \text{ peak area}} * IS_{\text{mass}} / IS_{\text{peak area}} \quad (2)$$

Where $PCB_{i \text{ mass}}$ is the mass of PCB_i in the sample extract (ng), RRF_{PCB_i} is given by Eq. (1), $PCB_{i \text{ peak area}}$ is PCB_i 's corresponding integrated peak area (arbitrary units) detected in the sample extract by GC–MS/MS, IS_{mass} is the known mass of the IS compound spiked into the sample extract (ng), and $IS_{\text{peak area}}$ is the IS compound's corresponding integrated peak area (arbitrary units) detected in the sample extract by GC–MS/MS. In the companion study [10–12], peak areas in sample extracts were determined with Agilent MassHunter Qualitative Analysis software using the “auto-integrate” function when peak height and resolution allowed and manual baseline integration when it did not. Each homolog transition in each sample extract was visually inspected and compared to PCB_i retention times in the cal to ensure correct peak identification.

Use Surrogate Standard (“SS”) recoveries to correct $PCB_{i \text{ mass}}$ values to monitor extraction efficiency and account for sample losses during the extraction and cleanup procedures resulting from volatilization or sorption to laboratory glassware. SS recoveries are calculated using a reference sample which is prepared with each batch of samples. The reference sample is prepared by spiking a known, exact mass of SS and IS into a GC-vial filled with approximately 1.5 mL hexane. SS recoveries are calculated for each sample by comparing the amount of SS spiked into the reference to the amount detected in the sample extract. SS recoveries (%) are given by Eq. (3).

$$SS_i \text{ Recovery} = \left(\frac{SS_i^{\text{sample}}}{SS_i^{\text{ref}}} \right) * 100 \quad (3)$$

Where $SS_i \text{ Recovery}$ is the recovery value for an individual surrogate standard (%), SS_i^{sample} and SS_i^{ref} are the masses of the individual SS compound in the sample extract or reference sample, respectively (ng; both calculated by Eq. (2)). Finally, the SS recovery corrected PCB_i mass can be calculated using Eq. (4):

$$PCB_{i \text{ mass}}^{SS_i\text{-corrected}} = PCB_{i \text{ mass}} / SS_i \text{ Recovery} \quad (4)$$

Quality assurance & quality control (QA/QC)

Assess extraction efficiency, reproducibility, and accuracy by using surrogate standards, method blank replicates, and analysis of standard reference materials. Mean and standard deviation percentage recoveries of PCB 14, PCB 65-d5 and PCB 166 in the companion study [10–12] were $93 \pm 15\%$, $86 \pm 11\%$ and $91 \pm 11\%$, respectively. Use percentage recoveries of surrogate standards less than 100% to correct congener mass as follows: use the surrogate recovery value for PCB 14 to correct PCB 1 to PCB 39, PCB 65-d5 to correct PCB 40 to PCB 127 and PCB 166 to correct PCB 128 to PCB 209. In the companion study [10–12], samples were processed in batches of five along with one method blank per batch. PCB mass detected in the method blanks were used to determine the limit of quantification (LOQ) as the upper limit of the 95% confidence interval (average mass plus two times the standard deviation).

All materials used in sample extraction should be triple rinsed with solvent (methanol, then acetone, followed by hexane) or combusted overnight at 450 °C to prevent background PCB contamination. Additionally, wash PUF and SPME passive samplers with solvent prior to deployment. Use the same ASE method that is used for extraction to clean raw PUF. Note that multiple PUF can be fit in a single ASE cell when cleaning. Place clean PUF in a fume hood on clean aluminum foil until excess solvent evaporated (~1 min), wrap individually in aluminum foil, and store at –10 °C until deployment. Clean SPME fiber samplers by sonication in hexane for 40 min, rinse with acetone, and envelope a bundle of fibers in clean aluminum foil, and store at room temperature until deployment.

Method validation

This method was validated in the companion study [10–12] by evaluating the ability of *Paraburkholderia xenovorans* LB400 to mitigate mass flux of PCB congeners from contaminated sediment to air. This validation was done by making relative comparisons between PCBs that had accumulated on PUF and SPME passive samplers deployed in bioaugmented treatments to those in non-bioaugmented controls. PCB profiles measured in the air and freely dissolved phases by PUF and SPME passive samplers, respectively, displayed the mass transport dynamics of labile PCBs within the bioreactor system. Any abiotic losses of analyte mass, however small, which may occur during sampler recovery are accounted for by making relative comparisons between control and treatment bioreactors. The congener profiles detected by each type of passive sampler depended on the inventory of PCB congeners present in the sediment, which had been previously determined (Fig. 7A) [8]. PUF passive samplers deployed in the companion study [10–12] primarily detected LC-PCBs that desorbed from sediment particles, entered the freely dissolved phase, and then volatilized to the air (Fig. 7B). SPME-fiber passive samplers that were directly immersed in the sediment slurry captured the fraction of labile PCB congeners that desorbed from sediment particles but had not yet volatilized at the time of sampling (Fig. 7C). Results of the analysis showed that LB400 significantly reduced the amount of total PCB mass that had accumulated on PUF passive samplers (Fig. 8 and Fig. 9). SPME samplers revealed how LB400 preferentially biodegraded freely dissolved lower chlorinated PCBs (LC-PCBs) as they desorbed from sediment (Fig. 9).

