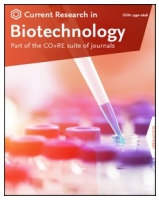
Current Research in Biotechnology 8 (2024) 100248



Contents lists available at ScienceDirect

Current Research in Biotechnology

journal homepage: www.elsevier.com/locate/crbiot





Biodegradation of (Aminomethyl)phosphonic acid (AMPA) by isolated microbial consortia extracted from biological filters at drinking water treatment plants

Laura Pickering [a], Miles Folkes [a], Barrie Holden [b], Peter Jarvis [a], Pablo Campo [a], Francis Hassard [a,*]

[a] Cranfield University, College Road, Cranfield, Bedfordshire MK43 0AL, UK
[b] UK Water Industry Research Limited, London, UK

ARTICLE INFO

Keywords:
Aminomethylphosphonic acid (AMPA)
Micropollutant removal
Pesticide
Selective Isolation
Enrichment
Drinking Water Treatment

ABSTRACT

The widespread use of glyphosate has significantly increased its presence in drinking water sources. Aminomethylphosphonic Acid (AMPA), a breakdown product of glyphosate, is challenging to remove from water using conventional treatment methods, posing risks to public health and environmental safety. This work investigates the biodegradation of AMPA by bacteria isolated from three environmental sources, with a focus on determining their potential application in water treatment systems. Two samples were collected from granular activated carbon (GAC) filters of different operational durations at a water treatment facility, and one sample was taken from soil that had historically been treated with glyphosate-based herbicides. Bacterial isolates capable of degrading AMPA were identified from these samples through selective enrichment, and kinetic degradation experiments were then conducted to assess their effectiveness. In environmental samples, after 48 hrs AMPA removal was > 70 % using GAC from an active treatment plant and soil samples removed 19 %. After bacterial isolation a consortium was isolated and from these four isolates were identified, comprising three species, including novel AMPA degraders M−S3 and M−SS (*Myroides sp*. mNGS23), and P-S92 (*Pseudochrobactrum saccharolyticum*). Within both minimal media supplemented with AMPA and raw untreated showing substrate concentrations above 10 mg/L whilst the specific degradation rates saw a decrease in substrate concentrations above 100 mg/L. AMPA removal occured in pilot scale sand filters augmented with P-S92 but removal was inconsistent. These findings show the potential of using biodegradation as an effective treatment strategy for AMPA removal from water. The identification of AMPA-degrading bacteria offers a promising solution for enhancing the removal of this persistent pollutant from contaminated waters. Further research is recommended to explore the full-scale application of these isolates in water treatment processes. This study contributes to the development of sustainable water treatment technologies by harnessing the natural degradative capacities of environmental bacteria.

Introduction

The intensification of agricultural activities has led to increased concentrations of xenobiotic and recalcitrant compounds in water ecosystems, challenging the safety and compliance of drinking water taken from these sources (Arora, 2020). Some groundwater and surface water can contain over 20 different pesticides and numerous organic micropollutants, often exceeding the regulatory threshold of 0.1 μg/L for individual pesticides applied in many countries (Ren et al., 2020; Gilliom et al., 1992). Specific pesticides such as glyphosate, clopyralid, metaldehyde, propyzamide, carbetamide, metazachlor, and quinmerac have widespread use and contribute to frequent non-compliance of drinking water standards. Traditional drinking water treatment processes such as coagulation-flocculation-clarification do not typically remove these compounds (Castro-Gutiérrez et al., 2022). Technologies like powdered and granular activated carbon (PAC and GAC) however, these methods significantly raise capital plant requirements (CAPEX) and operational (OPEX) costs and the carbon footprint of treatment (Rolph et al., 2018,

* Corresponding author at: Cranfield University, College Road, Cranfield, Bedfordshire MK43 0AL, UK.
E-mail address: francis.hassard@cranfield.ac.uk (F. Hassard).

https://doi.org/10.1016/j.crbiot.2024.100248
Received 6 June 2024; Received in revised form 22 July 2024; Accepted 29 August 2024
Available online 30 August 2024
2590-2628/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

2019). Advanced treatment processes, such as ozonation and advanced oxidation processes (AOPs) are capable of removing pesticides, but are expensive, difficult to deploy rapidly and lack long-term sustainability due to their high energy and chemical demands (Rolph et al., 2019). Disinfection processes such as chlorination can also transform some pesticides and their residues. An example of this is the herbicide glyphosate which has a recalcitrant transformation product known as Aminomethyl)phosphonic acid (AMPA, CH_6NO_3P) (Fauvelle et al., 2017). This product is transformed into chlorinated derivatives during chlorination. The health impacts of these residues and transformation products remain poorly quantified (Mesnage et al., 2015), highlighting the need for further investigation into alternative approaches (Han et al., 2021) such as biodegradation (Benner et al., 2013).

In the environment, glyphosate is initially metabolized into AMPA. The concentrations of glyphosate are typically lower than those of its metabolite AMPA, making AMPA a greater concern regarding water quality and environment (Lutri et al., 2020). AMPA may be converted into sarcosine by AMPA monoxygenase. Sarcosine oxidase then facilitates the degradation of sarcosine into glycine, a simple amino acid. In addition, microbial degradation of AMPA can involve enzymes such as C-P lyase or AMPA deaminase, which convert AMPA into methylamine and inorganic phosphate to further degrade AMPA (Ortiz-Hernández et al., 2013). This biotransformation process enables microorganisms to utilize glyphosate as a source of carbon and/or phosphorus. The biodegradation of AMPA can also occur through fungal metabolism, offering an alternative and non-specific pathway (Zhan et al. 2018) but this is not conducive for drinking water treatment applications.

To date, there has been limited research documenting AMPA removal across rapid gravity filters (RGF) or GAC (Yuan et al., 2022). The substantial diversity of bacterial groups capable of degrading glyphosate/AMPA includes the species *Sinomonas atrocyanea*, previously known as *Arthrobacter atrocyanea* (Kuhn & Starr, 1960). In batch tests, this bacterium achieved ~ 99 % glyphosate removal with a doubling time of 11.2 h (Pipke and Amrhein, 1988). Additionally, glycolate oxidase (GOX) from *Pseudomonas* sp. LBr is another key enzyme thought to be implicated in biodegradation. GOX oxidizes glyphosate to AMPA and glyoxylate, exhibiting a K_m value of 25 mM for glyphosate, a V_{max} of 0.8 U mg^{-1},and a k_{cat} of 0.006 sec^{-1}, suggesting substantial catalytic ability (Scott et al., 2008). The biodegradation potential for glyphosate and AMPA by bacteria has been demonstrated in a slow sand filter (SSF) (Hopman et al., 1995). Authors observed that glyphosate was regularly removed to below detectable levels, AMPA was significantly reduced, often by over 80 %, from initial concentrations of 0.48 μg/L to less than 0.05 μg/L. Post-filtration, AMPA concentrations further decreased from 0.7 μg/L to 0.08 μg/L, illustrating the efficacy of SSF for mitigating AMPA contamination. The primary aim of this study is to test the hypothesis that AMPA-degrading bacteria can be effectively isolated from environments with either current or historical exposure to AMPA, with the aim of then utilizing functional annotation to elucidate the specific bacteria involved and map their enzymatic pathways for AMPA degradation. A secondary aim is to explore the factors that influence the efficiency of bacterial AMPA degradation and the dynamics of their growth kinetics. To achieve this, we will test three AMPA-degrading isolates from GAC and soil environments to evaluate their degradation capabilities at both realistic environmental concentrations and elevated levels of AMPA, reflecting tests relevant to real-world water treatment scenarios. Additionally, these isolates will undergo comparative genomic analysis to uncover potential correlations between their genomic profiles and their efficiency in degrading AMPA.

Materials and methods

Chemicals and media

AMPA (99 % purity) and LC-MS (liquid chromatography-tandem mass spectrometry)-grade reagents including acetonitrile, ammonium acetate, methanol, and ammonium hydroxide were sourced from Fisher Scientific (UK). A stock solution of AMPA at 1 mg/L was prepared by dissolving AMPA in ultrapure water (PureLab option s7/15, 18.2 MΩ mg/L and TOC<3 ppb). This solution was sterilized using a 0.22 μm membrane filter and stored in the dark at 4–8 °C. Aliquots were diluted to the desired concentrations for each experiment. Ultrapure water was also utilized to prepare Luria-Bertani (LB) broth and Minimal Salt Media (MSM), both from Oxoid (UK). The minimal salt media was prepared using the following concentrations of reagents omitting glucose as a carbon source and depleting potassium phosphate (dibasic) (K_2HPO_4) and monopotassium phosphate (KH_2PO_4). Ammonium chloride (NH_4Cl) was added at a concentration of 1 g/L. Magnesium sulphate ($MgSO_4$) was incorporated at 0.2 g/L, and sodium chloride (NaCl) was added at 0.5 g/L. Calcium chloride ($CaCl_2$) was included at a concentration of 0.05 g/L, and ferrous sulphate (FeSO4) was added at 0.005 g/L. The MSM was supplemented with a trace mineral solution (MD-TMS from ATCC, Virginia, USA) according to Wolfe's formulation. The pH of all culture media was adjusted to 7.0 using 1 M HCl or NaOH and sterilized at 121 °C for 20 min in an autoclave. In selective media, traditional carbon sources were omitted and replaced. Growth plates for selective or rich media were prepared by dissolving 1.5 % agarose in either LB or MSM. Degradation experiments were undertaken in the dark.

