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Bioaugmentation of pilot-scale slow sand filters can achieve compliant levels for the micropollutant metaldehyde in a real water matrix

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ABSTRACT

Metaldehyde is a polar, mobile, low molecular weight pesticide that is challenging to remove from drinking water with current adsorption-based micropollutant treatment technologies. Alternative strategies to remove this and compounds with similar properties are necessary to ensure an adequate supply of safe and regulation-compliant drinking water. Biological removal of metaldehyde below the 0.1 $\mu g \bullet L^{-1}$ regulatory concentration was attained in pilot-scale slow sand filters (SSFs) subject to bioaugmentation with metaldehyde-degrading bacteria. To achieve this, a library of degraders was first screened in bench-scale assays for removal at micropollutant concentrations in progressively more challenging conditions, including a mixed microbial community with multiple carbon sources. The best performing strains, *A. calcoaceticus* E1 and *Sphingobium* CMET-H, showed removal rates of 0.0012 $\mu g \bullet h^{-1} \bullet 10^{7}$ cells^{-1} and 0.019 $\mu g \bullet h^{-1} \bullet 10^{7}$ cells^{-1} at this scale. These candidates were then used as inocula for bioaugmentation of pilot-scale SSFs. Here, removal of metaldehyde by *A. calcoaceticus* E1, was insufficient to achieve compliant water regardless testing increasing cell concentrations. Quantification of metaldehyde-degrading genes indicated that aggregation and inadequate distribution of the inoculum in the filters were the likely causes of this outcome. Conversely, bioaugmentation with *Sphingobium* CMET-H enabled sufficient metaldehyde removal to achieve compliance, with undetectable levels in treated water for at least 14 d (volumetric removal: 0.57 $\mu g \bullet L^{-1} \bullet h^{-1}$). Bioaugmentation did not affect the background SSF microbial community, and filter function was maintained throughout the trial. Here it has been shown for the first time that bioaugmentation is an efficient strategy to remove the adsorption-resistant pesticide metaldehyde from a real water matrix in upscaled systems. Swift contaminant removal after inoculum addition and persistent activity are two remarkable attributes of this approach that would allow it to effectively manage peaks in metaldehyde concentrations (due to precipitation or increased application) in incoming raw water by matching them with high enough degrading populations. This study provides an example of how stepwise screening of a diverse collection of degraders can lead to successful bioaugmentation and can be used as a template for other problematic adsorption-resistant compounds in drinking water purification.

1. Introduction

The European Union Drinking Water Directive (Council Directive 98/83/EC; DWD) defines the maximum concentration for individual and total pesticides in drinking water at 0.1 $\mu g \bullet L^{-1}$ and 0.5 $\mu g \bullet L^{-1}$, respectively. Pesticides such as clopyralid, metaldehyde, propyzamide, carbetamide, metazachlor and quinmerac have been identified as pollutants of concern as they are widespread in their application, pollute water resources and result in compliance failures in drinking water (Cooke et al., 2020; Cosgrove et al., 2019; Dillon et al., 2013).

These compounds share characteristics which present challenges to conventional drinking water treatment processes. Most are highly mobile in soil (log $K_{oc} < 2.7$ $mL \bullet g^{-1}$), with the exception of propyzamide, and they are polar molecules (log $K_{ow} < 3$) (Lewis et al., 2016). These properties result in micropollutant concentrations entering water sources through leaching or runoff from agricultural sources. For instance,

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for metaldehyde, studies in the UK have found peak concentrations to be 0.2–0.4 μg•L^{-1} in source waters, although greater concentrations of up to 2.7 μg•L^{-1} have been detected (Kay and Grayson, 2014); in samples taken from River Thames, Castle et al. (2018) identified concentrations of 0.009 - 4.2 μg•L^{-1}. In addition, these compounds have a small organic structure, and therefore are less likely to be adsorbed using conventional micropollutant treatments such as granular activated carbon (GAC) processes (Busquets et al., 2014; Cosgrove et al., 2019; Stuart et al., 2011). For the model pesticide tested here, metaldehyde, the adsorption to activated carbon is not constant, as its conformation and polarity can change due to its chemical structure (Anet, 1974). In addition, the efficacy of GAC for treating metaldehyde is in the order of a few months, thus, this strategy remains costly and ineffective for treating this pesticide (Busquets et al., 2014; Hall et al., 2011; Marshall, 2013).

More sophisticated treatments such as advanced oxidation and UV technologies have been extensively tested, however they are carbon intensive, require extensive capital expenditure and present treatment associated by-products with important implications for human health (Camm et al., 2014; Cooke et al., 2020). Catchment management initiatives in the UK (Get Pelletwise!, OSR Herbicides?: Think Water!) and application bans have reduced the number of failures in recent years. However, these compounds still represent a continued challenge to the supply of pesticide-free drinking water (Chief Inspector of Drinking Water, 2018; Cooke et al., 2020; Mohamad Ibrahim et al., 2019; The Voluntary Initiative, 2021). Clearly, more efficient and less carbon and energy-intensive treatments are required to ensure compliance and protect public health.

For metaldehyde, carbetamide, propyzamide and clopyralid, biodegradation is the main pathway for their removal from the environment, being mineralised by microorganisms and accelerated biodegradation upon repeated exposure has been observed (Arbeli and Fuentes, 2007; Hole et al., 2001; Rouchaud et al., 2000; Schütz et al., 1996; Simms et al., 2006; Walker and Welch, 1991). For metazachlor and quinmerac microbial metabolism occurs (Hart, 1995; Wang et al., 2018). Therefore, treatments focusing on biological removal (such as slow sand filtration) have been proposed as a promising strategy (Dillon et al., 2013).

Slow sand filters (SSFs) can be efficient in removing organic micropollutants through biodegradation and sorption and have been proposed as viable options for high levels of treatment in small drinking water treatment plants at community or household level (Escolà Casas and Bester, 2015; Scheytt et al., 2004). Previous studies have shown that the uppermost layer of the SSF (0–2 cm), known as the Schmutzdecke, is the most important in terms of active biomass, microbial diversity and range of functions, especially organic contaminant biodegradation (Chen et al., 2021; Haig et al., 2016; Oh et al., 2018). Co-occurrence of specific taxa and clustering of microbial communities and metabolic functions from samples taken from geographically-separated full-scale SSFs indicates that their microbial conformation is driven by environmental factors and has been linked to adequate SSF performance (Chen et al., 2021; Haig et al., 2016; Oh et al., 2018).

Only certain operational biofilters have been capable of removing specific recalcitrant polar, low molecular weight organic micropollutants. Other biofilters with similar features and source water characteristics have been unable to degrade these compounds, suggesting an important role of the resident microbial populations for undertaking this function (Rolph et al., 2018). The microbial community in SSFs might adapt to degrade specific micropollutants, however this usually happens after persistent elevated fluxes of the compound for extended periods of time (Rolph et al., 2019, 2020). However, the applicability of such an approach requires optimisation to reduce the acclimation period to practical durations so that the enrichment can occur during a closed-loop recycle period. A strategy such as bioaugmentation decouples this from active operation and enables the potential for continuous operation and is viewed as a more practical solution.

Bioaugmentation involves the addition of microorganisms capable of removing a specific compound to a natural or engineered system. This is a valuable strategy for SSFs since the indigenous microbial communities in biologically-active reactors seldom contain sufficient abundance of degrading populations capable of mineralising specific micropollutants when occurring in spikes in drinking water sources at the ng•L^{-1} to μg•L^{-1} range (Benner et al., 2013). Besides targeting a specific pollutant in the short term, this strategy might lead to the establishment of a stable degrading population within the filter (Samuelsen et al., 2017).

Most bioaugmentation candidate strains and consortia have only been shown to utilize their specific substrate at high concentrations (much higher than micropollutant levels). In most cases it remains unknown if they can degrade the compound when immersed in an environmental matrix with competing organisms and at very low initial concentrations (Benner et al., 2013).

Even if microorganisms capable of degrading the compound at low concentrations are added, many challenges remain. Environmental matrices often contain substances that inhibit microbial activity (toxins, enzyme inhibitors). The environment may lack cofactors necessary for enzymatic activity and the added organisms may use different organic compounds rather than the pollutant. Also, the inoculum may be hindered by competition with the indigenous microbial community or subject to predation by protozoa (Bouchez et al., 2000; Fuller, 2021; Goldstein et al., 1985; Moran et al., 1976). Finally, in sand filters, adhesion of the bioaugmentation agent to the sand particles can be important for preventing inoculum wash-out, allowing for its integration into the biofilm (Samuelsen et al., 2017). All these possible outcomes make the adequate selection, testing and monitoring of bioaugmentation candidates essential steps to ensure performance of the required function.