Detailed Protocol

Apparatuses & Materials:

- Apparatuses:
 - ThermoFisher / Dionex Accelerated Solvent Extractor (ASE) 350, 300, 200, 150, or 100
 - **Note:** An ASE 200 was the apparatus used for PLE in the companion study [10–12].
 - **Note:** Only ASE 150/350 models are still supported under ThermoFisher service contracts.
 - Biotage TurboVap II Concentration Evaporator Workstation (or similar nitrogen evaporation system)
 - 9" Pasteur Pipette Holder (a custom holder used in companion study) [10–12]
 - Thermolyne 30400 Furnace (or similar)
- Glassware, Materials, and Supplies
 - Fisherbrand™ Reusable Glass Narrow-Mouth Erlenmeyer Flasks; Cat. No. FB500250
 - Fisherbrand™ Tall-Form PTFE Evaporating Dish; Cat. No. 02-614-144
 - PYREX™ Narrow Mouth Erlenmeyer Flask with Standard Taper Stopper; Cat. No. 10-098G
 - 9" Fisherbrand™ Disposable Borosilicate Glass Pasteur Pipettes; Cat. No. 13-678-20D
 - 2 mL volume Fisherbrand™ Bulb for Small Pipets; Cat. No. 03-448-22
 - 2 mL capacity Fisherbrand™ 11 mm Glass Wide Opening Crimp Top Vials; Cat. No. 03-391-6
 - MilliporeSigma™ Supelco™ Certified glass inserts Large Opening Vials; Cat. No. 11-101-2128
 - Thermo Scientific™ Manual Crimpers, Decrimpers and Decrimping Pliers; Cat. Nos. 03-375-7 and 03-375-8A
 - Thermo Scientific™ Screw Top Vial Racks; Cat. No. 03-375-9
 - 11 mm closure-size Thermo Scientific™ Aluminum Crimp Seals with Prefitted Septa for 12 × 32mm Vials; Cat. No. 03-376-411
 - PYREX™ Glass Wool, borosilicate; Cat. No. 11-388
 - Thermo Scientific™ I-Chem™ Loose Septa for 24-414 Open Top Caps; Cat. No. 03-705-4
 - Dionex™ ASE™ 150/350 Stainless Steel Extraction Cells (10 / 11 mL, 22mL, or 33 / 34 mL recommended; ThermoFisher Cat. Nos., 068087, 068088, 068089 or Restek Cat. Nos., 25995, 25996, 26098, 26112, 26114, 26116, 26176)
 - Dionex™ 100/150/200/300/350 Extraction Cell Filters; Cat. No., 047017

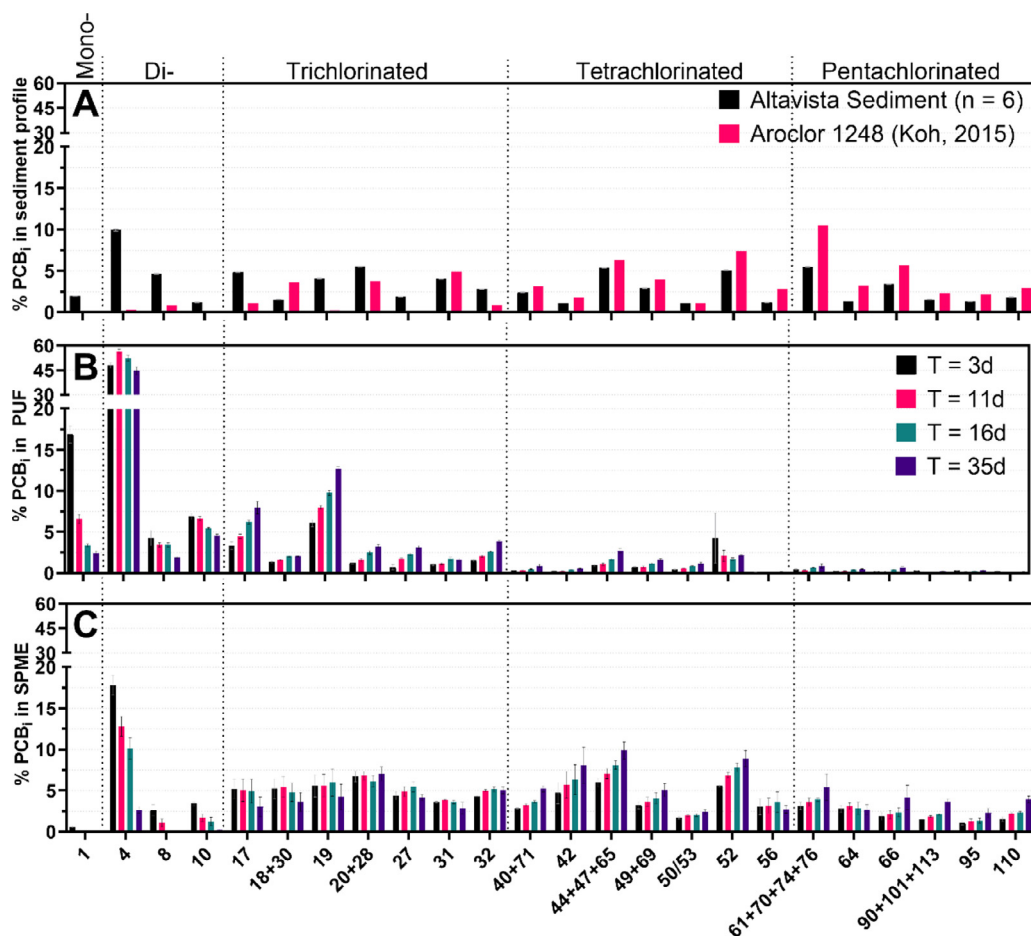


Fig. 7. – (A) PCB congener profile of Altavista sediment previously determined in Bako et al. (2021) [8] and used in companion study [10–12] compared to Aroclor 1248 [18]. For ease of reference, a condensed profile of individual PCB congeners is shown along the horizontal axis, although all 209 congeners (minus surrogate and internal standards) were measured. The condensed profile represents congeners $\geq 2\%$ of the total profile, by mass fraction, in either PUF or SPME samples at any time point in any treatment. (B) PCB congener profile detected in headspace of bioreactors using PUF passive samplers in the companion study [10–12]. Values shown are from the non-bioaugmented control group. Mass fractions of mono- and dichlorinated congeners trend lower or remain level as the relative contribution of the more slowly desorbing tri-, tetra-, and pentachlorobiphenyls trend higher with time. (C) Congener profile of freely dissolved PCBs detected in the aqueous compartment of the bioreactors using SPME passive samplers in the companion study [10–12]. $T = 3$ d samples capture LC-PCBs that rapidly desorbed from sediment before volatilizing to air whereas later samples capture the more slowly desorbing tri-, tetra-, and pentachlorobiphenyls that are still entering solution or have reached dynamic equilibrium. Error bars represent standard error of triplicate measurements ($n = 3$; except where otherwise indicated).