AMPA removal from acclimated biological-active GAC

Assimilable organic carbon (AOC)-free glassware was prepared according to the APHA-AWWA-WEF (2012) method and used for experiments. To evaluate the sorption capability of GAC and the role of biofilms for removal, control batch reactors with inactive biofilm GAC were run in parallel with non-acclimated reactors. The native GAC biofilm was deactivated by heating the media and accompanying water at 105 °C overnight, serving as the abiotic control. Batch experiments using inactive GAC abiotic controls were conducted in triplicate, each containing 100 mL of media and raw water spiked with AMPA at a concentration of 50 μg/L. Thirty grams of each media was added to conical flasks containing the spiked water. These samples were agitated at 150 RPM on a rotary shaker for 72 h. At predetermined intervals, samples were collected, immediately filtered through a 0.22 μm filter (Fisher Scientific, UK), chilled on ice, and stored at −20 °C until AMPA analysis by LC-MS/MS.

Analytical methods for AMPA

AMPA concentrations were analysed in water samples filtered through a 0.22 μm membrane, utilizing ultra high-performance liquid chromatography (ExionLC AD, AB Sciex) coupled with a triple quadrupole mass spectrometer (QTRAP 6500 plus, AB Sciex). Chromatographic separation occurred at 40 °C, injecting 10 μL of sample through a Thermofisher Hypercarb 5 μm 150 x 2.1 mm precolumn. The mobile phases consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in methanol (B), with a flow rate of 0.4 mL/min over 12 min. The gradient started at 0 % B, increasing to 10 % at 2 min and reaching 100 % B at 5 min, maintained for 1 min before returning to 0 % at 6.1 min. The electrospray capillary operated at a voltage of 4500 V and a desolvation temperature of 600 °C. Calibration was established using standards ranging from 0.01 to 100 μg/L (S 1), interpolating the sample peak areas on the calibration curve. An external standard at 10 μg/L was tested after every 10 samples, flanked by blanks to control for potential carryover. The limits of detection and quantification were established at 0.05 mg/L and 0.15 mg/L, respectively.

Selective enrichment for isolation of AMPA degrading strains

GAC samples were collected aseptically from active GAC filters at an operational full scale WTW in the North of England, where evidence of glyphosate/AMPA removal was prior documented (S-2). Samples from

GAC systems, operated for less than six months (GAC_3) and over six years (GAC_9) since their last regeneration, were transported to the laboratory on melting ice (4–8 °C) within 24 h. GAC was selected as the 'sink' as last asset in drinking water which could reasonably remove AMPA. Additionally, soil from an allotment in Bedfordshire (UK) with a prior history of glyphosate herbicide application 'potential source' was collected to serve as an alternative location to isolate specific glyphosate/AMPA degraders.

Upon collection, GAC and soil samples were divided; one half was stored at 4 °C and the other at −20 °C. For all bacterial isolation glassware was prepared to ensure it was free of AOC. A modified selective-enrichment procedure outlined by Castro-Gutiérrez et al. (2020) was used to isolate AMPA-degrading bacteria from the GAC/soil. For the initial enrichment passage (P1), 3 g of either soil or GAC was inoculated into 100 mL of MSM with 100 mg/L AMPA as the sole carbon and phosphate source in 250 mL Erlenmeyer flasks. All media containing AMPA was filter sterilized (0.22 μm) before use and supplemented with 2.0 mL L^{-1} phosphate-depleted trace elements solution (Vishniac & Santer, 1957). P1 was incubated at 25 °C with orbital shaking (150 rpm) in the dark for 72 h. Subsequently, 1.0 mL from P1 was transferred to fresh medium for the second (P2) and subsequent passages (P3 and P4). Aliquots from P3 and P4 were streaked onto solid MSM supplemented with 75 mg/L AMPA and 0.75 % agarose in triplicate, and incubated at 25 °C in the dark for 72 h. Different colony morphotypes that grew were purified by re-streaking onto rich agar. To differentiate AMPA degraders from potential agarose degraders and oligotrophs, pure cultures were also streaked onto MSM plates without added carbon. Strains unable to degrade AMPA were discarded, while those growing on AMPA-supplemented plates were presumptively identified as AMPA degraders. Isolates were preserved at −80 °C in glycerol for future analysis.

DNA extractions and 16 s rRNA analysis of bacteria

DNA was extracted from 0.4 g of GAC or soil using the NucleoSpin Soil DNA Extraction Kit (Macherey-Nagel, Germany), following the manufacturer's protocol. DNA purity and concentration were assessed using a Jenway Genova Nano spectrophotometer (Cole Parmer, UK) and an Invitrogen Qubit 3 fluorometer (Thermo Fisher Scientific, UK) with dsDNA High Sensitivity Assay (Invitrogen, UK). The V4-V5 regions of the 16S rRNA gene were amplified using 515F/806R primers (Walters et al., 2016) and polymerase chain reaction (PCR). Amplified products were sequenced on an Illumina MiSeq platform using 300 x 300 bp paired-end reads. Quality control of the sequencing data was performed with FastQC software (v. 0.11.9). Analysis was conducted using QIIME2 (v. 2020.6.0, Caporaso et al., 2010), employing the Divisive Amplicon Denoising Algorithm 2 (DADA2) for quality filtering (minimum Q score of 30), chimera removal, and construction of amplicon sequence variant (ASV) feature tables (Callahan et al., 2016). Sequences with an abundance of less than five were excluded. The forward reads were trimmed at 240 base pairs (bp) and the reverse reads at 200 bp selected based on quality scores of the reads. Taxonomic classification used the SILVA 138 database. DESeq2 was applied for normalization and differential abundance analysis, to adjust for library size variations and thus providing a robust statistical framework for identifying significantly enriched taxa across different sample conditions (Love et al., 2014). Beta diversity assessments used Bray-Curtis and UniFrac metrics, while alpha diversity was evaluated using Margalef's Richness index and Pielou's Evenness.

Whole genome sequencing of AMPA degrading isolates

Bacterial Culture and Sample Preparation: Each bacterial isolate was cultured in LB medium under controlled conditions (30 °C and 150 rpm) until reaching the late exponential phase. Post-culture, cells were centrifuged at 500 g for 10 min, and the supernatant was discarded, retaining the cell pellet. The pellet was resuspended in a cryoprotective solution and stored in Microbank bead tubes (Pro-Lab Diagnostics, UK) for further analysis. DNA Extraction and Quantification: For Illumina short-read sequencing, cell pellets underwent three washes with an extraction buffer containing lysozyme and RNase A, followed by incubation at 37 °C for 25 min. Proteinase K and additional Rnase A were added before a final incubation at 65 °C for 5 min. Genomic DNA was then isolated using Solid Phase Reversible Immobilization (SPRI) beads and eluted in an appropriate buffer. DNA concentration was determined in triplicate using the dsDNA HS kit as above. Library Preparation and Sequencing: Libraries for sequencing were prepared and sequenced on an HiSeq platform (Illlumina, USA), generating 250 bp paired-end reads. Sequencing adapters were trimmed using Trimmomatic 0.30 with a sliding window quality cut-off of Q15. For long-read sequencing with Oxford Nanopore, approximately 2 billion cells were processed to extract high molecular weight (HMW) DNA using a Nanobind CCB Big DNA Kit (Circulomics, USA) and concentration of HMW DNA assessed. Genomic DNA libraries were prepared using the SQK-RBK004 and SQK-LSK109 kits, and Native Barcoding EXP-NBD104/114 (Oxford Nanopore Technologies, UK), with 400–500 ng of DNA. Pooled libraries, containing twelve to twenty-four uniquely barcoded samples, were sequenced on a FLO-MIN106 (R.9.4) flow cell in a GridION device (Oxford Nanopore Technologies, UK). Data Analysis: De novo genome assembly and quality assessment were conducted using SPAdes (Bankevich et al., 2012) and QUAST (Gurevich et al., 2013), respectively. Annotation was performed automatically using Prokka (Seemann, 2014).

Identifying genes of glyphosate and AMPA biodegradation

Contigs assembled using the previously described methodology were viewed in SnapGene. This revealed several large contigs, indicating that the samples sent for sequencing were not pure isolates. Nucleotide sequences for the large contigs (>2MB) were extracted. The large contigs were automatically annotated using Prokka (Seemann, 2014).

Cell growth and AMPA degradation assays

Water was abstracted from Chicheley Brook (N 52.079045, E −0.627944), a lowland river source in England, and used in batch degradation experiments to test the efficacy of AMPA degradation in a real water matrix. This water was stored in a 30 m^3 tank at Cranfield University hosted UKCRIC National Research Facility for Water and Wastewater Treatment, with small volumes aliquoted for experiments. Three media types were used to assess bacterial growth rates: LB, MSM, and abstracted raw water. Each medium was inoculated with three different bacterial strains isolated from soils and two distinct GAC filters (3 and 9). Innocula were prepared by culturing each strain on nutrient agar for 72 h, followed by suspension in MSM to an initial optical density at 600 nm (OD_{600}) of 0.1. For the experiments, 0.7 mL of each inoculum was added to 100 mL of AMPA-supplemented MSM in five 250 mL Erlenmeyer flasks per replicate. The experiments utilized a BioScreen C MBR (Oy Growth Curves Ab Ltd, Finland), a microtiter plate reader with controlled temperature (25 °C) and agitation, allowing for the measurement of optical density at selected wavelengths. This setup enabled precise control over incubation conditions and facilitated the processing of many samples simultaneously. Measurement frequency was set to every 10 min over a period of 48 h to monitor bacterial growth. AMPA concentrations varied across cultures, including 0 μg/L, 2 μg/L, 10 μg/L, 50 μg/L, 100 μg/L, 1 mg/L, 10 mg/L, 100 mg/L, and 200 mg/L. Samples (1 mL) were collected from quintuplicate cultures at various time points for AMPA quantification. To maintain sterility, plates for the BioScreen C were prepared in a laminar flow hood, cleaned with 70 % ethanol, and exposed to UV light for 20 min prior to use.

The kinetic degradation of AMPA was assessed across a range of initial concentrations (2 μg/L to 200 mg/L) using three different media: LB, MSM, and raw untreated water. The experiments were conducted at a constant temperature of 30 °C and an agitation speed of 200 rpm.