Few studies have coupled bioaugmentation with sand filtration in the past. Bourne et al. (2006) evaluated the removal of the cyanobacterial toxin microcystin at 50 μg•L^{-1} for 15 d in pilot-scale SSFs fed with river water in which the strain *Sphingomonas* MJ-PV was added. Complete removal of microcystin LR was observed in all sand columns within 6 d, including within the uninoculated controls and it was inferred that contamination of the water reservoir which fed these columns by the degrading strain had occurred. McDowall et al. (2009) seeded laboratory scale SSF columns with a consortium of bacteria capable of removing the cyanobacterial metabolite geosmin and monitored them for up to 17 d Inoculated columns (~1×10^7 total active cells•mL^{-1}) reached a maximum of 38% additional removal of the compound in clarified water (0.1 μg•L^{-1} initial concentration) versus the controls. Lack of complete removal was attributed to a low contact time between consortium members and the micropollutant. Albers et al. (2015) bioaugmented pilot-scale rapid sand filters with *Aminobacter* sp. MSH1, a 2,6-dichlorobenzamide degrader, aiming to eliminate the pesticide from groundwater at a concentration of 0.2 μg•L^{-1}. In this study, ≤75% removal was attained. Degradation within this filter occurred for less than one month probably due to loss of the inoculated cells during backwashing, predation by protozoans or starvation.

Metaldehyde-degrading bacteria have recently been isolated for the first time and proven to use the compound as a sole source of carbon (Thomas et al., 2017). Since then, the diversity of known metaldehyde degraders has been expanded and a shared aerobic degradation pathway has been identified (Castro-Gutiérrez et al., 2020); however it remains unknown if they are capable of degrading the pesticide at micropollutant concentrations in natural environments.

The current paper posits that selection of the most efficient metaldehyde-degrading strains followed by its use as a bioaugmentation agents in a drinking water biofilm reactor will be effective in delivering metaldehyde compliant water while maintaining filter functionality, even when challenged with low pollutant concentrations in real water matrices. To test this, we aimed to (i) identify the most suitable bioaugmentation agents from a library of metaldehyde-degrading strains through bench-scale assays; (ii) evaluate if bioaugmentation of upscaled

systems with selected strains is an effective strategy for removing the contaminant from water collected from within an active drinking water treatment works; (iii) monitor the presence of degrading populations to better understand bioaugmentation outcomes; and (iv) determine if the background microbial community of the SSFs remains stable after being subject to bioaugmentation.

2. Materials and methods

2.1. . Reagents and water characteristics

Metaldehyde (99%) was purchased from Acros Organics (Morris, NJ), methanol (≥99.8%) was purchased from Fisher Scientific (Loughborough, UK), all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO.). 99% acetone was used to clean all equipment before use to prevent metaldehyde contamination. For the pilot-scale sand bioreactor studies, water was collected circa monthly from an operational site (South England) which abstracted water from the River Thames. Water pre-treatment consisted of reservoir storage, coagulation-flocculation using alum, and direct depth filtration. 20 m^3 of minimally-treated water were abstracted and transported to the UK National Research Facility in Water and Wastewater Treatment at Cranfield University and stored in a holding tank for not more than 1 month prior to use. In all cases, spiking of minimally-treated water was undertaken by adding 0.2 L of a stock solution of metaldehyde (10 mg•L^{-1}) dissolved in ultrapure water (PureLab Option s7/15, 18.2 MΩ cm and TOC <3 ppb) to 1 m^3 of minimally-treated water to obtain a 2 μg•L^{-1} final concentration. Water quality parameters are presented in Table S1.

2.2. Analytical methods for metaldehyde

Analyzes of metaldehyde concentrations were performed from filtered aqueous samples (0.22 μm) by using ultra high-performance liquid chromatography (ExionLC AD, AB Sciex, Framingham, MA) coupled to a triple quadrupole mass spectrometer (QTRAP 6500 plus, AB Sciex) (LC-MS/MS). Conditions for the chromatographic analyzes are included in Supplementary Methods - Chromatographic analyzes for metaldehyde. The limit of detection (LOD) and limit of quantification (LOQ) for metaldehyde were determined to be 0.01 μg•L^{-1} and 0.03 μg•L^{-1}, respectively using the signal to noise ratio approach (Wenzl et al., 2016); parameters for the calculations are presented in Table S2.

2.3. Bacterial strains and OD600nm calibration curve construction

Metaldehyde-degrading bacterial strains *Acinetobacter calcoaceticus* E1 (Thomas et al., 2017), *Acinetobacter bohemicus* JMET-C, *Acinetobacter lwoffii* SMET-C, *Pseudomonas vancouverensis* SMET-B, *Caballeronia jiangsuensis* SNO-D and *Sphingobium* sp. CMET-H (Castro-Gutiérrez et al., 2020) which were previously isolated by selective enrichment from metaldehyde-exposed soils were used in this study.

To ensure accurate dosing of degraders in the assays, calibration curves for OD_{600nm} vs number of cells grown in Luria Bertani (LB) broth at exponential growth phase were constructed for each individual metaldehyde-degrading strain using flow cytometry (Cheswick et al., 2019; Gatza et al., 2013). Details are included in Supplementary Methods - OD_{600nm} vs. number of cells for degrading strains and Supplementary Fig. S1.

2.4. Laboratory-scale batch assays for metaldehyde removal

For all bench-scale assays, assimilable organic carbon-free glassware was prepared beforehand (APHA-AWWA-WEF, 2012). Batch removal of metaldehyde was undertaken at an environmentally-relevant starting concentration of 2.0 μg•L^{-1} (Rolph et al., 2019). Removal was first tested in pure culture for all metaldehyde-degrading strains, in 0.22 μm filter-sterilized phosphate buffered saline (PBS) supplemented with trace elements solution (Vishniac and Santer, 1957). The purpose of this experiment was to indicate if bioavailability is a limiting factor for the removal compound at this low starting concentration. Strains were first grown in LB broth (200 mL, 30 °C, 150 rpm) for 20 h. Cells were then harvested by centrifugation (4000 g, 10 min.), washed twice with PBS, and resuspended in supplemented PBS to 5.0 × 10^8 cells•mL^{-1}. 2.5 mL of this inoculum were added to 250 mL Erlenmeyer flasks to obtain a microbial load of 1.0 × 10^7 cells•mL^{-1} in a 125 mL final volume of supplemented PBS (triplicate per strain). Duplicate abiotic controls (inoculum replaced with sterile PBS) were included in each analysis pipeline. Duplicate non-degrading strain controls (*A. calcoaceticus* RUH2202) were also prepared to account for any removal of metaldehyde due to sorption/absorption to/by the biomass of similar size, shape, and cell wall properties. Flasks were incubated at 30 °C and mixed at 150 rpm. At selected time points, 1.0 mL samples were withdrawn, immediately filtered (0.22 μm) into chromatography vials and then swiftly placed on ice and stored at −20 °C until metaldehyde analysis was undertaken using LC-MS/MS.

The strains which performed the best in pure culture tests were selected for trials in minimally-treated water, spiked with 2.0 μg•L^{-1} metaldehyde. Cultures were added to either clean sand (washed with deionized water and autoclaved) or operational sand with a biofilm (Schmutzdecke obtained from a full-scale SSF operated by Thames Water). The inocula were prepared as described previously, except that the wash and resuspension steps were performed using sterile 0.85% NaCl solution instead of supplemented PBS and 4.0 × 10^7 degrading cells•mL^{-1} per flask as inoculum. Triplicate 250 mL Erlenmeyer flasks were established per strain with a liquid volume of 100 mL and 33 g of either clean sand or sand with a biofilm. Duplicate abiotic controls were included in the analysis. Flasks were incubated and samples were taken exactly as described previously.

2.5. Trials of bioaugmentation strains in pilot-scale flow through SSF

Six covered Perspex columns were constructed with at total height of 1.29 m, an internal diameter of 0.15 m and 22.8 L total internal volume each (Supplementary Fig. S2). The sand bed depth was 0.80 m. Quartz sand (Specialist Aggregates, UK) was sorted to 0.1–0.3 mm size and a uniformity coefficient of 1.35, thoroughly washed with tap water and placed on a 0.20 m gravel bed which had a particle size of 1–5 mm. Given the total diameter of the SSF bed, wall effects on the hydraulics were considered minimal (Martin et al., 2013).