- Dionex™ ASE™ Extraction Filter Insertion Tool; Cat. No., 056929
- Biotage® Evaporation Tube TurboVap® II, 50 mL, 1 mL EndPoint; Cat. No. C128511
- Kimberly-Clark Professional™ Kimtech™ Purple Nitrile™ Gloves; Cat. No. 19-149-863
- Boardwalk BWK 7116 1000' Length, 18" Width, 16 Micron Thickness Extra Standard Aluminum Foil Roll
- Fisherbrand™ General-Purpose Student Forceps; Cat. No., 10-280
- Fisherbrand™ General-Purpose Extra-Long Forceps; Cat. No., 10-316
- Fisherbrand™ High Precision Straight Slender Fine Point Tweezers/Forceps; Cat. No., 12-000-127
- Kimberly-Clark Professional™ Kimtech Science™ Kimwipes™ Delicate Task Wipers, 1-Ply; Cat. No., 06-666
- Hamilton™ Gastight™ Syringe, 25 μ L, Sold by MilliporeSigma™ Supelco™, Cat. No. 11-101-1076
- Hamilton™ 1700 Series Gastight™ Syringe, 100 μ L; Cat. No. 12-012-401
- Fisherbrand™ Easy-Squeeze Wash Bottles; Cat. No. 02-897-12
- Fisherbrand™ Petri Dishes with Clear Lid (150 \times 15 mm); Cat. No. FB0875714

• Chemicals & Solvents

- Fisher Chemical™ Silica Gel Sorbent (70-230 Mesh, Grade 60); Cat. No. 2826-1
- Hexanes (Pesticide Grade), Fisher Chemical; Cat. No. H300-4
- Methylene Chloride (Pesticide Grade), Fisher Chemical; Cat. No. D142-4

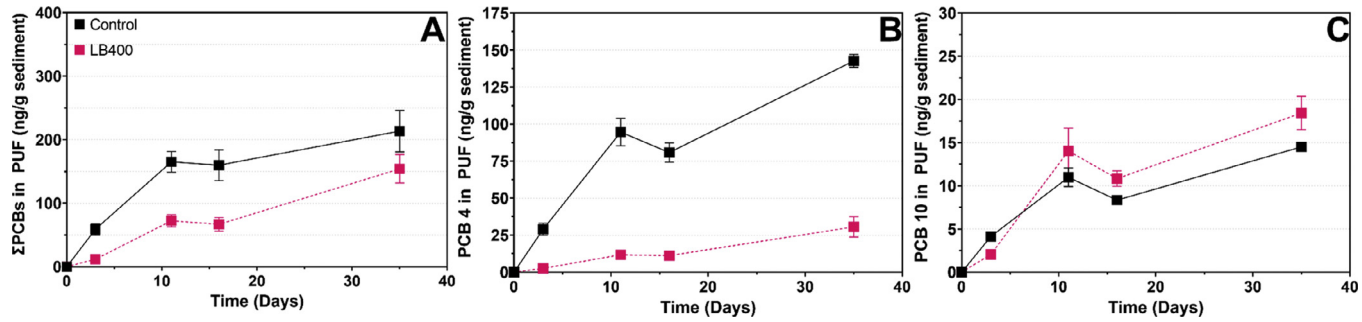


Fig. 8. – (A) Σ PCBs in PUF over 35 days in treatment and control from the companion study [10–12]. Significant differences ($p < 0.05$) were observed between bioaugmented and non-bioaugmented groups at all timepoints. On average, Σ PCBs accumulation in the bioaugmented treatment was decreased by 47% across all timepoints, relative to the non-bioaugmented control. (B) PCB 4 (2,2'-dichlorobiphenyl) in PUF over 35 days in the companion study [10–12]. On average, PCB 4 accumulation was reduced by 85% in the bioaugmented treatment. (C) PCB 10 (2,6-dichlorobiphenyl) in PUF over 35 days in the companion study [10–12]. There was no significant reduction in PCB 10 accumulation in PUF in the bioaugmented treatment, relative to the control.

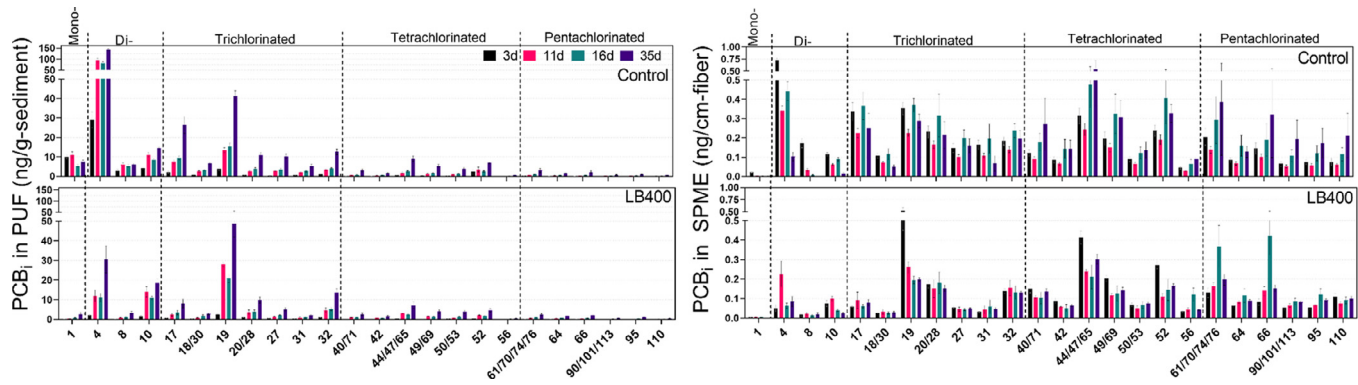


Fig. 9. - PCB₁ accumulation in PUF (left) and SPME (right) over a 35-day incubation period in the companion study [10–12]. PUF profiles reveal that LB400 mitigated nearly all PCB mass from volatilizing in earlier timepoints except for congeners that were particularly resistant to biodegradation (e.g., PCBs 10,19, 32). SPME profiles show a decrease in freely dissolved LC-PCB mass that corresponds with PUF measurements. The $T = 16$ d SPME measurement in the control is likely elevated due to small-scale sediment heterogeneity in one replicate.