Sampling was performed at 24-hour intervals. After sampling, the samples were filtered and centrifuged at 10,000 rpm for 5 min to separate the bacteria from the AMPA residues. The AMPA concentrations in the supernatants were quantified using LC-MS/MS, as previously described. To further understand the degradation process, kinetic parameters were analysed. This analysis included the calculation of the microbial growth rate (μ) using Equation (1) and the doubling time (t_d) using Equation (2). This approach allowed for the examination of how varying AMPA concentrations influence bacterial activity, providing insights into the relationships between substrate concentration, bacterial growth rates, and degradation efficiency.

Equation (1). Growth rate of AMPA degrading bacteria

$$\mu = \frac{(\ln x2 - \ln x1)}{(t2 - t1)} \quad (1)$$

Equation (2). Doubling time of AMPA degrading bacteria

$$t_d = \frac{\ln 2}{\mu} \quad (2)$$

Statistical analysis

Statistical analyses were conducted using PRIMER7 (Primer-E, Auckland, New Zealand) and IBM SPSS Statistics for Windows, version 29.0.1.0 (IBM Corp., Armonk, NY, USA). Abundance data for the 16S rRNA gene at the genus level was normalized to the total sample count, square root transformed, and used to construct Bray-Curtis resemblance matrices. From these resemblance matrices, Principal Coordinate Analysis (PCoA) plots were generated for data ordination. To assess the impact of enrichment on microbial communities and isolated strains, Permutational Multivariate Analysis of Variance (PERMANOVA) was employed. This analysis used a Type III (partial) sum of squares with residuals permuted under a restricted model (999 permutations) to compute P-values. For the AMPA removal data from GAC and soil, values were log-transformed (base 10) before performing a one-way ANOVA to compare treatment effects.

Phylogenetic analysis

To identify the species of the contigs extracted from the enriched culture, 16 s rRNA sequences were extracted from the contigs using barrnap (https://github.com/tseemann/barrnap, Version 0.8) and aligned against the NCBI database using BLASTn to identify highly similar sequences. Sequences with a similarity score ≥ 95 % were considered highly similar. The results are shown below in Table 1, below. To construct the phylogenetic tree, these sequences were aligned against 16 s rRNA sequences of known and hypothesised degraders of AMPA using MUSCLE (Edgar, 2004). The evolutionary history of these sequences was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). This tree, drawn to scale, shows the sum of the branch length branch lengths (1.3654) that correspond to the evolutionary distances computed using the Jukes-Cantor method (Jukes and Cantor, 1969) representing the number of base substitutions per site. Bootstrap values over 70 %, based on 1000 replicates, are displayed next to the branches to assess node reliability (Felsenstein, 1985). The analysis, which involved 12 nucleotide sequences, Analysis was performed in MEGA6 (Tamura et al., 2013).

Table 1
The main bacterial species isolated and identified from each of the different isolation sample sources using 16 s rRNA.

Isolate	Species	Percentage
GAC_S9	*Ochrobactrum anthropi*	61.54
	Ochrobactrum anthropi ATCC 49188	15.38
	Ochrobactrum quorumnocens	15.38
	Alcaligenes faecalis	7.69
GAC_S3	*Bacillus altitudinis*	86.81
	Bacillus aerophilus	0.69
	Bacillus pumilus	4.86
	Bacillus safensis	2.08
	Myroides odoratus DSM 2801	0.69
	Alcaligenes faecalis	0.69
Soil_S1	*Stenotrophomonas maltophilia*	60
	Ochrobactrum anthropi ATCC 49188	20
	Myroides odoratus DSM 2801	20

Pilot-Scale AMPA removal

Four Perspex columns were constructed, each 1.29 m high with an internal diameter of 0.15 m and a total volume of 22.8 L (Castro-Gutiérrez et al., 2022). Columns were covered with thick black fabric to prevent light exposure to the sand bed. The bed comprised a 0.2 m deep gravel layer (particle size 1–5 mm, uniformity coefficient Cu = 1.35) and a 0.8 m sand layer (particle size 0.1–0.3 mm, Cu = 1.35). One column was modified for water recycling with a 3 L holding tank overflowing into the waste. Water was supplied to the columns using a peristaltic pump (530 U, Watson Marlow, UK) from a 1 m^3 holding tank dosed with AMPA to a final concentration of 50 μg/L validated using LC-MS/MS. Flow rates for the three straight-through columns were 1.8 L h^{-1} and for the recycling column 1.4 L h^{-1} initially with a recycling rate of 0.8 L h^{-1}. The filters were left to ripen and develop a schmutzdecke for 4 weeks before dosing with continuous operation to allow turbidity (SM 2130) and water quality parameters, including total and dissolved organic carbon (TOC, SM 5310), to stabilize at < 0.1 nephelometric turbidity units (NTU). Column one served as a control with no bioaugmentation but with continual AMPA dosing. Column two received a single dose (1X), 1.0 x 10^7 cells mL^{-1}, of bacteria, column three a double dose of bacteria (2X), 2.0 x 10^7 cells mL^{-1}, and column four used water on a closed loop recycle with a 1X dose of bacteria. The dose of bacteria was determined by CFU. The experiment lasted 4 weeks with dosing in column two at the start of week one and columns two and three at the start of week two. GAC_S9 was the chosen bacterial consortium for pilot-scale dosing as an earlier study suggested that it had the greatest performance in bench scale systems possibly due to complete Pho operon.

Results and discussion

GAC and soil AMPA removal

Over a 48-hour period, AMPA removal was observed from systems containing GAC and soil samples that had been collected from the full scale water treatment works and an allotment site, respectively (Fig. 1). The GAC_9 and GAC_3 samples exhibited an increasing but eventually stabilizing removal rate of AMPA, suggesting potential saturation of adsorption sites or stabilization of microbial degradation processes. Specifically, GAC_9 achieved a removal efficiency of 55.39 % (SD=2.94) at 8 h, which rose to 72.16 % (SD=2.89) by 48 h. Meanwhile, GAC_3 showed a removal of 40.20 % (SD=1.87) at 8 h, reaching a peak of 57.42 % (SD=2.14), a result which differed statistically from GAC_9 (one-way ANOVA F (2, 117) = [4.502], p = 0.013)). The soil sample exhibited a lower removal rate, starting at 18.64 % (SD=1.24) and slightly increasing to 19.79 % (SD=2.20) by 48 h. In contrast, negative removal was noted in abiotic GAC samples, possibly due to AMPA release from the GAC after heat treatment (Sasi et al., 2021). Abiotic soil samples saw a decline in AMPA removal from 10.4 % (SD = ±0.42) to 4.7 % (SD = ±0.04). The AMPA negative samples showed no change (Fig. 1). GAC_9′s treatment was the most effective, consistently increasing to over 70 % removal, showing the significant role of biological activity in AMPA degradation, as opposed to physical or chemical interactions alone, which resulted in negative removal rates in the abiotic GAC_9 controls. In well-established GAC systems such as GAC_9, several factors enhance AMPA biodegradation. Over time, microbial communities develop extensive biofilms, which are complex aggregates

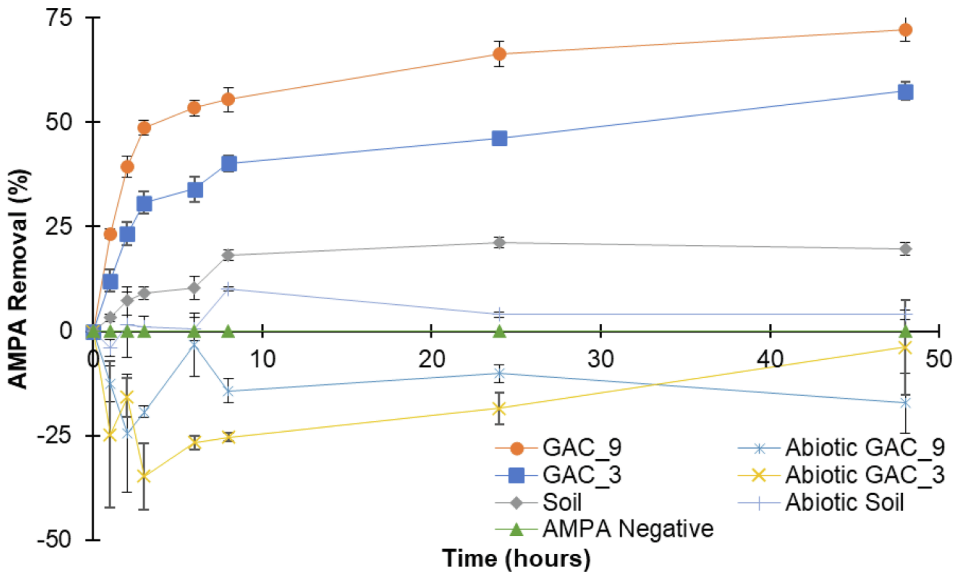


Fig. 1. The removal of AMPA from minimal media using GAC and soil obtained from different sources. AMPA negative and Abiotic controls were used to identify physical or biological removal pathways.

of microorganisms within a self-produced matrix (Flemming & Wingender, 2010). These biofilms stabilize the bacterial environment, boosting their capacity to produce enzymes important for biodegradation of contaminants, such as AMPA, to which they are exposed (Singh et al., 2015). Additionally, prolonged AMPA exposure promotes genetic adaptations in these microbes, enhancing their metabolic capabilities (Cases & de Lorenzo, 2005). The probable increase in specific degrader populations could contribute to the adaptability and biodegradation efficiency of these biofilms (Sviridov et al., 2015).