Water was supplied to each column using a peristaltic pump (530 U, Watson Marlow, UK) from a 1 m^3 holding tank at a flowrate of 1.8 L•h^{-1}. The empty bed contact time (EBCT) was 9.5 h and the HRT was 3.5 h. A Schmutzdecke was permitted to form, and filters were ripened for a period of ~3 weeks of continual operation prior to the start of the assay until water turbidity stabilised to <0.1 nephelometric turbidity units (NTU) in the filtrate.

The SSF pilots were monitored weekly for water quality parameters including total and dissolved organic carbon (TOC) SM 5310 and turbidity SM 2130 (APHA-AWWA-WEF, 2012), which were within the expected thresholds for operational SSFs (15–25% removal and <1 NTU, respectively) (Collins, 1998).

2.6. SSF treatments

Pilot-scale SSFs were operated for 72 d in total to assess the efficiency of bioaugmentation for metaldehyde removal. Phase 1 (days 1–55): on day 1 metaldehyde dosing was started in the test columns; bioaugmentation with *A. calcoaceticus* E1 was first performed on day 16 at 1 × and then the amount of inoculum added was increased to 2× on day 42. Phase 2 (days 56–72): aiming to enhance metaldehyde removal, on day 56 *A. calcoaceticus* E1 was added at 3× to filter 3 and *Sphingobium* CMET-H was added at 2× to filters 4 and 5. Details of the treatments,

inoculation times and the resulting amount of bioaugmentation strains in the aqueous phase of the SSFs are presented in Table 1. Inoculum preparation details are included in Supplementary Methods – SSF Inoculum preparation procedure.

2.7. DNA extraction from SSFs

Sand was sampled from each SSF on d 1, 16, 18, 23, 30, 37, 42, 44, 51, 56, 58, 65 and 72 from the Schmutzdecke of each SSF and from the top, middle and bottom layers of the SSFs upon filter decommissioning using a sterile spatula and stored at −20 °C until DNA extraction. Genomic DNA was extracted from 0.4 g of sand using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The quality of nucleic acid extractions was verified in SYBR Safe (Thermo Fisher Scientific, Paisley, UK) stained 1.0% agarose gels. DNA concentration and purity were quantified using a Jenway Genova Nano spectrophotometer (Cole Parmer, St. Neots, UK).

2.8. Quantification of total bacteria and metaldehyde-degrading gene copies in SSFs

To track the genetic determinants of metaldehyde degradation, the abundance of genes *mahY* from *A. calcoaceticus* E1 and *mahS* from *Sphingobium* CMET-H was determined using qPCR. Gene *mahY* is part of a shared metaldehyde-degrading cluster in Proteobacteria (Castro-Gutiérrez et al., 2020), while heterologous expression of *mahS* in *E. coli* confers the ability to degrade metaldehyde (Fuller, 2021). Total bacteria in SSF samples were quantified by qPCR using primer pair 341F/534R (Muyzer et al., 1993; Petrić et al., 2011). Oligonucleotides used in this study for degrader quantification are presented in Table S3. All oligonucleotides were synthesized by Merck Life Science (Dorset, UK). Details are included in Supplementary Methods – qPCR for metaldehyde-degrading genes.

2.9. 16S rRNA gene amplicon sequencing for microbial community analyzes

The V4-V5 region of the 16S rRNA gene was amplified for each sand sample using 515F/806R primers (Walters et al., 2016). PCR conditions and purification steps are described elsewhere (Castro-Gutiérrez et al., 2018). 16S rRNA amplicons were sequenced on an Illumina MiSeq instrument using 300-bp paired-end sequencing at the Bioscience Technology Facility of the University of York.

Whole-community 16S rRNA V4-V5 region amplicon sequence information was quality checked using Fastqc software v. 0.11.9. Read sequences were analyzed using QIIME2 v2020.6.0 (Caporaso et al., 2010). Demultiplexed paired-end sequences were imported and the Divisive Amplicon Denoising Algorithm 2 was implemented for quality filtering (Q score ≥30), chimera removal, and feature table construction (Callahan et al., 2016). Taxonomy was assigned to the feature table using the Greengenes 13.8 reference database (McDonald et al., 2012). Feature tables were rarefied to equal number of reads, and the associated taxonomy was extracted for further analysis. Raw reads for 16S rRNA amplicons were deposited in the European Nucleotide Archive under study PRJEB40595.

2.10. Statistical analyzes

All statistical analyzes were performed in PRIMER7 (Primer-E, Auckland, New Zealand). 16S rRNA gene abundance data for the genus level was standardized by sample total, transformed by square root and Bray-Curtis resemblance matrices were constructed. PERMDISP was used to test for homogeneity of multivariate dispersions. Permutational MANOVA (PERMANOVA) was used assess the influence of different factors on the microbial community composition (9999 permutations). Principal coordinates analysis (PCoA) was used for data ordination. The DIVERSE function was used to analyze the richness and diversity (Shannon's H' index), calculated from the abundance for each distinct genus in the samples.

3. Results and discussion

3.1. Performance of metaldehyde-degrading strains in laboratory-scale batch experiments

First, it was necessary to determine if isolated metaldehyde-degrading strains could remove the compound at environmentally-relevant initial concentrations (2 μg•L^{-1}) in pure culture, in defined

Table 1
Operational characteristics and treatments for the pilot-scale SSF.

SSF number	Metaldehyde in inlet	Phase 1 (days 1–55) Bioaugmentation	Purpose	Phase 2 (days 56–72) Bioaugmentation	Purpose
1	No	No	Non-treated control	No	Non-treated control
2	No	Yes*	Persistence of bioaugmentation agent *A. calcoaceticus* E1 without metaldehyde input	No	Persistence of bioaugmentation agent *A. calcoaceticus* E1 without metaldehyde input
3	Yes	Yes*	Effect of bioaugmentation with *A. calcoaceticus* E1 (1 × / 2 ×) on metaldehyde removal – replicate 1	Yes†	Effect of bioaugmentation with *A. calcoaceticus* E1 (3 ×) on metaldehyde removal
4	Yes	Yes*	Effect of bioaugmentation with *A. calcoaceticus* E1 (1 × / 2 ×) on metaldehyde removal – replicate 2	Yes‡	Effect of bioaugmentation with *Sphingobium* CMET-H on metaldehyde removal
5	Yes	No	Removal of metaldehyde without bioaugmentation – replicate 1	Yes‡	Effect of bioaugmentation with *Sphingobium* CMET-H on metaldehyde removal
6	Yes	No	Removal of metaldehyde without bioaugmentation – replicate 2	No	Removal of metaldehyde without bioaugmentation

The strain concentrations were determined by dilution plate counts of the inoculum in LB agar and the estimated water volume in the SSFs.
* Bioaugmentation with *A. calcoaceticus* E1 at 1 × concentration (2.40×10^7 c.f.u.•mL^{-1}) and 2 × concentration (4.84×10^7 c.f.u.•mL^{-1}) were performed on days 16 and 42, respectively.
† Bioaugmentation with *A. calcoaceticus* E1 at 3 × concentration (8.11×10^7 c.f.u.•mL^{-1}) was performed on day 56.
‡ Bioaugmentation with *Sphingobium* CMET-H at 5.00×10^7 c.f.u.•mL^{-1} was performed on day 56.

medium with metaldehyde as the only carbon source, and at a fixed inoculum level (1×10^7 cells•mL^{-1}). As these strains have been previously shown to degrade the compound at much higher starting concentrations (150 mg•L^{-1}) in the exact same medium (Castro-Gutiérrez et al., 2020), the purpose of this experiment was to indicate if bioavailability is a limiting factor for the removal compound at this low starting concentration. Side by side comparison of the different strains under identical conditions permitted characterisation of the removal efficiency (Fig. 1a).