- Acetone (Pesticide Grade), Fisher Chemical; Cat. No. A40-4
- Methanol, OmniSolv™, Pesticide Residue Grade, MilliporeSigma™; Cat. No. MMX04841
- Sulfuric Acid (Certified ACS Plus), Fisher Chemical™; Cat. No. A300-212
- Sodium Sulfate Anhydrous, Certified ACS, Suitable for Pesticide Residue Analysis (Granular, 10-60 mesh), Fisher Chemical™, Cat. No. S415-500
- Water, Optima™ LC/MS Grade, Fisher Chemical™; Cat. No. W6500
- Passive Samplers
 - TE-1015, 1.5-inch x 3-inch Polyurethane Foam Substrate 10/pk; Cat. No. TE-1015
 - SPME fibers manufactured by Fiberguide Industries, Inc. with a 210 μm inner diameter and a 10 μm-thick layer of PDMS coating
 - **Note:** Custom order required
- PCB Surrogate Standards
 - 3,5-Dichlorobiphenyl (PCB 14)
 - Deuterated 2,3,5,6-Tetrachlorobiphenyl (PCB D65)
 - 2,3,4,4',5,6-Hexachlorobiphenyl (PCB 166)
- PCB Internal Standards
 - 2,2',3,4,4',5,6,6'-Octachlorobiphenyl (PCB 204)
- PCB Calibration Standard
 - PCB Congener Mix 1, Accustandard; Cat. No. M-1668A-1-0.01X
 - PCB Congener Mix 2, Accustandard; Cat. No. M-1668A-2-0.01X
 - PCB Congener Mix 3, Accustandard; Cat. No. M-1668A-3-0.01X
 - PCB Congener Mix 4, Accustandard; Cat. No. M-1668A-4-0.01X
 - PCB Congener Mix 5, Accustandard; Cat. No. M-1668A-5-0.01X

Preparation of acidified silica

1. Combust silica gel sorbent overnight at 450 °C and allow it to cool.
 - a. **Note:** Do not overfill container in which silica is being combusted (\leq halfway).
 - b. **Note:** Loosely cover mouth of container with aluminum foil.
2. Weigh and pour a portion of the silica into a clear glass container that can be securely closed (e.g., Erlenmeyer flask with cap or stopper).
3. Carefully add enough concentrated sulfuric acid to the same container such that a 2:1, silica:acid mass ratio is achieved.
4. Securely close the container and thoroughly mix the silica and acid by shaking vigorously.
 - a. **Note:** If using Erlenmeyer flask with sliding stopper, use your thumb to hold the stopper down during shaking.
 - b. **Note:** Shake the flask until no lumps can be seen.

PUF sampler preparation

1. Thoroughly triple rinse (methanol, acetone, then hexane) and/or combust all laboratory materials immediately before use to prevent background PCB contamination.
 - a. **Note:** *Do not* combust ASE cell components; ASE cells, caps, collection vials, and collection vial caps with septa).
 2. Cut 3" × 1.5" PUF cylinders to desired dimensions ($h \approx 1" \times r = 1.5"$ in the companion study) [10–12] using scissors.
 3. Clean PUF cylinders by pressurized liquid extraction (PLE) in an accelerated solvent extractor (ASE):
 - a. Screw the bottom cap onto the ASE cell.
 - b. Use tweezers to place an ASE filter in the cell and push it to the bottom with the insertion tool.
 - c. Place the PUF passive sampler in the ASE cell and gently push it to the bottom with the insertion tool.
 - d. Place a top ASE filter in ASE cell and gently push down to PUF cylinders with insertion tool.
 - e. Screw the top cap onto the ASE cell.
 - f. Place the ASE cell on the top carousel and place a collection vial in the corresponding position on the bottom carousel.
 - g. Load or create the ASE method with the following parameters:
 - i. Heat: 5 min
 - ii. Static Time: 5 min
 - iii. Flush Volume: 60%
 - iv. Purge Time: 200 s
 - v. Pressure: 1500 psi
 - vi. Temperature: 100 °C
 - vii. Cycles: 1
 - viii. Solvents: 1:1, acetone:hexane
1. **Note:** For ASE models 300 and below, you must pre-mix solvents before beginning extraction. For ASE 350, you can select the solvent types to be mixed inside the extraction cell from up to 3 different solvent reservoirs at a time.

- h. Place empty collection vials in the rinse position(s) on the bottom carousel.
 - i. Run the rinse function to clear ASE solvent lines of any residual PCB contamination.
 - j. In the 'Load Method' menu, specify the cell position at which the run will start, and press start.
4. Let the ASE cell(s) cool before handling.
 5. Remove any potential sources of PCBs from fume hood and place a sheet of clean aluminum foil inside.
 6. Open both sides of ASE cell and use insertion tool to push clean PUF cylinders onto aluminum foil.
 7. Let the residual solvent evaporate from PUF (~ 1 min).
 8. Wrap the PUF samplers individually in clean aluminum foil.
 9. Place the wrapped PUF samplers in Ziploc bag; label, date, and initial.
 10. Store at -10 °C until deployment.