Microbial community from soil and GAC

Comparative analysis of microbial communities from two GAC filter beds, GAC_9 and GAC_3, and soil samples, taken prior to any experimental AMPA exposure was investigated to understand the environmental microbial communities, revealed distinct differences. The GAC samples exhibited similar microbial profiles, attributed to their exposure to identical environmental conditions (same water source and process conditions at the WTW). In contrast, the soil sample contained a different consortium of microbes. For the GAC, this similarity was explained by their exposure to the same environmental conditions (same water source and process conditions at the WTW). Notably, both GAC

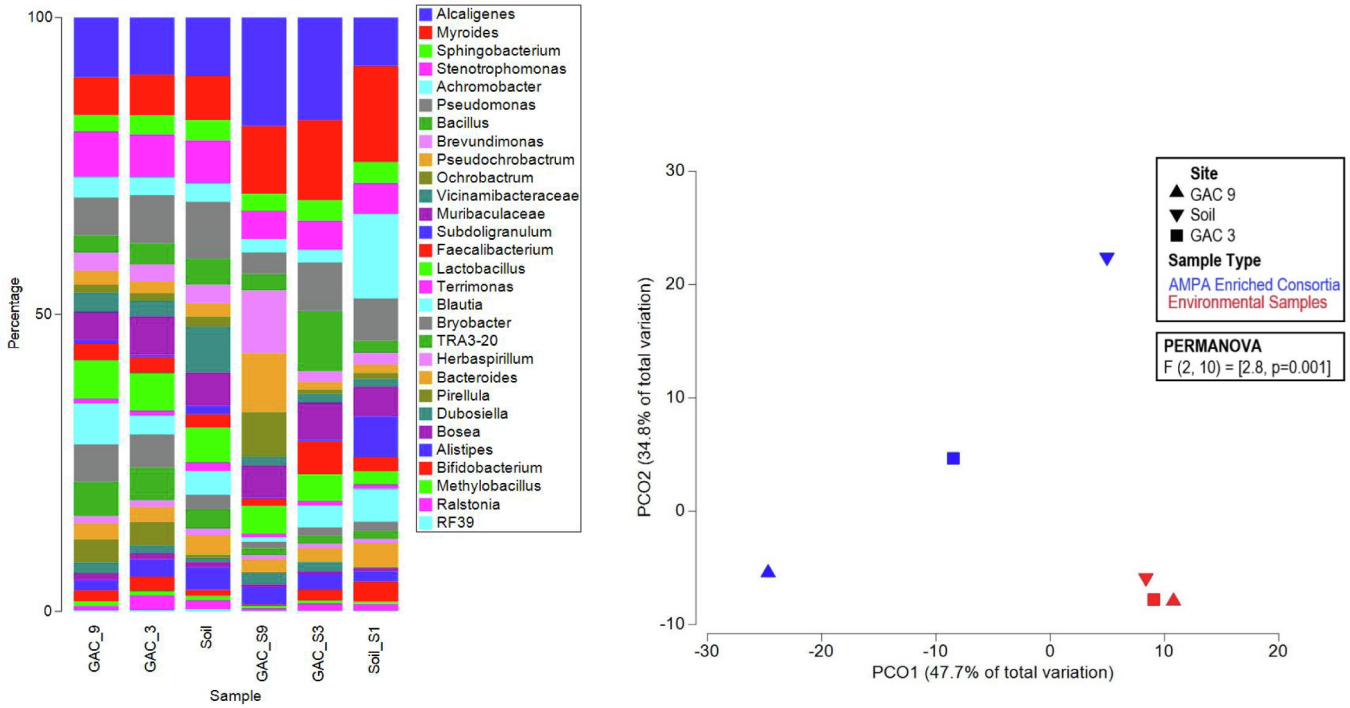


Fig. 2. A) Community of bacteria in the isolates and environmental samples at genus level showing the relative abundance of 16 s rRNA gene copies limited to the top 30. B) Principal ordinate analysis (PCoA) plot of weighted UniFrac distance of the environment.

adsorbers were characterized by a high presence of *Alcaligenes*, though they diverge in the abundance of other genera. For instance, GAC_9 was dominated by *Brevundimonas*, *Pseudochrobactrum*, and *Ochrobactrum*, whereas GAC_3 was rich in *Pseudomonas*, *Bacillus*, and *Muribaculaceae*. Soil_S1 had higher levels of *Achromobacter*, *Pseudomonas*, and *Subdoligranulum* (Fig. 2A). The soil-derived isolates feature a dynamic array of species, including *Stenotrophomonas maltophilia* JV3, *Ochrobactrum anthropi* ATCC 49188, and *Myroides odoratus* DSM 2801, each contributing to the community's function. After enrichment with AMPA, the GAC_S3 consortium increased its diversity of *Bacillus*, including *Bacillus altitudinis* complex, *Bacillus pumilus*, and *Bacillus safensis* FO-36b, alongside *Myroides odoratus* DSM 2801 and *Alcaligenes faecalis*, suggestive of a diverse metabolic biodegradation capacity. Following enrichment by AMPA, the GAC_S9 consortium saw increased relative abundance of *Ochrobactrum anthropi* ATCC 49188, *Ochrobactrum quorumnocens*, and *Alcaligenes faecalis* (Table 1).

Isolation of specific AMPA degrading consortia

Following AMPA enrichment in batch culture conditions, significant divergence was observed in the microbial populations. The PCO1 axis of the PCoA plot highlighted the impact of AMPA enrichment on isolates compared to environmental samples, while PCO2 revealed variations between sample sites and their effects on both environmental samples and their AMPA-enriched counterparts (PERMANOVA level F (2, 10) = 2.8, p = 0.001, Fig. 2B). Isolating specific bacterial strains from environmental samples is complicated by the presence of other microorganisms like fungi, protozoa, and viruses. Contamination risks during collection, transport, and processing add to the complexity. The targeted bacteria, often not predominant in their communities, face intense competition for resources and have specific growth requirements such as pH, temperature, and nutrient availability. Despite these challenges, successful isolation of AMPA tolerant organisms was achieved from GAC samples and soil. Long-term exposure to micropollutants encourages bacteria to develop degradation mechanisms, which may become dormant if pollutant levels fall but can reactivate with new exposures or enriched through elevated levels.

The PCoA plot (Fig. 2B) provides insight into the variation in microbial communities across different samples and treatments. The x-axis (PCO1) and y-axis (PCO2) account for 47.7 % and 34.8 % of the total variation, respectively. The statistical analysis (PERMANOVA) indicates a significant difference in microbial community composition among the groups (F(2, 10) = 2.8, p = 0.001). This result suggests that both the origin of the samples and the enrichment process significantly influences the microbial community structure. In the plot, the AMPA-enriched isolates (blue symbols) form distinct clusters separate from the environmental samples (red symbols). This separation highlights the differences in microbial communities that develop under selective enrichment conditions aimed at degrading AMPA. The isolates from GAC 9 (blue triangles) and GAC 3 (blue squares) are notably distinct from their respective environmental counterparts, indicating enhanced AMPA degradation capabilities. The soil samples (inverted triangles), both enriched and environmental, also show distinct separation from the GAC-derived samples, suggesting inherent differences in microbial communities between soil and GAC environments. The clustering of enriched isolates indicates successful adaptation for AMPA degradation, highlighting the potential for using these microbial communities in practical applications to remove AMPA from contaminated water.

Identity and genomic analysis of AMPA-degrading strains

Two pathways hypothesized to be involved in AMPA biodegradation have been previously identified: the well-characterized Phn Operon pathway (*phnCDEFGHIJKLMNOP*) and a less understood transaminase-phosphonatase pathway (Stosiek et al., 2010; Sviridov et al., 2012; Sviridov et al., 2015; Fig. 3A). Four isolates were identified, comprising three species, including novel AMPA degraders M−S3 and M−SS (*Myroides sp*. mNGS23), and P-S92 (*P. saccharolyticum*), as depicted in Fig. 3B, (Table 2). Previous studies have noted the ability of the *Alcaligenes* genus to be able to biodegrade AMPA (Lerbs et al., 1990). Prokka was used to identify genes that encode proteins implicated in AMPA biodegradation. Isolate P-S92 is believed to biodegrade AMPA via both identified pathways. It possesses a nearly complete Phn Operon (*phnAFGHIJKLMN*) and genes for a hypothesized transaminase pathway

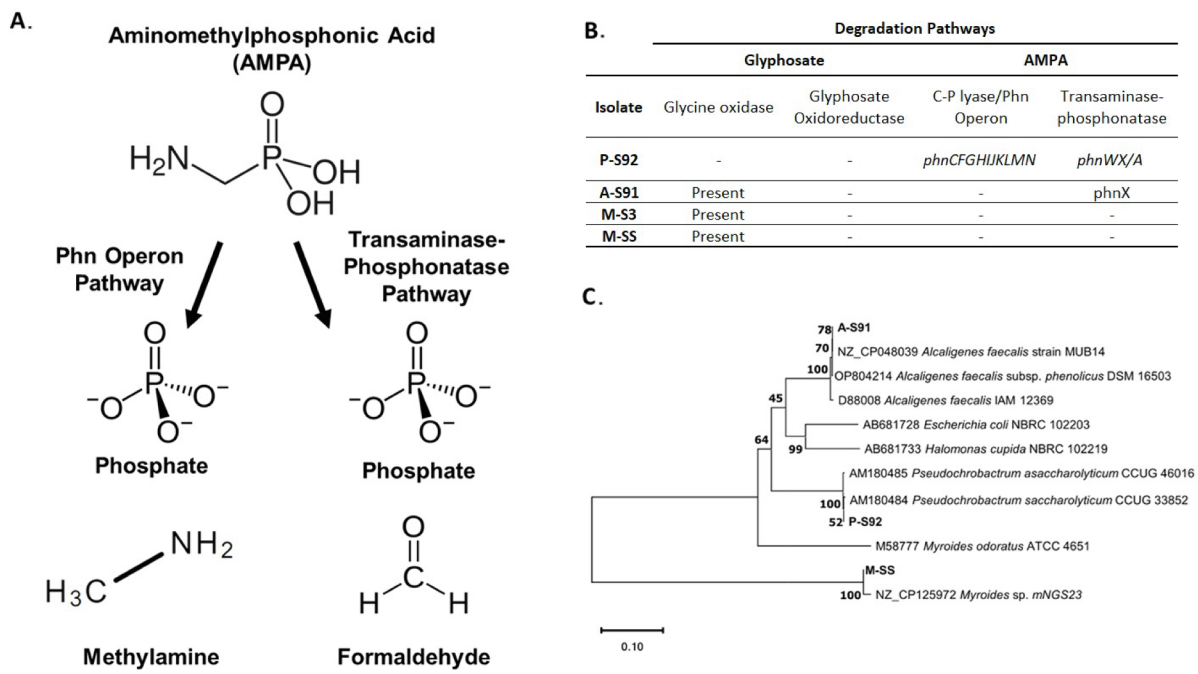


Fig. 3. A) Enzymatic degradation pathways of (Aminomethyl)phosphonic Acid − AMPA. B) Degradation pathways present for each of the isolates. C) Phylogenetic tree of each of the isolate.