The strains had varying ability to remove metaldehyde at this low initial concentration. *Sphingobium* CMET-H showed the fastest removal, with 56% removed after 2 min and undetectable amounts of the compound after 15 min. Two of the *Acinetobacter* strains (*A. calcoaceticus* E1 and *A. bohemicus* JMET-C) were next in terms of removal efficiency. *C. jiangsuensis* SNO-D showed a slower utilization of the compound, while negligible removal was observed for *A. lwoffii* SMET-C and *P. vancouverensis* SMET-B. There was no metaldehyde removal by the control non-degrading strain. Removal at the final time point ranged from none to below the LOD, even though most (except *Sphingobium* CMET-H) share the same metaldehyde-degrading gene cluster and have been shown to degrade it at similar rates at higher concentrations (150 mg•L^{-1}) (Castro-Gutiérrez et al., 2020).

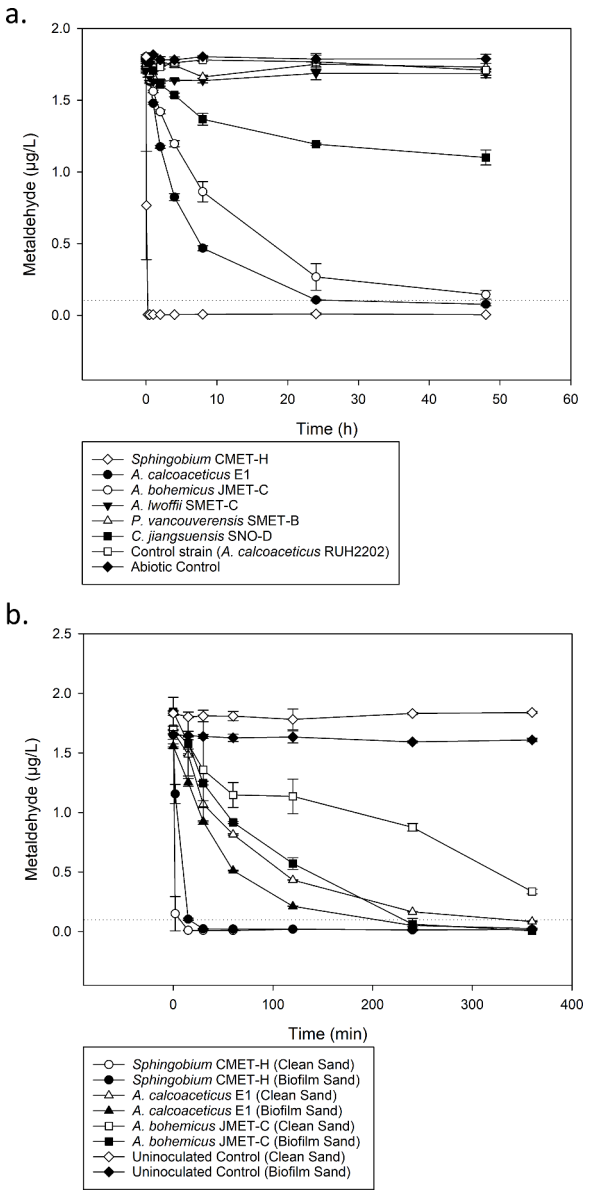

Fig. 1. Batch metaldehyde removal assays for degrading strains in **a.** pure culture systems (1×10^7 degrading cells•mL^{-1}) with supplemented PBS and **b.** minimally-treated water and sand systems (4×10^7 degrading cells mL^{-1}). 2 µg•L^{-1} nominal starting concentration was used. The dashed line indicates the regulatory limit (0.1 µg•L^{-1}).

Downregulation of catabolic enzymes or limited bioavailability are the main factors that can hinder degradation at very low concentrations (Bosma et al., 1996; Kundu et al., 2019). For recently evolved xenobiotic degradation pathways, it is unlikely that an efficient gene expression regulation mechanism has had a chance to emerge, so the expression of enzymes involved in these catabolic reactions would be predominantly constitutive (Sørensen et al., 2009). For the model metaldehyde-degrading bacteria, *A. calcoaceticus* E1, a constitutive nature of the degrading pathway is supported by the observation that the onset of metaldehyde degradation proceeds very rapidly after inoculum addition to a metaldehyde-containing aqueous matrix, even if the inoculum has been pre-grown in nutrient rich media without the contaminant at elevated (150 mg•L^{-1}) (Fuller, 2021) or low concentrations (2.0 µg•L^{-1}; this study). Only a slight increase in metaldehyde-degrading activity after pre-culturing with metaldehyde vs. acetate as sole carbon sources was found (Thomas et al., 2017). These observations suggest that in *A. calcoaceticus* E1 the degrading pathway is constitutive, though it can be slightly upregulated. Since horizontal gene transfer is responsible for the dissemination of the shared degrading pathway across these strains (Castro-Gutiérrez, 2020), the limited degradation rates at these low concentrations for specific strains would likely be due to low bioavailability instead of decreased enzyme expression.

Micropollutant bioavailability can be influenced by factors such as phase distribution and mass transfer, as well as associated physiological factors including membrane permeability, active uptake systems, and excretion of surfactants and enzymes (Cirja et al., 2008). Studies researching other micropollutants such as atrazine have applied compound-specific isotope fractionation and demonstrated that mass transfer across the cell membrane becomes the rate-limiting step below 60 µg•L^{-1} in a chemostat (Ehrl et al., 2019) with complete growth rate control evidenced in a retentostat at circa 12 µg•L^{-1} where substrate is sufficient to maintain viability but not cell growth (Kundu et al., 2019). On the other hand, transport of micropollutants can be influenced by membrane transporters. For instance, whole genome sequencing of glyphosate-resistant mutants of *Bacillus subtilis* showed that its incorporation into the cell is mediated by the high-affinity glutamate/aspartate symporter GltT (Wicke et al., 2019). It is possible that bioavailability limitations of metaldehyde are related to these factors, and similar approaches could be used in subsequent studies to determine this.

The best performing strains, *A. calcoaceticus* E1, *A. bohemicus* JMET-C and *Sphingobium* CMET-H, were selected for further validation in batch tests that more closely mimic a SSF environment. Conditions included the use of non-sterile minimally-treated water and presence of sand with and without an active microbial biofilm from a Schmutzdecke. It was posited that these conditions would expose the inocula to different sources of organic compounds, inhibitory/antimicrobial agents, and interactions with other microorganisms in the water and sand. Metaldehyde removal below regulatory limits (0.1 µg•L^{-1}) in a time frame of less than 3–6 h (estimated contact time in the sand bed for SSF) was desirable. Since degrading enzyme expression is likely constitutive it was proposed that a moderate increase of the initial inoculum (from 1×10^7 to 4×10^7 cells•mL^{-1}) could effectively and rapidly increase the contact between the degrading enzymes and the pollutant, therefore reducing the treatment time needed to ensure good removal performance and compliance.

Metaldehyde removal shown in Fig. 1b. *Sphingobium* CMET-H reduced metaldehyde concentrations to levels below the instrument LOD in less than 15 min. for the clean systems and in less than 30 min for

the systems with a biofilm. *A. calcoaceticus* reduced concentrations to below the regulatory limit in under 4 h for the systems with a biofilm and under 6 h for the clean systems. For *A. bohemicus*, this occurred in less than 4 h in the systems with a biofilm, however the desired removal level had not been reached in the clean sand system at the end of the assay. Calculated removal rates for the best strains, *Sphingobium* CMET-H and *A. calcoaceticus* E1, in the systems with a biofilm were 0.019 $\mu g \bullet h^{-1} \bullet 10^7$ cells^{-1} and 0.0012 $\mu g \bullet h^{-1} \bullet 10^7$ cells^{-1}.

The additional carbon sources present here can have a positive or negative effect on contaminant degradation. Positive effects can result from increased microbial biomass; however, this is usually the case in longer experiments that allow for more extensive microbial growth.