SPME sampler preparation

1. Triple rinse (methanol, acetone, then hexane) and/or combust all laboratory materials to prevent background PCB contamination.
2. Cut ~10–20 cm lengths of SPME fiber.
3. Clean the cut lengths of fiber by sonication in hexane (or, alternatively, by soaking in hexane; see step 4).
 - a. Place the cut fibers in small, glass graduated cylinder.
 - b. Fill the cylinder with hexane such that SPME fibers are completely submerged.
 - c. Cover the mouth of the cylinder with a small piece of aluminum foil.
 - d. Fill the sonicator with reverse osmosis filtered water.
 - e. Secure the cylinder on ring stand with a clamp and lower the cylinder into sonicator such that most of the cylinder is submerged in the water.
 - f. Sonicate for 40 min.
 - g. Decant hexane from the graduated cylinder to a solvent waste jug.
 - h. Rinse the fibers with acetone and decant.
 - i. Rinse the fibers with deionized or Optima water.
 - j. Wipe the fibers dry with a Kimwipe™.
 - k. Carefully wrap the dry bundle of fibers in an envelope of clean aluminum foil; label, date, and initial
 - i **Note:** Do not let fibers puncture foil.
 - l. Store at room temperature until deployment.
4. Alternatively, clean fibers by soaking in hexane for 24 h.
 - a. Repeat steps 3a-c.
 - b. Place cylinder in fume hood for 24 h (label, date, initial).
 - c. Repeat steps 3 g-l.
5. At the time of deployment, cut the cleaned fibers into segments of the desired length to be deployed inside the bioreactor (~2.5 cm in companion study [10–12] to fit inside a 2 mL capacity GC-vial).
 - a. Inside a fume hood, establish a working area by laying down clean aluminum foil on which clean fibers can be set.
 - b. Place the envelope of fibers on the separate piece of clean aluminum foil.
 - c. Carefully open the envelope and remove a single clean fiber.
 - d. Place the fiber on clean aluminum foil alongside a small ruler.
 - e. Use flathead tweezers to securely grasp the fiber ~2.5 cm from the end
 - i **Note:** It can be difficult to grasp a fiber with tweezers that is lying on a flat surface. To make it easier, use one hand to slightly raise the aluminum foil sheet on which the fiber is resting so that the foil is no longer touching the surface of the fume hood and, with the other hand, use the tweezers to grasp the fiber laying on the aluminum foil but be careful not to puncture the foil itself. Lay the foil back down before step 5f.
 - f. Use your (sterile-gloved) index finger or another set of tweezers to press down on the longer end of the fiber
 - g. Slowly bend the fiber upwards with the tweezers until it snaps.
 - i **Note:** The 2.5 cm fiber segment should be controlled by the flathead tweezers but sometimes segments are lost after breaking the fiber.
 - h. Repeat steps 5c-g until the total desired deployed length is achieved (~30–40 cm per bioreactor in companion study [10–12]).

Bioreactor setup

1. Triple rinse (methanol, acetone, then hexane) and/or combust all laboratory materials to prevent background PCB contamination.
2. Label, date, initial each bioreactor flask.
3. Add aqueous solution to bioreactor flask (In companion study [10–12], 33.3 mL or 100 mL of liquid K1 microbiological medium to a 250 mL Erlenmeyer flask for treatment and control, respectively).
4. Place flask on calibrated balance inside a fume hood and tare.

5. Add 10 g of contaminated sediment gravimetrically (wet weight, in companion study) [10–12]; record exact mass.
6. Add SPME fiber segments cut to desired length (~30–40 cm of ~2.5 cm segments in companion study).
7. To treatment bioreactor, add 77.7 mL of bioaugmentation solution (*Paraburkholderia xenovorans* LB400 in K1 microbiological medium in companion study) [10–12].
8. Insert PUF sampler in neck of flask.
9. Cover neck of flask with clean aluminum foil.
10. Place flask on platform shaker table (set at 150 rpm in companion study) [10–12].

PUF sampling

1. Remove bioreactor flask from shaker table and place in fume hood.
2. Remove PUF sampler from neck of flask and wrap in clean aluminum foil; label, date, and initial.
3. Store at –10 °C until PCB extraction, sample extract cleanup, and analysis.

SPME sampling

1. Inside a fume hood, establish a working area by laying down clean aluminum foil upon which recovered fibers can be set (Fig. 5A).
2. Transfer the volume of sediment slurry from the bioreactor flask to a secondary container (ceramic evaporating dish in companion study) [10–12].
3. Carefully inspect the flask to see if any SPME fiber segments can be easily retrieved with fine point tweezers.
 - a. **Note:** Deployed fiber segments may bunch together, stick to the side of containers, or become covered by sediment that has settled to the bottom of the flask during the incubation period (Fig. 5C).
 - b. **Note:** It can be easier to locate the fibers that have stuck to the side of the flask by looking along the inside wall through the neck from the top, rather than from the side.
4. Recover SPME fiber segments with tweezers and set them in a designated area on the clean aluminum foil sheet so as not to lose them.
5. Transfer the sediment slurry volume from the secondary container back to the flask.
6. Repeat steps 3 and 4 but by inspecting the secondary container instead of the flask.
7. Repeat steps 2–6 until the target length of fiber is recovered (~20 cm in the companion study) [10–12].
8. Using a squeeze bottle, rinse the segments of recovered fiber as they are lying flat with a small volume of ultrapure deionized water to remove any sediment debris (Fig. 5D).
9. Dry the recovered fibers by wiping / rolling them on the aluminum foil working area with a Kimwipe™.
10. Measure each recovered segment with a small ruler, record the length of recovered fiber, and place the measured fiber inside a glass GC-vial insert.
 - a. **Note:** Use a marker to record the length of individual segments inside the fume hood to shorten the time it takes to recover the entire target length of fibers from each flask but do not forget to transfer the recorded measurements to permanent records (lab notebook or spreadsheet).
11. Once all recovered segments are inside the GC-vial insert, use the point of the tweezers to push the fibers down into the insert so that no fibers are overhanging (Fig. 5E).
12. Using a squeeze bottle, carefully fill the GC-vial insert with hexane.
 - a. **Note:** Fill the GC-vial insert enough so that most of the fibers' length is submerged but leave enough room so that the desired volume of internal standard can also be added (19.6 ng 2,2',3,4,4',5,6,6'-octachlorobiphenyl suspended in 25 µL hexane in the companion study) [10–12].
13. Place the insert inside the GC-vial and spike with internal standard.
 - a. **Note:** Be sure to rinse the internal standard syringe between samples to avoid carryover of excess standard (3 × dichloromethane followed by 3 × hexane in companion study) [10–12].
14. Cap the GC-vial; label, date, and initial.
15. Store at –10 °C until analysis (no additional cleanup steps necessary therefore, no surrogate standards required).