Table 2
AMPA-degrading strains isolated by enrichment culture procedure and identification based on 16S rRNA gene sequences against the NCBI database.

Environmental Site	Strain Code	Closest relative	Closest relative GenBank Accession Number	Similarity (%)	No. of bases compared	Identification of isolate
GAC9	A-S91	*Alcaligenes faecalis* strain MUB14	CP048039.1	100 %	1533	*Alcaligenes faecalis*
	P-S92	*Pseudochrobactrum saccharolyticum* strain CCUG 33852	GCA_008801715.1	100 %	1400	*Pseudochrobactrum saccharolyticum*
GAC3	M−S3	*Myroides* sp. mNGS23_01	CP125972.1	99.7 %	1514	*Myroides* sp. mNGS23_01
Soil	M−SS	*Myroides* sp. mNGS23_01	CP125972.1	99.7 %	1514	*Myroides* sp. mNGS23_01

(*phnWX/phnWA*). Unlike A-S91, which likely utilizes the transaminase-phosphonatase pathway without any Phn Operon genes, P-S92 does not possess genes linked to glyphosate biodegradation. This specificity in substrate degradation has been observed in other studies (Balthazor & Hallas, 1986; Dick & Quinn, 1995). Neither M−S3 nor M−SS show genes associated with AMPA biodegradation. A phylogenetic analysis in MEGA revealed that isolate A-S91 is 100 % similar to *A. faecalis* strain MUB14, P-S92 is identical to *P. saccharolyticum*, and both M−S3 and M−SS are 99.7 % similar to *Myroides* sp. mNGS23 (Fig. 3C).

Growth kinetics

In this study, distinct growth characteristics emerged among isolates across the range of growth media and AMPA exposure conditions, highlighting the diversity in microbial responses to environmental stressors. Soil_S1, in nutrient-rich LB media, exhibited a significantly higher growth rate (μ = 0.205) at 0 μg/L AMPA compared to GAC_S3 (μ = 0.084) and GAC_S9 (μ = 0.156) (Table 3). This suggests that Soil_S1 has a competitive advantage in environments lacking AMPA, potentially due to its adaptation to nutrient-dense conditions. However, as AMPA concentrations increased, Soil_S1′s growth rate decreased more sharply than that of GAC_S9, indicating a greater sensitivity to elevated AMPA levels. The specific degradation rates of AMPA varied significantly with substrate concentration and media type. For instance, in LB media, the GAC_S9 isolate consistently showed the highest growth rates across all concentrations, whereas GAC_S3 performed the lowest except at the highest concentration of 200 mg/L (Fig. 4). In minimal media supplemented with AMPA, the degradation rate for GAC_S9 started at 0.14 d^{-1} (SD=0.003) at 0.01 mg/L AMPA and slightly decreased to 0.13 d^{-1} (SD=0.008) at 200 mg/L. In contrast, Soil_S1 started at a degradation rate of 0.15 mg/L (SD=0.012) and dropped to 0.029 mg/L (SD=0.011) under the same conditions (Fig. 5A, Table 3). The degradation rates after acclimatization align with those from batch kinetic trials using non-acclimatized sand taken directly from an active WTW deploying SSF, and are about half those seen with acclimatized sand at 50 μg L^{-1} (Rolph et al., 2019). These rates are, however, significantly lower than those from isolated culture trials at high pollutant concentrations (Thomas et al., 2017), highlighting the role of microbial adaptation in degradation efficiency. Degradation follows Michaelis-Menten kinetics, with a half-rate constant of 25–26 μg L^{-1} in an environmental batch system, and 8.8 mg/L in isolated pure cultures with a pesticide as the sole substrate (Thomas et al., 2017).

Interestingly, the degradation patterns in raw untreated water further highlighted these dynamics, where GAC_S9 showed a relatively stable degradation rate from 0.20 d^{-1} (SD=0.025) at 0.01 mg/L AMPA, decreasing only slightly to 0.18 d^{-1} (SD=0.074) at 200 mg/L. This stability suggests a resilience or tolerance developed by GAC_S9 to AMPA. This contrasted with the sharp decrease in degradation rates observed for GAC_S3 and Soil_S1 at similar concentrations (Fig. 5C). The observed growth and degradation trends could be attributed to several factors, including enzymatic adaptations to AMPA, the availability of AMPA as a nutrient source, and the toxic effects of higher pesticide concentrations. Enzymatic adaptations are crucial as microorganisms evolve to metabolize AMPA efficiently, possibly utilizing it as an alternative nutrient source (Kepler et al., 2020). Additionally, AMPA can serve both as a carbon and phosphorus source, which may enhance microbial activity at lower concentrations but lead to toxicity at higher levels due to cellular stress and metabolic disruption (Rueppel et al., 1977). The variation in microbial response might also suggest hormesis, where low levels of AMPA stimulate growth, enhancing microbial activity, whereas higher concentrations prove inhibitory (Calabrese and Blain, 2011). This hormetic response, as opposed to the straightforward substrate inhibition suggested by classic Michaelis-Menten kinetics, reflects a complex interaction between AMPA concentration and bacterial metabolism, indicating adaptive responses within microbial communities to varying AMPA levels (Yao et al., 2012).

Table 3
Growth rate (μ) and doubling time (t_d) in hours for each of the bacterial isolates at different concentrations in different types of media.

	AMPA concentration	**0 μg/L**		**2 μg/L**		**10 μg/L**		**50 μg/L**			
Isolate	**Media**	**μ**	**t_d**	**μ**	**t_d**	**μ**	**t_d**	**μ**	**t_d**		
GAC_S9	LB	0.156	4.439	0.150	4.629	0.148	4.692	0.136	5.079		
	Minimal Media	0.028	24.460	0.143	4.836	0.141	4.922	0.143	4.843		
	River Water	0.225	3.083	0.181	3.831	0.205	3.379	0.236	2.934		
GAC_S3	LB	0.084	8.274	0.085	8.111	0.085	8.137	0.083	8.357		
	Minimal Media	0.267	2.595	0.240	2.884	0.239	2.906	0.242	2.865		
	River Water	0.097	7.161	0.062	11.221	0.117	5.907	0.118	5.884		
Soil_S1	LB	0.205	3.387	0.210	3.304	0.190	3.644	0.195	3.546		
	Minimal Media	0.176	3.931	0.169	4.093	0.151	4.584	0.153	4.544		
	River Water	0.136	5.101	0.186	3.718	0.199	3.478	0.206	3.361		
	AMPA concentration	**100 μg/L**		**1 mg/L**		**10 mg/L**		**100 mg/L**		**200 mg/L**	
Isolate	**Media**	**μ**	**t_d**	**μ**	**t_d**	**μ**	**t_d**	**μ**	**t_d**	**μ**	**t_d**
GAC_S9	LB	0.137	5.053	0.193	3.593	0.188	3.688	0.167	4.164	0.160	4.338
	Minimal Media	0.141	4.905	0.055	12.660	0.115	6.044	0.127	5.439	0.131	5.307
	River Water	0.220	3.155	0.253	2.741	0.221	3.143	0.270	2.564	0.177	3.910
GAC_S3	LB	0.083	8.328	0.116	5.970	0.113	6.155	0.130	5.333	0.136	5.102
	Minimal Media	0.251	2.757	0.323	2.147	0.322	2.152	0.314	2.206	0.342	2.024
	River Water	0.136	5.080	0.141	4.901	0.117	5.923	0.195	3.547	0.043	16.203
Soil_S1	LB	0.217	3.190	0.183	3.793	0.188	3.689	0.172	4.038	0.167	4.143
	Minimal Media	0.182	3.815	0.083	8.330	0.081	8.607	0.079	8.780	0.029	23.671
	River Water	0.170	4.079	0.236	2.935	0.218	3.182	0.250	2.770	0.018	38.396