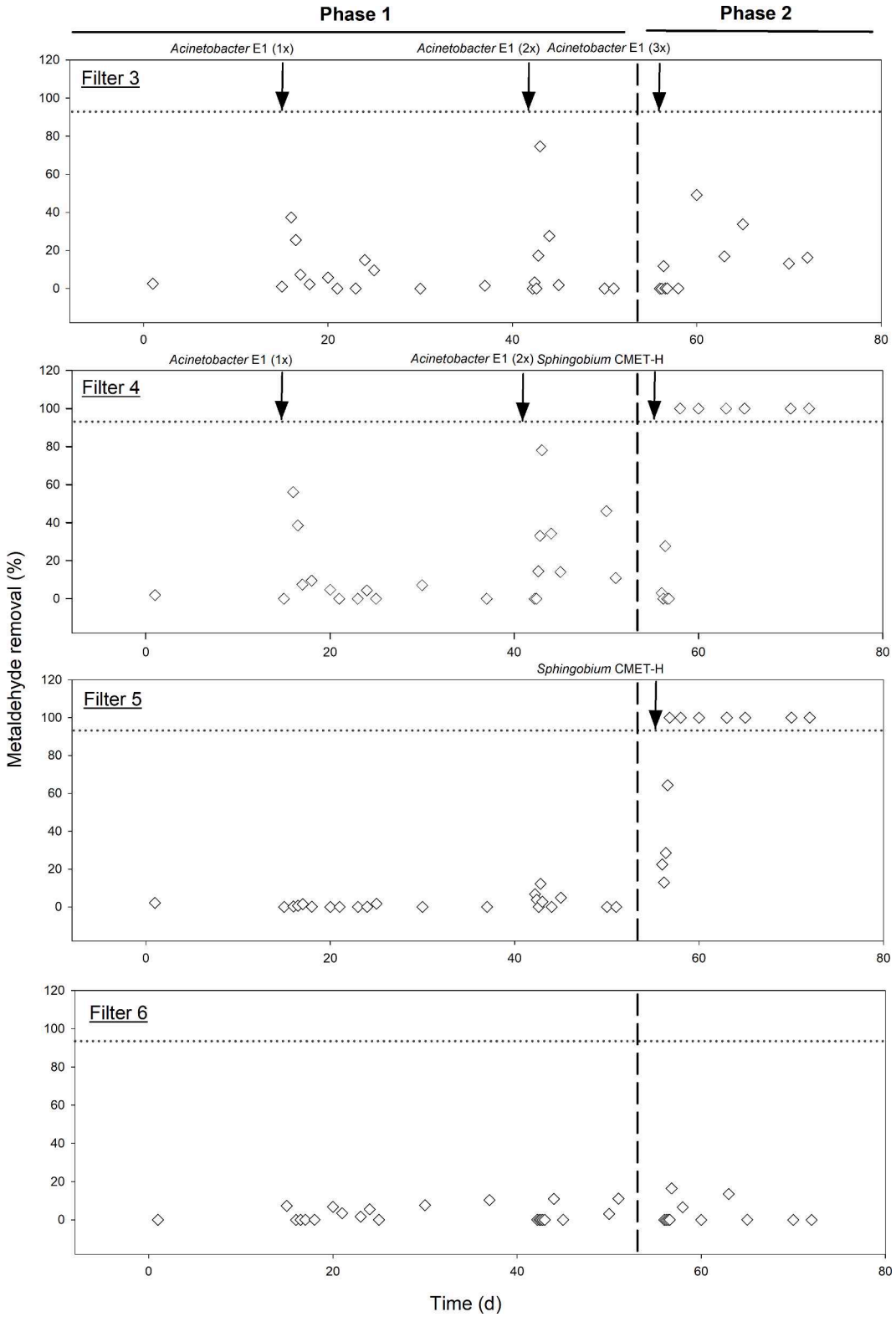

Fig. 2. Percentages of metaldehyde removal calculated as 100 – {([*metaldehyde*]$_{outlet}$ – [metaldehyde]$_{inlet}$)/[metaldehyde]$_{inlet}$}*100 in SSFs with metaldehyde addition (3 to 6). Arrows indicate the inoculation events of bioaugmentation agents. Dotted lines indicate 95% metaldehyde removal.

Negative effects may occur if specific compounds other than the pesticide are present at high enough concentrations that cause catabolite repression (Kalisky et al., 2007) or toxicity. Should a strain provide evidence of metaldehyde removal under these conditions, it would be more likely to be active under continuous flow in a SSF. Even in this challenging scenario, with mixed indigenous microbial communities and multiple substrates in a complex matrix, metaldehyde levels were brought below the regulatory limits and even below the LOD within a reasonable time frame using a practical inoculum size.

A. calcoaceticus E1 was used first for inoculation of the pilot-scale SSF because,at the time of the experiments, only the genes involved in metaldehyde catabolism for this strain (*mahX, mahY*) had been identified and characterised (Castro-Gutiérrez et al., 2020), and primer sets had been validated for tracking them in complex microbial communities (Castro-Gutiérrez, 2020). *Sphingobium* CMET-H was trialled second, as, at that point, no verifiable information was available regarding the genes responsible for the metaldehyde degradation. Subsequent work identified a gene, *mahS*, as responsible for metaldehyde degradation in *Sphingobium* CMET-H (Fuller, 2021).

3.2. Effect of bioaugmentation on metaldehyde removal in a continuous flow-through SSF treating real water

Six pilot-scale SSFs (22.8 L total volume each) were set up with a continuous flow (1.8 L•h^{-1}) of minimally-treated water, either spiked (filters 3–6) or not spiked (filters 1 and 2) with metaldehyde at a concentration of 2.0 μg•L^{-1} (Table 1) and a HRT of 3.5 h. No detection of metaldehyde above the LOD occurred in the inlet of non-spiked filters. Metaldehyde removal was quantified through time and is shown in Fig. 2. A 95% metaldehyde removal was required to obtain water below the regulatory limit of 0.1 μg•L^{-1} (dotted line).

In Phase 1 of the trial (days 1 to 55), metaldehyde removal in the non-bioaugmented (control) SSFs (5 and 6) was very low, with negligible removal detected at most time points and with a maximum removal of 12.3% at time 42.8 d in filter 5 and 11.2% at time 51 d in filter 6.

In filters 3 and 4 *A. calcoaceticus* E1 was first inoculated at 16 d If the removal rates from the batch assays were directly extrapolated, addition of 2.1 × 10^7 cells•mL^{-1} water of *A. calcoaceticus* E1 would be needed to remove all metaldehyde from the water column (12.66 μg), as well as the additional metaldehyde input from the inlet flow (3.6 μg) in a 1 h period. However, the SSF columns were operated at 18.5–20.0 °C (vs. 30 °C in batch assays), without shaking, and the effect of washout, predation and die-off would have to be accounted for during extended operation. Therefore, additional cells were added to the reactor such that × 9 more cells were used in these experiments (a × 3 increase was selected for each of the effects of temperature, mixing and cell losses). The aim was to attain the establishment of a degrading population without having a deleterious impact on the indigenous microbial community of the SSF and hence influence the function of this system (e.g., for turbidity removal). This calculation yielded a target cell concentration of 1.9 × 10^8 cells•mL^{-1} water. Nevertheless, plate counts after inoculation revealed that an inoculation density of 2.4 × 10^7 colony forming units (c.f.u.)•mL^{-1} was achieved in the filters. The cause of this apparent cell loss is discussed later in the paper. In the next 12 h after inoculation, metaldehyde removal increased to a maximum of 37.3% and 56.1% in filters 3 and 4. However, the effect appeared transient, and the removal returned to pre-inoculation levels at 18 and 20 d, respectively.

At 42 d, double the original amount (2×) of *A. calcoaceticus* E1 was added to filters 3 and 4. Here, plate counts for the inoculum revealed that the filters had 4.84 × 10^7 c.f.u.•mL^{-1} of strain E1. Peaks in removal were observed after 43 d, reaching values of 74.6% and 78.1% for filters 3 and 4. Metaldehyde elimination returned to pre-inoculum levels at 45 and 51 d, respectively. The removal process therefore lasted for longer than during the first inoculation. Thus, doubling the inoculum increased the peaks of removal by a factor of 2 and 1.5, respectively, and increased the effect duration. This suggested that removal could be controlled by the amount of strain dosed. However, this was not sufficient to achieve compliance.

Given these results, in Phase 2, two different strategies were applied to enhance metaldehyde elimination. Triple the original amount (3×) of *A. calcoaceticus* E1 was added to filter 3 only, while inoculation with *Sphingobium* CMET-H was carried out in filters 4 and 5. Filter 4 had been previously treated with *A. calcoaceticus* E1, while filter 5 had not been subjected to any bioaugmentation.

After inoculation with strain E1 at 56 d, filter 3 reached a peak of 49.1% removal at 60 d (8.11 × 10^7 c.f.u.•mL^{-1} water in the filter), nonetheless the effect seemed to last for longer than before, with some residual removal still present even at the end of the trial.