Preparation of reference sample

1. Prepare a clean workstation in the fume hood by placing a piece of aluminum foil down to cover the entire area in which you intend to conduct the PCB extraction/cleanup procedure(s).
 - a. **Note:** Items to have inside the fume hood at an arm's reach for the duration of the PCB extraction / cleanup procedures include:
 - i. Sharpie / marker for labeling
 - ii. Small glass container (Erlenmeyer flask or beaker) with pure hexane covered in aluminum foil to prevent contamination
 - iii. Small glass containers with DCM and hexane for rinsing syringes (covered in aluminum foil)
 - iv. Tweezers / forceps

- v. Squeeze bottles containing methanol, acetone, hexane, and dichloromethane for triple rinsing
 - vi. Medium / large size beaker to collect waste solvent during triple rinsing
 - vii. Trays / racks for TurboVap tubes
2. Remove surrogate standards from storage location and allow them to warm to room temperature inside of the fume hood.
 3. Clean the gas tight syringe used for surrogate standard spiking (100 μ L in companion study) [10–12] by fully rinsing it 5 times each with dichloromethane followed by 5 rinses with hexane.
 - a **Note:** Allow the solvent to be fully taken up into the syringe before extruding solvent into waste container to ensure complete rinsing.
 4. Create the reference sample:
 - a. Fill a GC-vial with \sim 1.5 mL hexane.
 - b. Spike the vial with the same amount of surrogate that will be added to samples.
 - i. **Note:** The reference samples will be spiked with internal standard at the conclusion of the cleanup procedure (see *Final Concentration & Internal Standard Spike*: section)
 - c. Immediately cap the GC-vial; label, date, and initial.

PCB extraction from PUF

Time-saver tip: If extracting more than 6 samples in a batch, you can make the most of your time by first prepping, loading, surrogate spiking, and beginning extraction of the first 3 or 4 samples on the ASE (steps 1–8 of this section). While those are running on the ASE, you should have plenty of time to finish the prepping and surrogate spiking for the remaining samples. If using an ASE 350, you will have to stop the run by pressing the “Stop” button on the ASE and selecting option 1, “Abort the current run”, before you can open the plastic shield door and load the final samples on the carousel. Wait until a sample and the subsequent rinse have finished before stopping the run. You will then have to load the method again and specify which cell you want the method to start from.

1. Thoroughly triple rinse (methanol, acetone, then hexane) and/or combust all laboratory materials immediately before use to prevent background PCB contamination.
 - a. **Note:** *Do not* combust ASE cell components; (ASE cells, caps, collection vials, and collection vial caps with septa)
2. Prepare PUF sample(s) and method blanks for extraction on ASE:
 - a. Screw the bottom cap onto the ASE cell.
 - b. Use tweezers to place an ASE filter in the cell and push it to the bottom with the insertion tool.
 - c. Place the PUF passive sampler in the ASE cell and gently push it to the bottom with the insertion tool.
 - d. Rinse the gas tight syringe used for surrogate standard spiking (100 μ L in companion study) [10–12] 5 times with dichloromethane followed by 5 times with hexane.
 - e. Spike the PUF passive sampler in the ASE cell with surrogate standard.
 - i. **Note:** Accurate results require precise standard spiking. It is easiest to ensure an accurate spike volume by first using a Pasteur pipette to transfer a small aliquot (\sim 1 mL) of the standard solution to an appropriately labeled, capped GC-vial.
 1. Puncture the GC-vial septa with the syringe needle and invert the vial with the needle still inside.
 2. Draw standard solution into the syringe and inspect for air bubbles.
 3. If air bubbles are present, draw up slightly more volume than the syringe capacity but be careful to not pull the plunger out of the syringe.
 4. Use a short, quick push of the plunger to force the air bubbles back into the GC-vial and slowly adjust the volume in the syringe to the appropriate spike volume.
 5. Being careful not to bump the plunger, turn the syringe right-side-up (needle down) and remove it from the GC-vial.
 6. Extrude the spike volume onto the PUF sample within the ASE cell with a slow, steady push of the syringe plunger.
 - f. Use tweezers to place a top ASE filter in the cell and gently push down to PUF sampler with the insertion tool.
 - g. Screw the top cap onto the ASE cell.
 - h. Place the ASE cell on the top carousel and place a collection vial in the corresponding position on the bottom carousel.
3. Turn on ASE and make sure it is receiving at least 150 psi nitrogen gas pressure to ensure proper function.
4. Check that there is enough solvent in reservoir(s) to run an entire batch of samples
5. Load or create the ASE method with the following parameters:
 - a. Heat: 5 min
 - b. Static Time: 5 min
 - c. Flush Volume: 60%
 - d. Purge Time: 200 s
 - e. Pressure: 1500 psi
 - f. Temperature: 100 °C
 - g. Cycles: 1
 - h. Solvents: 1:1, acetone:hexane
 - i. **Note:** For ASE models 300 and below, you must pre-mix solvents before beginning extraction. For ASE 350, you can select the solvent types to be mixed inside the extraction cell from up to 3 different solvent reservoirs at a time.

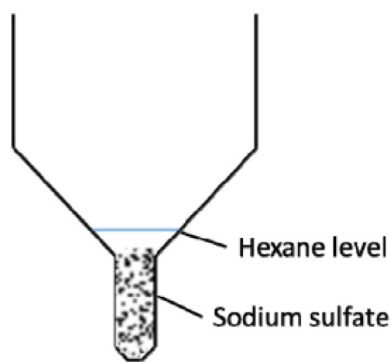


Fig. 10. – Schematic showing appropriate hexane level following concentration and water removal with sodium sulfate in a TurboVap tube.

6. Place empty collection vials in the rinse position(s) on the bottom carousel.
7. Run the rinse function to clear ASE solvent lines of any residual PCB contamination.
8. In the 'Load Method' menu, specify the cell position at which the run should start, and press start
 - a **Note:** When the samples have finished running on the ASE, the collection jars can be capped with new triple rinsed septa and the sample extract can be stored in the freezer overnight, if necessary. However, it is best practice to complete the extraction process in one day, if possible.
9. Under a steady stream of nitrogen gas, concentrate sample extracts to 1 mL.
10. Check if the samples contain water by adding a small amount of hexane to each TurboVap tube.
 - a. If hexane is completely miscible with the sample extract, you can proceed to the section of this protocol on column cleanup.
 - b. However, if a distinct phase layer forms, the sample extract turns cloudy, and/or a precipitate forms at the bottom of the tube, there is water in your samples which must be removed before they can be run through the cleanup columns.
 - c. **Note:** Neglecting to remove water will result in a reaction between the water in the sample and the silica in the cleanup column that will not allow the sample extract to flow through which may cause a significant loss of sample extract, poor cleanup performance, and low surrogate recoveries.
11. If sample extracts contain water, carry out the following steps; if not, skip to acidified silica gel column cleanup in the next section.
 - a. Add 5 ml of hexane to each sample that contains water.
 - b. Vigorously but carefully swirl the TurboVap tubes to ensure complete mixing of the water and hexane layers.
 - c. Slowly add sodium sulfate to the TurboVap tube such that all the water in the sample is absorbed.
 - i. **Note:** The sodium sulfate will form a single semi-solid mass as it absorbs water.
 - ii. **Note:** Add enough so that there is some free sodium sulfate remaining in the sample.
 - iii. **Note:** If there is only one sample in the batch that contains water, add sodium sulfate to the blank, even if the blank does not contain water itself to ensure that it is treated the same as the most handled sample.
 - d. Concentrate excess hexane under steady stream of nitrogen gas.
 - i. **Note:** If using TurboVap or another automated nitrogen evaporation system, you will not be able to use the sensor setting because of interference by sodium sulfate, so monitor the instrument closely because the hexane will evaporate very quickly.
 - e. Stop the concentration when the hexane layer is ~5 mm above the level of the sodium sulfate (Fig. 10).