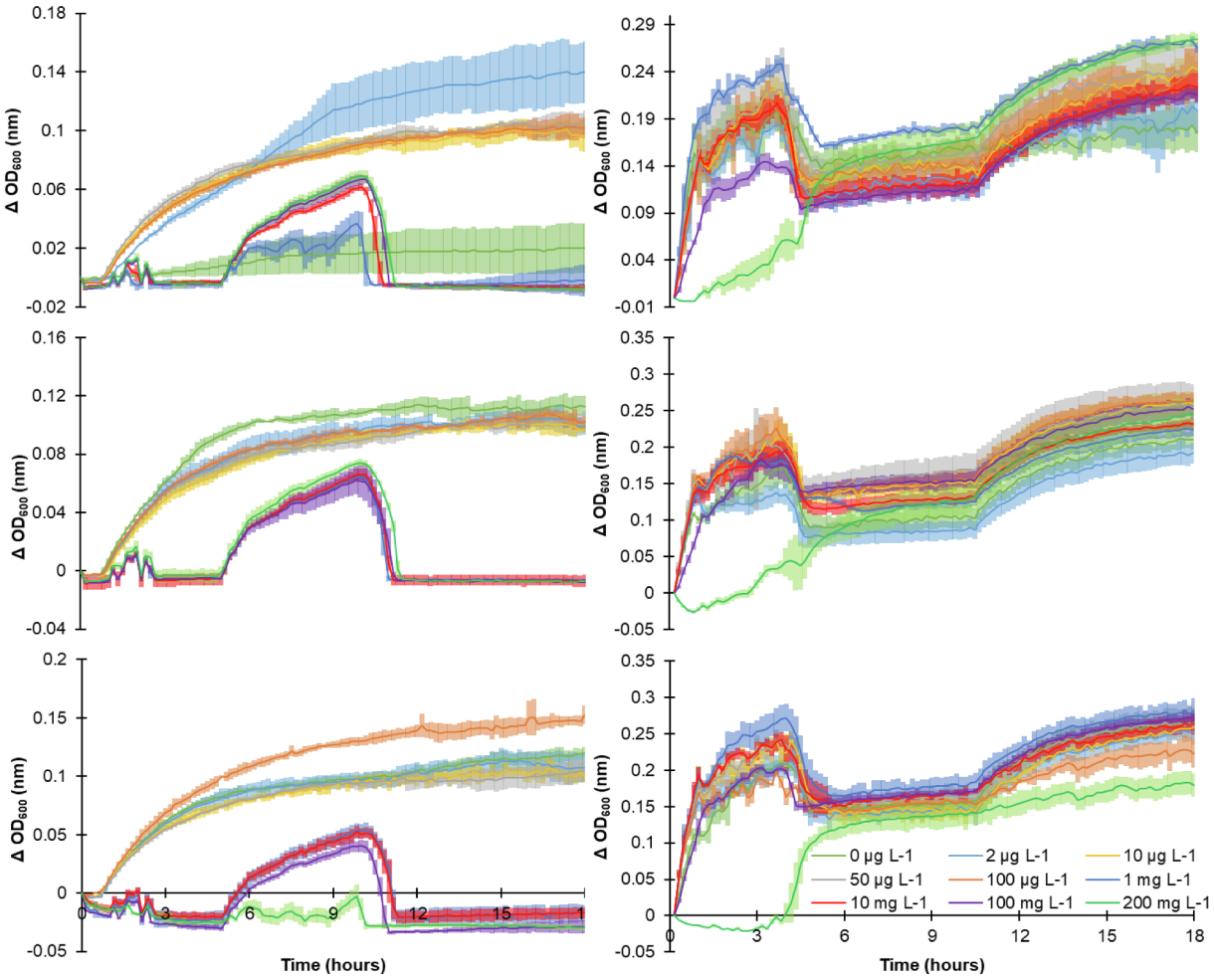


Fig. 4. OD_{600} showing the growth of each of the isolates in minimal media and untreated water, with concentrations of 0 µg/L, 2 µg/L, 10 µg/L, 50 µ g/L, 100 µg/L,1 mg/L, 10 mg/L, 100 mg/L, 200 mg/L. 1. A. GAC_S9 MSM B. GAC_S9 Untreated water, C. GAC_S3 MSM D. GAC_S3 Untreated water, E. Soil_S1 MSM F. Soil_S1 Untreated water. Data obtained every 10 min with measurements represented as averages of 5 replicates ± standard deviation.

This study supports the efficacy of biological processes for AMPA treatment using acclimatized strains isolated from real WTW and soil. Similar approaches have effectively treated pollutants like linuron, pesticide mixtures, and 4-nitrophenol (Horemans et al., 2014; Kim Tiam et al., 2016). A biofilm developed in high galactose concentrations (300 mg/L) maintained degradation below a substrate concentration of 0.3 mg/L for nearly 200 days, indicating that adaptation to oligotrophic conditions and nutrient presence can sustain biofilm activity (Rittmann and Brunner, 1984). However, acclimatization at high concentrations may not always adapt populations to low-level compound removal, potentially reducing degradation efficiency (Gözdereliler et al., 2013), something especially apparent for AMPA which has reported cytotoxicity even at relatively low concentrations of 50 µg/L (Fig. 3). Glyphosate inhibits the shikimate pathway essential for aromatic amino acid synthesis in plants and some microorganisms, while its breakdown product AMPA, though less toxic, can still disrupt microbial communities by altering soil chemistry and microbial competition. To promote success in future studies a long isolation/acclimatization period is recommended for AMPA, similar to findings for other pollutants such as nitrophenol (Spain and Van Veld, 1983). Further research should aim to optimize acclimatization times, particularly when dealing with previously exposed populations that might reactivate their enzyme activity upon encountering higher concentrations of recalcitrant substrates (Wingender and Jaeger, 2003). Previous results from continuous large scale processes suggest that this can be quickly achieved. For example, full-scale slow sand filters rapidly initiate metaldehyde degradation once concentrations surpassed 0.2 µg L^{-1}, indicating the potential for a rapid start-up based on early detection of elevated pesticide concentrations (Rolph et al., 2019).

Pilot scale AMPA removal

To further explore the practical applications of these findings, we conducted a pilot-scale experiment to assess the efficacy of AMPA removal under more realistic conditions. Throughout the duration of the study at pilot scale, there was some fluctuation in the temperature of the water, with the influent being slightly cooler than the effluent (15.3–19.7 °C vs. 18.1–20.1 °C, respectively). Turbidity for the influent (0.28–1.65 NTU) and effluent (0.174–1.13 NTU) varied, but never exceeded The World Health Organization (WHO) turbidity recommendation > 5 NTU, and only 8 % of the time > 1 NTU (Table 4). The changes and differences in influent and effluent suggest that the different bioaugmentation events and water recycling did not impact or compromise the efficiency of the filters at removing turbidity (Huisman and Wood, 1974). The influent and effluent data indicate that bioaugmentation events and water recycling did not compromise the filters' efficiency in removing turbidity (Huisman and Wood, 1974). No AMPA was detected in the source water, suggesting that AMPA dosing into the columns was likely the bacteria's first exposure, hence they lacked the necessary enzymes for biodegradation (Rolph et al., 2019). During the pilot scale experiment, average AMPA removals were calculated weekly and for the entire study duration across different sand filters (Table 5).

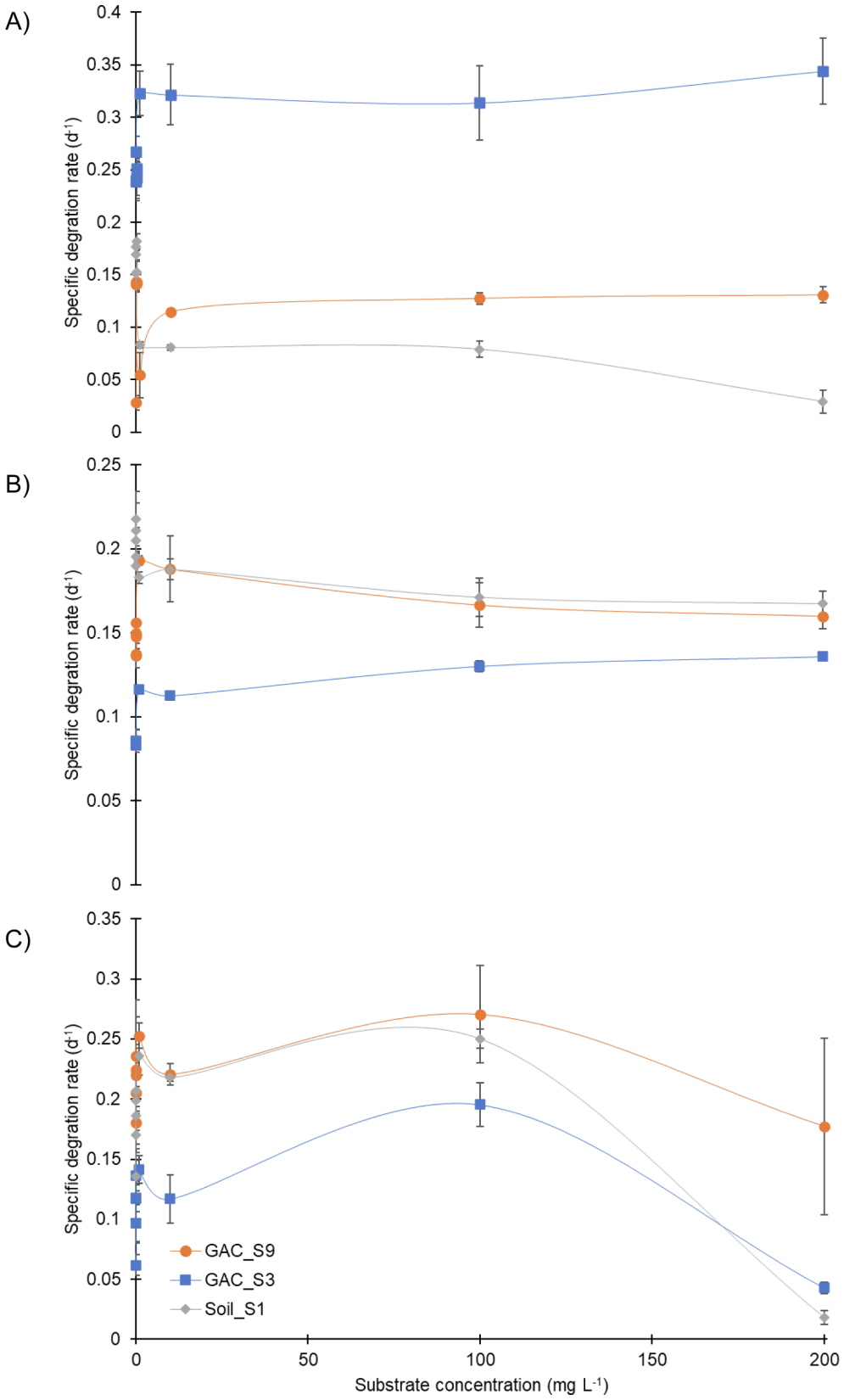


Fig. 5. Specific degradation rate per over different substrate concentrations for each isolate and substrate media used. A) AMPA supplemented MSM, B) LB, C) Raw Untreated water.

Table 4
Water paraments for both influent and effluent of each of the filters.