For *Sphingobium* CMET-H, if directly extrapolating from batch assays, 1.4 × 10^6 cells•mL^{-1} water would be needed to remove all metaldehyde in the water column plus the incoming load in a 1 h period without any of the previously mentioned corrections. However, given the previous results with strain E1, where compliance was not achieved (even after increasing its concentration), *Sphingobium* CMET-H was inoculated in excess at 5.6 × 10^7 cells•mL^{-1} water. Contrary to *A. calcoaceticus*, there was very little discrepancy between this calculated dose and the effectively detected concentration in the water (5.0 × 10^7 c.f.u.•mL^{-1} water). Bioaugmentation with this strain in filters 4 and 5 at 56 d led to a rapid and significant increase in the degradation of metaldehyde, achieving removal of the compound to below the LOD (0.01 μg•L^{-1}) after 58 and 56.8 d for filters 4 and 5. Removal of the compound at these levels persisted until 72 d when the SSFs were decommissioned. The maximum metaldehyde removal rate for this strain occurred shortly after inoculation in SSF 5 (0.67 μg•L^{-1}•h^{-1}), whereas for filter 4 this was 0.61 μg•L^{-1}•h^{-1}. Thereafter, since no contaminant breakthrough was detected, removal capacity was calculated to be 0.57 μg•L^{-1}•h^{-1} in both vessels following a HRT of 3.5 h.

Comparatively, smaller laboratory-scale through-flow columns with previously acclimated sand (50 μg•L^{-1} metaldehyde, 5 d pre-exposure) have been used and a maximum removal rate of 0.17 μg•L^{-1}•h^{-1} from an inlet concentration of 0.5 μg•L^{-1} and 13.8 h contact time was obtained (Rolph et al., 2019). A fluidised bed reactor (100 L) with acclimated sand and non-controlled temperature has also been tested and reached a removal rate of up to 0.2 μg•L^{-1}•h^{-1} from an inlet concentration of 0.1–0.9 μg•L^{-1}, with 67 min HRT and a recycle ratio of 28.6 (Rolph et al., 2020). Therefore, *Sphingobium* CMET-H can remove the compound at high rates under these conditions for at least two weeks without any prior acclimatisation to the target pesticide *in situ*. This suggests that targeted bioagumentation solutions might be suitable even for ephemeral and highly variable micropollutants by matching them with a high enough degrading population.

3.3. Microbial community in the SSF

Quality statistics for 16S rRNA gene amplicon sequencing are shown in Table S4. 78 samples were sequenced, obtaining an average of 173,142 reads per sample after quality control. The sample from filter 5 at 23 d was removed due to a low number of reads after quality control. All remaining samples were rarefied to 61,312 reads. Rarefaction curves (Supplementary Fig. S3) indicated that for all samples the richness of bacterial taxa had already reached a plateau at this depth.

3.3.1. Fate of bioaugmentation agents

An assessment of the relative amounts of the bioaugmentation agents in the Schmutzdecke of the SSFs was performed. The percentages of the community corresponding to the genera *Acinetobacter* and *Sphingobium* for the filters that underwent bioaugmentation (2 to 5) is shown in Fig. 3.

A Python script was written which identified and counted the 16S rRNA sequences for strains *A. calcoaceticus* E1 and *Sphingobium* CMET-H

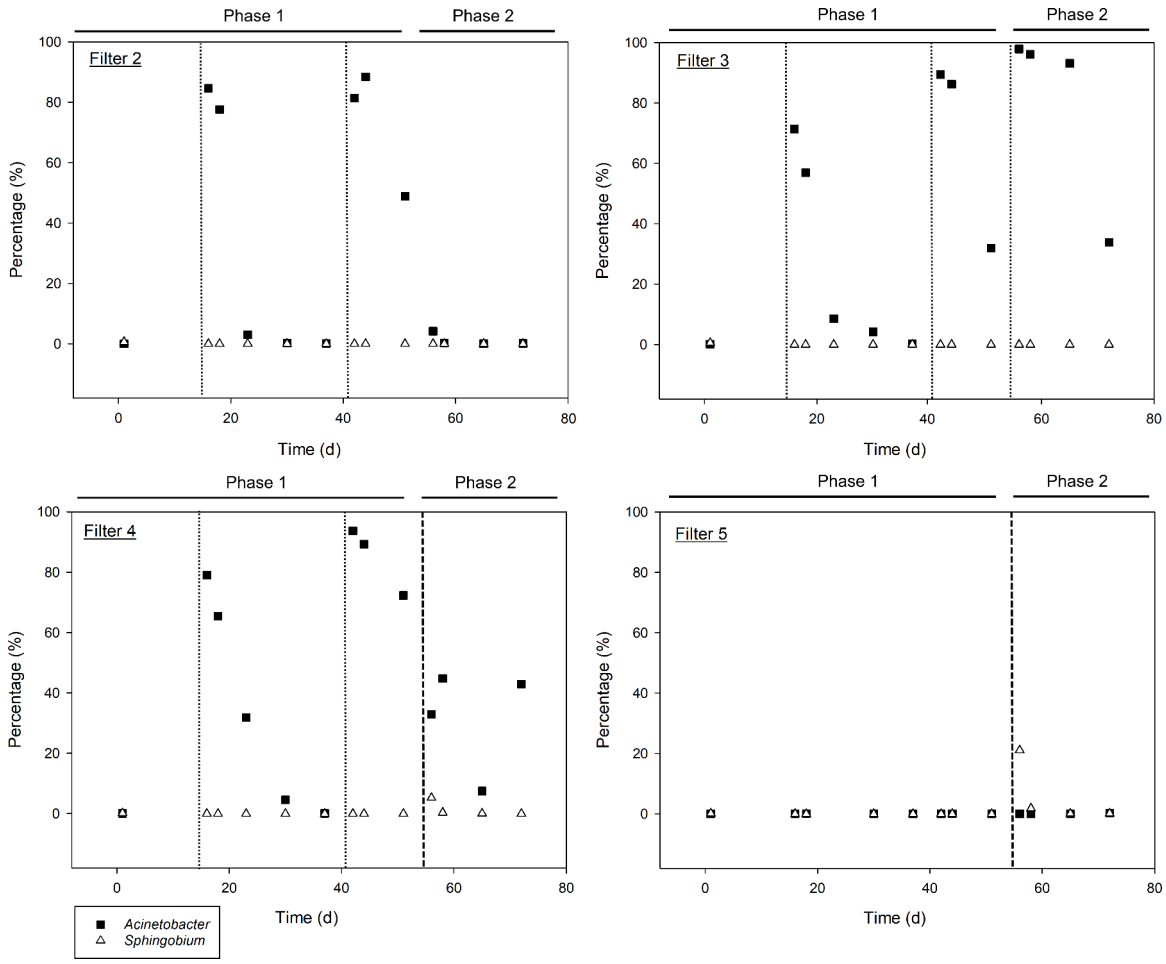

Fig. 3. Percentage of the microbial population at genus level composed of the genera *Acinetobacter* (black squares) and *Sphingobium* (clear triangles) in pilot-scale SSFs throughout time according to 16S rRNA gene amplicon sequencing. Dotted vertical lines indicate time of inoculation with *A. calcoaceticus* E1 and while dashed vertical lines indicate time of inoculation with *Sphingobium* CMET-H.

from the reads files for the SSF sequencing. 87.8% of all *Acinetobacter* raw reads had at least a 99% sequence similarity with *A. calcoaceticus* E1, including 66.9% exact matches. For *Sphingobium* CMET-H, 52.6% of all *Sphingobium* raw reads had a 99% sequence similarity with *Sphingobium* CMET-H, including 37.4% of exact matches.

For phase 1, before inoculation with *Acinetobacter* E1 in SSFs 2, 3 and 4, the genus was absent at this sequencing depth in filter 2, while the percentage detected was 0.1% for both filters 3 and 4 (Fig. 3). After the first bioaugmentation (1×) at time 16 d, the *Acinetobacter* population peaked in the next sampling at 84.6%, 71.4% and 79.0% for filters 2, 3 and 4, respectively. Nonetheless, the population returned to pre-inoculation levels at 30–37 d regardless of the presence of metaldehyde in the inlet, suggesting *Acinetobacter* was not actively growing at a rate sufficient for a sustainable population in the reactor. The second bioaugmentation with this same strain (2×) at time 42 d resulted in higher relative abundance (88.4–93.7%) and the percentage of the augmented genus in the community decreased more slowly with time, indicating greater persistence in the reactor. However, once again, the permanence of the inoculum was not markedly different between filters that had metaldehyde input (filters 3 and 4) and the one that did not (filter 2). This suggests that this metaldehyde concentration in the inlet is insufficient to maintain the E1 strain degrading population in the SSFs, and that the die-off rate is independent of this factor. Approximately 3 × the original amount of strain *A. calcoaceticus* E1 was added to filter 3 for Phase 2 of the experiment at time 56 d, reaching a peak of 97.9% of the community, however at the end of the assay at time 72 d the population had already dropped to 33.8%.