Time-saver tip: If processing more than 6 samples in a batch, begin concentrating sample extracts as soon as the first 6 samples have completed their extraction process on the ASE. You will be able to concentrate the first 6 sample extracts (step 9 of this section) and begin the sample extract cleanup procedure before the other samples have finished running on the ASE. However, do not concentrate the first 6 samples unless you plan on finishing the entire batch. It is best practice to process all samples in the sample batch in sequence with one another.

Acidified silica gel column setup

Time-saver tip: If you are planning to conduct the PCB extraction and cleanup procedures in the same day (which you should do if possible), prepare the columns immediately after all the samples are loaded onto the ASE. However, do not wet the columns with hexane (step 7, this section) until you are ready to begin column cleanup (next section).

1. Place a column holder in the fume hood working area.
 - a **Note:** The column holder apparatus in Fig. 6 is custom made from a ring stand, two pieces of 2 × 4 lumber, and 12 small spring-loaded roller clamps. See Fig. 11 for closer detail.

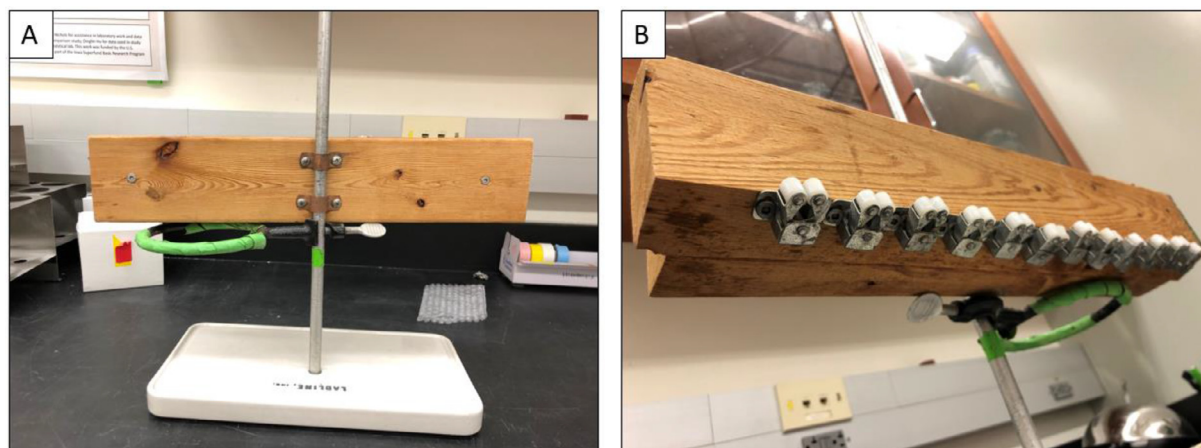


Fig. 11. – (A) Custom column holder apparatus from the rear. Two pieces of plywood are drilled together, fastened to the ring stand with metal brackets, and supported by the ring such that the height of the holder can be adjusted. (B) Custom column holder from below. The columns are securely held with small, spring-loaded roller clamps. Columns are held by sliding them into the clamps from the above.

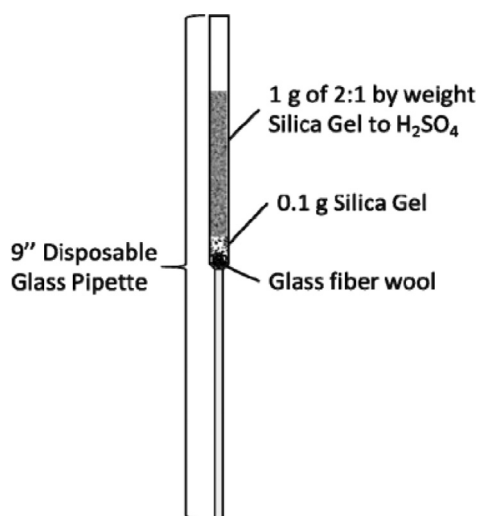


Fig. 12. – Schematic showing appropriate proportions of acidified and non-acidified silica gel in cleanup column setup.

2. Using fine-point tweezers, gently pull apart a small amount (~2–3 cm) of combusted glass wool and place it inside a 9" Pasteur pipette.
3. Using the tip of another 9" Pasteur pipette, push the glass wool down to where the pipette constricts and gently pack the wool by lightly pressing along the circumference of the pipette.
 - a. **Note:** Do not use too much wool or pack it too tightly because it can restrict sample flow through the column.
4. Using a small glass funnel, carefully add ~0.1 g of combusted silica gel.
 - a. **Note:** As a rule of thumb, 0.1 g in a 9" Pasteur pipette is approximately the height of your pinky nail (≤ 1 cm)
5. Using the funnel, add ~1 g of acidified silica gel (Fig. 12).
 - a. **Note:** Adding 1 g of acidified silica gel will result in about an inch of space remaining at the top of the pipette.
 - b. **Note:** The masses of silica do not need to be exact. The objective is to add enough acidified silica gel to adequately clean sample extracts prior to GC–MS/MS analysis. The non-acidified silica gel is present to capture the reaction products from the acidified column cleanup such that they do not wash through to the sample eluent.
6. Place the prepared columns into a pipette holder such that the tips of the columns can be inserted inside a 50 mL TurboVap evaporation tube (Fig. 6) but do not place the TurboVap tubes under the columns yet.
7. Before the samples are added to the columns, wet the columns and perform a final column cleaning step by running pure hexane through each column at least twice.
 - a. Allow the hexane to drip onto the aluminum foil in the fume hood – it will quickly evaporate.