	Effluent				Influent			
Filter	**Control**	**1X**	**2X**	**Recycle**	**Control**	**1X Inlet**	**2X**	**Recycle**
Temp. (° C)	18.4–20.1	18.1–19.9	18.3–20.0	18.4–19.8	15.3–19.7	15.7–19.6.	16.1–18.9	15.6–19.6
pH	7.67–8.16	7.91–8.23	8.05–8.78	7.99–8.64	8.03–8.64	8.12–8.77	8.11–8.59	8.03–8.67
Redox (mV)	195.6—548.1	204.1–548.1	178.9–497.7	167.5–531.2	176.6–571.3	189.7–588.6	134.7–601.2	145.4–597.1
UV_{254} (abs)	0.011–0.031	0.013–0.041	0.018—0.047	0.014–0.053	0.021—0.064	0.018–0.074	0.022–0.077	0.27–0.063
Turbidity (NTU)	0.134–1.13	0.127–0.764	0.241–0.846	0.174—1.13	0.348–1.65	0.297–0.995	0.311–1.21	0.278–1.34
NO_3 (mg/L)	0.2–0.8	0.1–0.7	0.2–0.7	0.3–0.9	0.4–1.1	0.5–1.2	0.5–1.2	0.4–1.3
NH_4 (mg/L)	< 0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
DOC (mg/L)	3.28–4.16	2.98–3.77	3.13–3.97	2.79–4.11	3.54–4.91	3.61–4.81	3.22–5.15	3.44–5.27

Table 5
Average removal of Aminomethyl(phosphonic) acid, (AMPA) over the duration of the study in the different sand filters ± Standard Deviation.

Filter	Control	1X	2X	Recycle
Average removal (%)	4.7 ± 9.9	13.3 ± 13.9	14.7 ± 8.5	−10.1 ± 15.4
Week 1 (%)	10.6 ± 13.3	0.4 ± 9.9	–	–
Week 2 (%)	3.3 ± 13.2	19.7 ± 3.9	14.2 ± 12.1	0.1 ± 12.7
Week 3 (%)	1.2 ± 5.3	12.5 ± 6.5	17.4 ± 3.7	−3.2 ± 13.4
Week 4 (%)	3.6 ± 4.8	25.9 ± 16.2	13.9 ± 7.4	−20 ± 11.2

These averages illustrate each filtration system's efficiency in removing AMPA from the four pilot columns over four weeks, comparing different treatment intensities: Control, 1X, 2X, and Recycle. The control filter, without AMPA-removing bacteria, served as a baseline to measure removal due to physical effects alone. As expected, minimal removal was observed, with an average efficiency of 4.7 ± 9.9 % over four weeks. Weekly efficiency fluctuated, peaking at 10.6 ± 13.3 % in Week 1, decreasing to 1.2 ± 5.3 % in Week 3, and slightly increasing to 3.6 ± 4.8 % in Week 4. The initial high removal could be due to AMPA degraders weakly adhering to sand particles, while the subsequent decrease in performance may result from washout, allowing more AMPA to pass through (Samuelsen et al., 2017).

A single dose of bacteria (1X) represented the standard treatment level. The overall average removal efficiency was significantly higher at 13.3 ± 13.9 % compared to the Control (4.7 ± 9.9 %), suggesting that the treatment was effective and that the removal of AMPA from the water can be directly attributed to biological action. The weekly removal rates varied, with the lowest rate in Week 1 at 0.4 ± 9.9 % and the highest in Week 4 at 25.9 ± 16.2 %, indicating an overall increasing trend in removal efficiency over time (Figure S3). To observe the effects of bacteria concentration on removal, the third column received a double dose of bacteria (2X), resulting in a slightly higher average removal efficiency (14.7 ± 8.5 %) compared to the 1X treatment (13.3 ± 13.9 %), with the peak removal of 17.4 ± 8.5 % in Week 3. This suggests that while increasing treatment intensity can enhance removal efficiency, it was inconsistent. The fourth column, which involved recycling the medium, showed a negative average removal rate of −10.1 ± 15.4 %, indicating an overall increase in AMPA concentration. This suggests reintroduction of AMPA or hydraulic washout of bacteria (Herrero and Stuckey, 2015).

The cumulative removal of AMPA (μg) over a 25-day period was determined. The control group, without bacterial treatment, showed a minimal and consistent increase in cumulative AMPA removal, reaching about 50 μg by day 25, indicating baseline removal due to physical and possibly biological processes. The 1X treatment, with a single bacterial dose, exhibited a significantly higher cumulative removal. Initially similar to the Control, a marked increase began around day 10, peaking at approximately 250 μg by day 25, demonstrating effective bacterial enhancement of AMPA degradation over time. The 2X treatment, also followed the initial trend of the Control and 1X treatments. However, its removal rate accelerated around day 10, reaching a cumulative removal of about 200 μg by day 25 (Fig. 6).

The 1X treatment showed the most consistent improvement in removal efficiency, peaking in the last week (Table 2). However, efficiency remains below regulatory compliance, highlighting AMPA's stability and resistance. Challenges like competitive pressures in real water likely influenced degradation rates (Jones et al., 2009). These findings show bioaugmentation can enhance AMPA removal in slow sand filters, offering a sustainable and cost-effective alternative to conventional methods, improving drinking water quality, and meeting regulations. Microbial dynamics in sand filters are key to AMPA degradation. Future research should refine dosing schedules, explore different bacterial strains, and test different conditions to improve performance. Studies on immobilisation have also demonstrated a performance benefit for other recalcitrate pesticide residues (Horemans et al., 2017). Long-term studies on the stability of bioaugmented systems would help biological informed engineering efforts (Bouchez et al., 2000). Effective AMPA removal benefits public health and the environment by ensuring safer water supplies. Advanced analytical methods like LC-MS/MS are critical for accurate detection and quantification of AMPA (Jamin et al., 2014).

Exploring the reusability of microbial strains in bioremediation aids in understanding their long-term effectiveness for drinking water treatment and catchment management. Future studies incorporating batch reuse experiments within continuous flow systems will help assess how well these strains maintain their degradation efficiency over time. Further analysis of their genetic stability under repeated use conditions will provide insights into potential genetic changes that could affect their biodegradation capabilities, ensuring their safe application in bioremediation (Hassard et al., 2024). This includes examining how specific microbial species or consortia contribute to the degradation process and how they interact under varying environmental conditions expected within drinking water treatment processes. Further examination of the genetic stability of these strains under repeated use is recommended. Such studies could reveal genetic adaptations that might affect their biodegradation capabilities, particularly relevant when these strains are used in drinking water systems (e.g., treatment and catchment management scenarios) where public health cannot be compromised. To mitigate potential risks to both public health and local ecosystems, implementing verified disinfection processes and conducting regular environmental monitoring are important. These measures will help maintain the effectiveness and sustainability and realise the benefits bioremediation technologies in water treatment applications, ensuring both public safety and environmental integrity. Future studies should focus on identifying and characterizing these enzymes and genetic markers to provide a clearer understanding of the biodegradation mechanisms at a molecular level.

Conclusion

At the outset of this experiment three aims were stipulated with

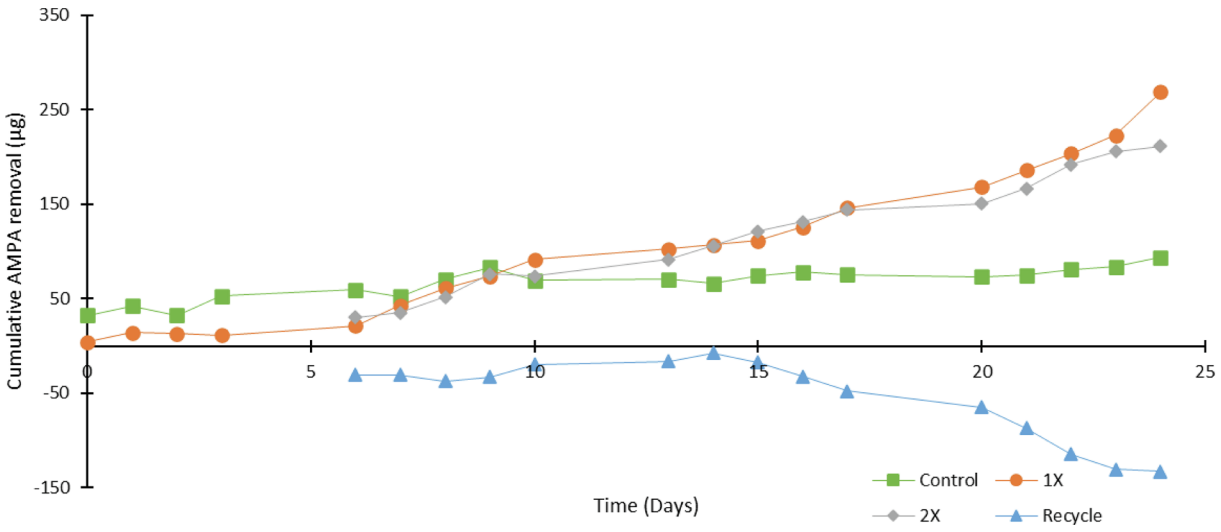


Fig. 6. Cumulative AMPA removal (g) over the duration of the pilot scale study.

regards to the biodegradation of the pollutant AMAP. The first aim was that it was possible to successfully isolate AMPA-degrading bacteria from various environments known to be exposed to AMPA. This was proven correct as within this study, three strains and consortiums of AMPA degrading bacteria were successfully identified, two from GAC biofiltration units of differing ages and one from soil taken from an allotment with known glyphosate usage.

The second aim was that identifying these isolated bacteria could enhance our understanding of enzyme pathways for degradation processes. The isolated consortiums of bacteria have shown that they are able to grow and use AMPA as a carbon source across a variety of different concentrations, ranging from 0.1 µg/L to 200 mg/L.