Overall, these data for *Acinetobacter* indicate that a larger inoculum extended the persistence of the bioaugmentation agent and produced higher, although transient, peaks in abundance. However, removal of metaldehyde (>25%) was mainly observed when *Acinetobacter* populations exceeded c.70% of the total number of 16S reads present in the Schmutzdecke (Fig. 3 and Supplementary Fig. S4) and the required 95% elimination was not reached.

To investigate further, the amount of *mahY* gene copies (metaldehyde-degrading gene from *A. calcoaceticus* E1) was assessed via qPCR in the different levels of the bioaugmented SSFs at the end of the trial to ascertain the final distribution of *A. calcoaceticus* E1 (Table 2). Interestingly, strain retention was dramatically higher in the top layer than in the lower layers, with a top:middle:bottom (T:M:B) ratio of 9658:5:1 and 573:1:1 in filters 3 and 4, respectively. Given these results, and the fact that the concentration of strain E1 detected in the filters was much lower than the pre-calculated inoculum amounts for this strain, it appeared that aggregation of *A. calcoaceticus* E1 cells during the inoculum preparation procedure reduced the number of c.f.u. detected by the culture method and hindered the adequate distribution of the bacteria throughout the filter by causing retention of aggregates in the top layer. Bacterial autoaggregation can be triggered as a form of protection from environmental stress and has been reported for *Acinetobacter baumanni/calcoaceticus* complex (Ishikawa et al., 2012; Trunk et al., 2018). This phenomenon would have reduced the overall contact time between the pesticide and the bacteria and contributed to the failure to obtain an efficient and continued metaldehyde removal.

Sphingobium CMET-H was added to filters 4 and 5 in Phase 2 to reach

Table 2
mahY and *mahS* gene copies per g of sand in the different SSF layers (T: top, M: middle, B: bottom) upon decommissioning at 72 d.

		Filter 2		Filter 3		Filter 4		Filter 5	
		Av. (g.c./ g sand)	S.D.	*Av.* (g.c./ g sand)	S.D.	*Av.* (g.c./ g sand)	S.D.	*Av.* (g.c./ g sand)	S.D.
mahY	**T**	1.1×10^5	2.1×10^3	5.9×10^9	2.8×10^8	7.1×10^7	3.9×10^6	N.D.	N.A.
	M	7.0×10^4	3.3×10^3	3.1×10^6	7.6×10^4	1.3×10^5	5.5×10^3	N.D.	N.A.
	B	3.3×10^4	1.3×10^3	6.1×10^5	4.4×10^4	1.2×10^5	4.7×10^3	N.D.	N.A.
mahS	**T**	N.D.	N.A.	N.D.	N.A.	5.7×10^5	1.7×10^4	2.9×10^5	4.8×10^3
	M	N.D.	N.A.	N.D.	N.A.	3.7×10^5	8.5×10^3	1.6×10^5	3.1×10^3
	B	N.D.	N.A.	N.D.	N.A.	1.4×10^5	7.2×10^3	3.4×10^4	9.3×10^2

g.c.: gene copies.
S.D.: standard deviation.
N.D.: not detected.
N.A.: not applicable.

a cell concentration of 5.0×10^7 in the aqueous phase of both filters, very close to the precalculated concentrations (no aggregation detected). Before inoculation, a relative average population of only 0.02 and 0.03% of the community belonged to the genus *Sphingobium* in filters 4 and 5. Peaks of 5.3% and 21.1% in the upper layer of filters 4 and 5 were promptly reached but the abundance decreased rapidly to much lower levels due to decay, retention at lower levels or washout from the filters. Nonetheless, in this case, metaldehyde removal was persistent until the end of the trial.

Given that despite the relatively low percentages of *Sphingobium* CMET-H detected in the Schmutzdecke pesticide elimination was efficient, we aimed to determine whether the *mahS* metaldehyde-degrading gene had been transferred horizontally to the indigenous microbiota during the trial. qPCR was used to quantify the number the *mahS* gene copies in the bioaugmented SSFs and compare them against the *Sphingobium* population, determined from 16S rRNA amplicon sequencing and total bacterial rRNA gene copies. If dissimilar patterns arise through time, this suggests horizontal gene transfer of *mahS* to the indigenous microbial population. However, Fig. 4 shows that *mahS* gene and *Sphingobium* abundances in the Schmutzdecke follow a similar pattern, making this hypothesis unlikely.

It was also hypothesized that the bulk of the *Sphingobium* CMET-H inoculum was being retained further down the SSF instead of in the Schmutzdecke. To determine this, the abundance of *mahS* genes was quantified at the different depths of the bioaugmented filters 4 and 5 at the end of the trial (Table 2). Data showed that *mahS*-carrying degraders were being retained at an approximate T:M:B ratio of 9:5:1 and 4:3:1 for filters 4 and 5, respectively. Even though this scattering was much more homogeneous than for *A. calcoaceticus* E1, this last strain represented an extreme case of uneven distribution. Thus, no preferential retention in the lower levels for *Sphingobium* CMET-H was observed.

The absolute numbers of *Sphingobium* CMET-H that remained in the filters were lower than for *A. calcoaceticus* E1, yet metaldehyde was efficiently removed in filters inoculated with the former. As shown in batch scale assays, *Sphingobium* CMET-H removed metaldehyde at a faster rate than any of the other strains which were tested at an identical cell density. It was posited that this characteristic, along with the lack of inoculum aggregation upon addition, were the main drivers behind its

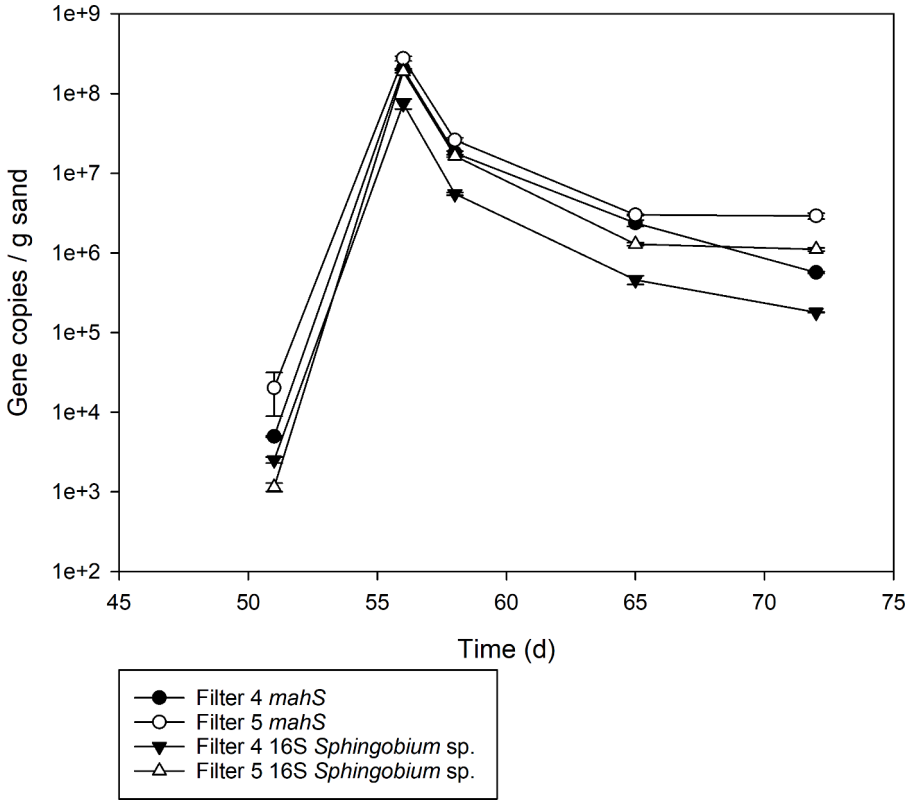

Fig. 4. *mahS* gene copies and 16S rRNA gene copies for *Sphingobium* sp. over time in the Schmutzdecke of bioaugmented SSFs.

superior performance at pilot-scale. Ruling out horizontal gene transfer and preferential inoculum retention at lower-levels through careful experimental work strengthened this position.