- b. For the second column wetting, overfill the columns such that hexane overflows the column capacity and completely washes the outside of the columns. This is so if a column gets accidentally overfilled or if otherwise unintended solvent gets into the sample eluent it is less likely to contain unwanted contaminants.
- c. As hexane washes through the columns, inspect the section just below the glass wool where the pipette constricts. A small air bubble may form there that greatly restricts the flow of sample through the column. If so, add hexane to the column, lightly attach a pipette bulb to the column, and gently push the air bubble out by gently forcing hexane through. The air bubble should become dislodged and slowly make its way out of the pipette. Be careful to remove the bulb without sucking up any hexane back up through the column – doing so may ruin the integrity of the column.
 - i. **Note:** To make sure you can remove the bulb without sucking up any hexane, the bulb should be barely attached to the column. When removing it, try to “peel” it off rather than lifting straight up.

Cleanup of PUF sample extract

1. After the columns have been washed with hexane, place a freshly triple rinsed and appropriately labeled 50 ml TurboVap tube under each column.
2. Using a 9” Pasteur pipette, transfer each sample extract from the TurboVap tubes in which they were concentrated to their respective columns.
3. Rinse each TurboVap tube that contained a sample at least three times each with ~1 ml of hexane and transfer each rinse volume to the cleanup column.
 - a. **Note:** Thoroughly rinse the inside wall of the tube up to the height of the pre-concentration solvent level. If you added sodium sulfate to the tube, thoroughly rinse that too.
 - b. **Note:** Before adding more hexane to each column, allow the previous rinse to completely vacate the head space of the cleanup column pipette to avoid continually diluting a small amount of the initial sample addition; the column cleanup is more efficient the closer to a plug flow model the sample flow approximates.
4. After the samples and the 3 × rinses of each tube have been run through the column, rinse each column by pipetting pure hexane until the small TurboVap tubes collect a volume of at least 10 ml of column eluent.
 - a. **Note:** At this point, TurboVap tubes can be capped with freshly triple-rinsed caps/septa and stored in the freezer overnight, if necessary.
 - b. **Note:** A column may stop flowing if a sample containing water is mistakenly run through. If this happens, you will have to add hexane to the column and gently force the sample through with a pipette bulb. Repeat this process until an eluent volume of 10 mL is collected in the 50 mL TurboVap tube. Concentrate the sample to ~1 mL, check for water, remove the water if necessary (see step 11 in PCB Extraction from PUF: section), and run it through a fresh column.

Final concentration & internal standard spike

1. Remove the internal standard (IS) from storage location and allow it to come to room temperature inside the fume hood.
2. Rinse the gas tight syringe used for internal standard spiking (25 µL in companion study) [10–12] 5 times with dichloromethane followed by 5 times with hexane.
3. Concentrate the sample extracts to 0.75–1 mL under a steady stream of nitrogen gas.
4. Check that the samples are completely clean (free of any color and cloudiness after concentration). If the samples are at all cloudy or have any color, run them through cleanup columns again.
5. Transfer samples to appropriately labeled GC vials with Pasteur pipettes
6. Use a separate Pasteur pipette to rinse the TurboVap tubes with 0.5–1 mL of hexane up to the height of the solvent level before the final concentration. Transfer the rinse volume to the corresponding GC-vial.
 - a. **Note:** Do not immediately cap the GC-vial. Leave the pipette inside the TurboVap tube and allow any residual solvent to be taken up by capillary action while transferring other samples / rinse volumes to their respective vials. Once all solvent is taken up, perform the final transfer.
 - b. **Note:** At this stage, be very careful not to accidentally transfer a concentrated sample extract to a GC-vial already containing sample volume. It is an easy mistake to make which will undo hours of your work. To ensure this does not happen, turn each GC-vial 180° immediately after it receives the initial transfer volume such that the label is facing away from you and turn back around when making the final transfer.
7. Spike each sample (including the reference) with internal standard and cap the vials immediately.
 - a. **Note:** Remember to rinse the syringe with DCM and then hexane between every sample (see step 2e from PCB Extraction from PUF: section for detailed spiking tips)
8. After spiking with internal standard, the samples are ready for analysis on GC–MS/MS.
 - a. **Note:** Clean hexane is run at the beginning of each batch sequence to assess baseline quality, instrumental interference, and/or residual contamination. Additionally, clean hexane is run directly after the calibration standard as well as at the end of the batch sequence to control for carryover effects between runs. Remember to regularly replace the inlet septa and liner because their degradation over time is responsible for most instrument interferences.
 - b. **Note:** An example sequence for a standard batch is shown below:
 - i. Hexane Vial Position #1

- ii. Cal Vial Position #2
- iii. Hexane Vial Position #1
- iv. Hexane Vial Position #1
- v. Reference Vial Position #3
- vi. Method Blank Vial Position #4
- vii. Sample 1 Vial Position #5
- viii. Sample 2 Vial Position #6
- ix. Sample 3 Vial Position #7
- x. Sample 4 Vial Position #8
- xi. Sample 5 Vial Position #9
- xii. Hexane Vial Position #1
- xiii. Hexane Vial Position #1

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Christian M. Bako: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Andres Martinez:** Conceptualization, Methodology, Software, Validation, Writing – review & editing, Funding acquisition. **Rachel F. Marek:** Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Keri C. Hornbuckle:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jerald L. Schnoor:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Timothy E. Mattes:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Underlying data for this work and the “R” code for the PCB reactive transport model have been deposited in the Iowa Research Online (IRO) institutional data repository for future reuse under an Open Data Commons Attribution License (ODC-By) and an Open Software License (OSL 3.0), respectively. There are no user registration or fee requirements to download the underlying dataset or mass transport model developed for this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.mex.2023.102039](https://doi.org/10.1016/j.mex.2023.102039).

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