3.3.2. Overall microbial community

Regarding the wider microbial community, the rarefied dataset for all filters contained 37 different phyla and 528 genera of Bacteria and Archaea. To identify factors influencing the microbial community composition of the SSFs, analysis of the inherent microbial communities (i.e., not including *Acinetobacter* and *Sphingobium*) was carried out first at phylum level and subsequently at genus level. Phylum Proteobacteria dominated the microbial community composition in the filters (Fig. 5a) as has been observed in the other SSF systems (Chen et al., 2021; D'Alessio et al., 2015; Haig et al., 2014), followed by Planctomycetes and Bacteroidetes.

At genus level, increasing diversity (Shannon Diversity Index (H')) with time can be recognized in the microbial community (Supplementary Fig. S5), however no marked differences were present between individual SSFs. This observation goes in hand with an enhancement of SSF performance with maturity (Pompei et al., 2017).

The PCoA plot for SSF microbial community distribution at genus level is shown in Fig. 5b. For Phase 1, sampling time was a significant determinant of community dissimilarity (PERMANOVA Pseudo-F = 12.01, p = 0.0001, df = 8). Conversely, neither metaldehyde addition

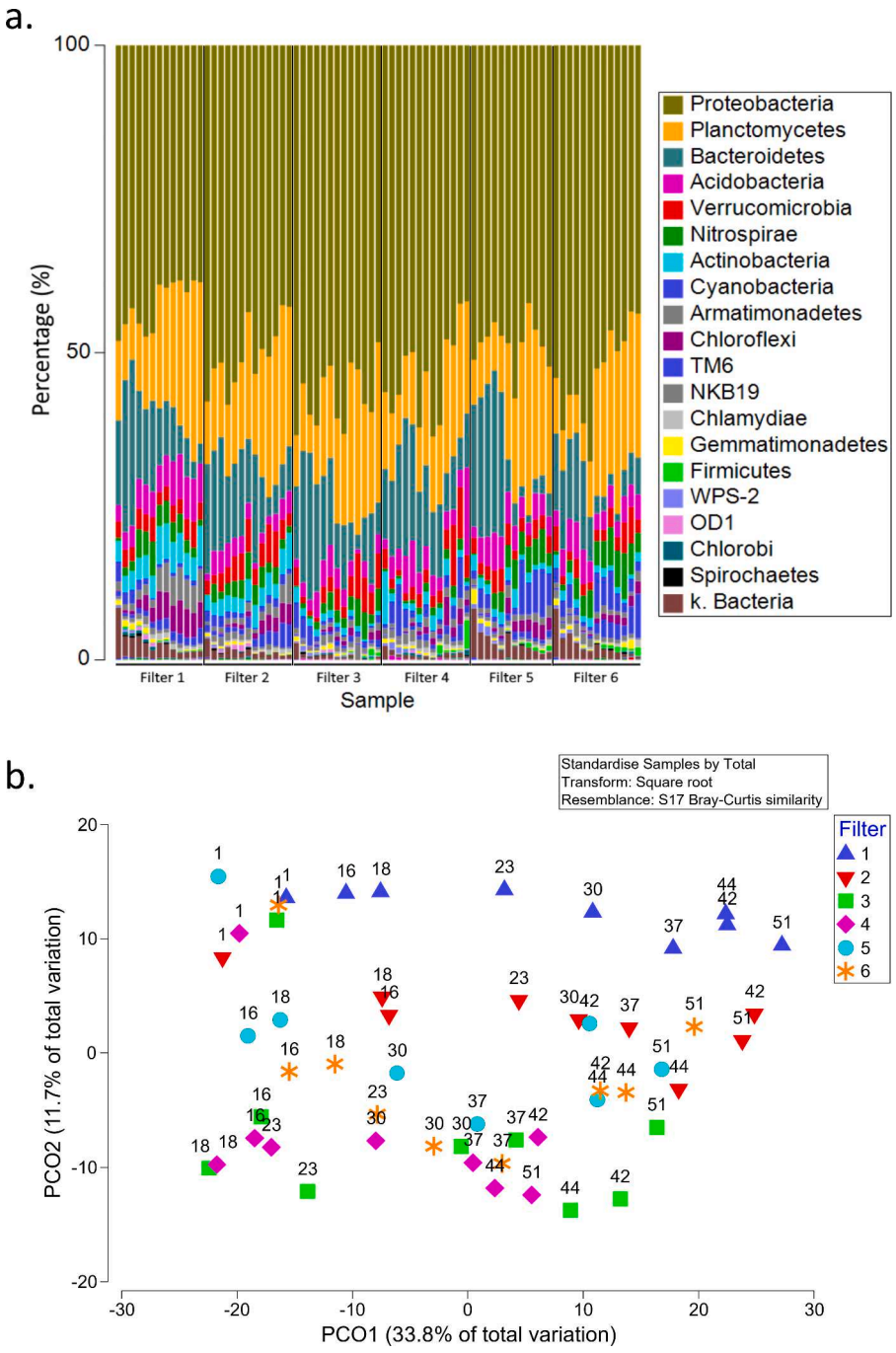

Fig. 5. Background microbial community of the SSF Schmutzdecke**. a.** Phylum level relative abundance of 16S rRNA gene copies in through time (restricted to the top 20 taxa). Samples are ordered in chronological order for each filter. **b.** PCoA plot at genus level of bacterial community 16S rRNA gene amplicons. Labels indicate sampling time; symbols indicate different filters.

(PERMANOVA Pseudo-F = 3.1849, p = 0.1587, df = 1) nor bioaugmentation with *A. calcoaceticus* E1 (PERMANOVA Pseudo-F = 1.985, p = 0.1568, df = 1) were found to be factors that explained the differences between the communities. Similarly, in Phase 2 no significant impact of bioaugmentation with *Sphingobium* CMET-H (PERMANOVA Pseudo-F = 1.3127, p = 0.37, df = 1) or metaldehyde input (PERMANOVA Pseudo-F = 1.9021, p = 0.1712, df = 1) was found in the remaining microbial communities.

The low impact of metaldehyde on the microbial community (whether inoculated or indigenous) could be explained by the fact that the compound is present here at micropollutant levels ($\mu g \bullet L^{-1}$ vs. $mg \bullet L^{-1}$ for bulk natural organic matter in the water). Growth of pure cultures on single substrates requires a minimum level of the compound (threshold concentration), which has typically been reported to range between 1 and 100 $\mu g \bullet L^{-1}$ for different compounds (Egli, 2010). This threshold has not been determined for metaldehyde, nevertheless no significant changes in the communities were detected with the concentrations used here. In contrast, significant effects have been found at elevated concentrations of 50 $\mu g \bullet L^{-1}$ (Rolph et al., 2019).

Our data indicate that bioaugmentation with metaldehyde degraders, dosing of metaldehyde, or both simultaneously did not significantly impact the remaining microbial community in the SSF and, as reinforced by adequate water parameters of the filter effluents (Table S1), it is unlikely that this strategy will disturb the microbial function of a SSF. Further work in our laboratory will include testing the performance of *Sphingobium* CMET-H over extended reactor operation times (i.e., months) in more challenging scenarios such as lower inoculum, lower temperatures, and varying metaldehyde concentrations, as well as different dosing approaches.

4. Conclusion

Here, it has been demonstrated for the first time that a bioaugmentation strategy can provide removal of metaldehyde from water to below the regulatory limit in an upscaled continuous-flow system in a real environmental matrix. Having a diverse collection of degraders at the start of the study was vital to finally achieve successful bioaugmentation. Aggregation of *A. calcoaceticus E1* in the upscaled filters caused its retention almost exclusively in the top layer which limited its removal efficiency due to poor contact between pesticide and degrader. The distribution of *Sphingobium* CMET-H was more homogeneous, which, coupled with its high degrading capacity, led to efficient compound removal for an extended period. This study provides a template for similar stepwise screening and upscaling strategies which could be used for other problematic adsorption-resistant compounds in drinking water purification. Future work is required to ascertain the persistence, longevity, and growth of *Sphingobium* CMET-H in upscaled systems with transient peaks in substrate concentrations. Also, it should be investigated if, once established in the microbial community, it can still respond in reduced time periods when challenged with the compound.

Data Access

Data associated with this manuscript is available at: 10.17862/cranfield.rd.18412244.

Declaration of Competing Interest

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118071.

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