The final aim was that an examination of growth patterns in response to varying AMPA concentrations will increase understanding of the relationship between AMPA concentration and the effect on growth of the bacterial strains. The growth kinetics study showed an optimum concentration for growth, where concentrations above or below this threshold negatively affected growth, and hence performance, of the bacteria.

This work highlights the potential of using bioaugmentation in slow sand filters as a sustainable and cost-effective method for reducing AMPA levels in drinking water. While regulatory compliance was not achieved in this initial study, the findings lay a strong foundation for future research aimed at optimizing and developing biologically-based water treatment methods for recalcitrant micropollutants like AMPA. Implementing these biological treatments could significantly improve water quality, aligning with regulatory requirements and safeguarding public health.

This research has shows that bacterial degradation can be utilised for the removal of AMPA from drinking water. However, the full industrial application has not yet been fully explored, therefore this study has the potential to lead on to further research with the strains and consortium that have been identified.

Data access

Data associated with this manuscript is available on Figshare at 10.6084/m9.figshare.26885539.

CRediT authorship contribution statement

Laura Pickering: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Miles Folkes:** Formal analysis. **Barrie Holden:** Resources, Writing – review & editing. **Peter Jarvis:** Supervision, Writing – review & editing. **Pablo Campo:** . **Francis Hassard:** Conceptualization, Supervision, Methodology, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition.

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.

Data availability

data shared through link

Acknowledgements

The authors gratefully acknowledge financial support from the Engineering and Physical Sciences Research Council (ESPRC), United Kingdom through their funding of a Doctoral Training Allocation Award to LP (EP/R513027/1) and from the project sponsors UKWIR Ltd. The authors acknowledge United Utilities for providing samples and site access.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crbiot.2024.100248.

References

APHA-AWWA-WEF, 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. Washington.
Arora, P.K., 2020. Bacilli-mediated degradation of xenobiotic compounds and heavy metals. Front. Bioeng. Biotechnol. 8, 1–28.
Balthazor, T.M., Hallas, L.E., 1986. Glyphosate-degrading microorganisms from industrial activated sludge. Appl. Environ. Microbiol. 51, 432–434.
Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.
Benner, J., Helbling, D.E., Kohler, H.P.E., Wittebol, J., Kaiser, E., Prasse, C., Ternes, T.A., Albers, C.N., Aamand, J., Horemans, B., Springael, D., 2013. Is biological treatment a viable alternative for micropollutant removal in drinking water treatment processes? Water Res. 47, 5955–5976.
Calabrese, E.J., Blain, R.B., 2011. The hormesis database: the occurrence of hormetic dose responses in the toxicological literature. Regul. Toxicol. Pharm. 61, 73–81.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Cases, I., de Lorenzo, V., 2005. Genetically modified organisms for the environment: stories of success and failure and what we have learned from them. Int. Microbiol. 8, 213–222.

Castro-Gutiérrez, V., Pickering, L., Cambronero-Heinrichs, J., Holden, B., Haley, J., Jarvis, P., Jefferson, B., Helgason, T., Moir, J., Hassard, F., 2022. Bioaugmentation of pilot-scale slow sand filters can achieve compliant levels for the micropollutant metaldehyde in a real water matrix. Water Res. 211, 1–12.

Dick, R.E., Quinn, J.P., 1995. Control of glyphosate uptake and metabolism in pseudomonas sp. 4ASW. FEMS Microbiol. Lett. 134, 177–182.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Fauvelle, V., Montero, N., Mueller, J.F., Banks, A., Mazzella, N., Kaserzon, S.L., 2017. Glyphosate and AMPA passive sampling in freshwater using a microporous polyethylene diffusion sampler. Chemosphere 188, 241–248.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution (n y) 39, 783–791.

Flemming, H.C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–633.

Gilliom, R.J., Barbash, J.E., Crawford, C.G., Hamilton, P.A., Martin, J.D., Nakagaki, N., Nowell, L.H., Scott, J.C., Stackelberg, P.E., Thelin, G.P., Wolock, D.M., 1992. The quality of our nation's waters—Pesticides in the nation's streams and ground water, 1992-2001.

Gözdereliler, E., Boon, N., Aamand, J., De Roy, K., Granitsiotis, M.S., Albrechtsen, H.J., Sørensen, S.R., 2013. Comparing metabolic functionalities, community structures, and dynamics of herbicide-degrading communities cultivated with different substrate concentrations. Appl. Environ. Microbiol. 79, 367–375.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075.

Han, J., Zhang, X., Jiang, J., Li, W., 2021. How much of the total organic halogen and developmental toxicity of chlorinated drinking water might be attributed to aromatic halogenated DBPs? Environ. Sci. Tech. 55, 5906–5916.

Hassard, F., Curtis, T.P., Dotro, G.C., Golyshin, P., Gutierrez, T., Heaven, S., Horsfall, L., Jefferson, B., Jones, D.L., Krasnogor, N., Kumar, V., 2024. Scaling-up engineering biology for enhanced environmental solutions. ACS Synth. Biol. 13 (6), 1586–1588.

Hopman R., Beerendonk E. F., Puijker L. M., 1995. Glyphosate and AMPA in the production of drinking water.

Horemans, B., Hofkens, J., Smolders, E., Springael, D., 2014. Biofilm formation of a bacterial consortium on linuron at micropollutant concentrations in continuous flow chambers and the impact of dissolved organic matter. FEMS Microbiol. Ecol. 88, 184–194.

Jamin, E.L., Bonvallot, N., Tremblay-Franco, M., Cravedi, J.P., Chevrier, C., Cordier, S., Debrauwer, L., 2014. Untargeted profiling of pesticide metabolites by LC-HRMS: an exposomics tool for human exposure evaluation. Anal Bioanal Chem 406, 1149–1161. https://doi.org/10.1007/s00216-013-7136-2.

Jukes, T.H., Cantor, C.R., 1969. Evolution of Protein Molecules. In: Munro, H.N. (Ed.), Mammalian Protein Metabolism. Academic Press, pp. 21–132.

Kepler, R.M., Epp Schmidt, D.J., Yarwood, S.A., Cavigelli, M.A., Reddy, K.N., Duke, S.O., Bradley, C.A., Williams, M.M., Maula, J.E., 2020. Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate. Appl. Environ. Microbiol. 86, 1–16.

Kim Tiam, S., Fauvelle, V., Morin, S., Mazzella, N., 2016. Improving toxicity assessment of pesticide mixtures: the use of polar passive sampling devices extracts in microalgae toxicity tests. Front. Microbiol. 7, 1388.

Kuhn, D.A., Starr, M.P., 1960. *Arthrobacter atrocyaneus*, n. sp., and its blue pigment. Archiv Fur Mikrobiologie 36, 175–181.

Lerbs, W., Stock, M., Parthier, B., 1990. Physiological aspects of glyphosate degradation in *Alcaligenes spec*. strain GL. Arch. Microbiol. 153, 146–150.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 1–21.

Lutri, V.F., Matteoda, E., Blarasin, M., Aparicio, V., Giacobone, D., Maldonado, L., Becher Quinodoz, F., Cabrera, A., Giuliano Albo, J., 2020. Hydrogeological features affecting spatial distribution of glyphosate and AMPA in groundwater and surface water in an agroecosystem. Córdoba. Argentina. Science of the Total Environment 711, 1–12.

Mesnage, R., Defarge, N., Spiroux de Vendômois, J., Séralini, G.E., 2015. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem. Toxicol. 84, 133–153.

Ortiz-Hernández, L., Snchez-Salinas, E., Dantn Gonzlez, E., Luisa, M., 2013. Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process. Biodegradation - Life of Science. Intech 251–287.

Pipke, R., Amrhein, N., 1988. Degradation of the phosphonate herbicide glyphosate by *Arthrobacter atrocyaneus* ATCC 13752. Appl. Environ. Microbiol. 54, 1293–1296.

Ren, H., Tröger, R., Ahrens, L., Wiberg, K., Yin, D., 2020. Screening of organic micropollutants in raw and drinking water in the Yangtze River Delta, China. Environ. Sci. Eur. 32, 1–12.

Rittmann, B.E., Brunner, C.W., 1984. The nonsteady-state-biofilm process for advanced organics removal. Journal (water Pollution Control Federation) 56, 874–880.

Rolph, C.A., Jefferson, B., Hassard, F., Villa, R., 2018. Metaldehyde removal from drinking water by adsorption onto filtration media: mechanisms and optimisation. Environ. Sci. Water Res. Technol. 4, 1543–1552.

Rolph, C.A., Villa, R., Jefferson, B., Brookes, A., Choya, A., Iceton, G., Hassard, F., 2019. From full-scale biofilters to bioreactors: engineering biological metaldehyde removal. Sci. Total Environ. 685, 410–418.

Rueppel, M., Brightwell, B., Schaefer, J., Marvel, J., 1977. Metabolism and degradation of glyphosate in soil and water. J. Agric. Food Chem. 23, 517–528.

Sasi, P.C., Alinezhad, A., Yao, B., Kubátová, A., Golovko, S.A., Golovko, M.Y., Xiao, F., 2021. Effect of granular activated carbon and other porous materials on thermal decomposition of per- and polyfluoroalkyl substances: mechanisms and implications for water purification. Water Res. 200, 1–10.

Scott, C., Pandey, G., Hartley, C.J., Jackson, C.J., Cheesman, M.J., Taylor, M.C., Pandey, R., Jeevan, Khurana, L., Teese, M., Coppin, C.W., Kahli, Weir, M., Rakesh, Jain, K., Lal, R., Russell, R.J., Oakeshott, J.G., 2008. The enzymatic basis for pesticide bioremediation. Indian J. Microbiol. 48, 65–79.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069.

Singh, R.L., Singh, P.K., Singh, R.P., 2015. Enzymatic decolorization and degradation of azo dyes–A review. Int. Biodeter. Biodegr. 104, 21–31.

Spain, J.C., Van Veld, P.A., 1983. Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure time, inoculum, and chemical structure. Appl. Environ. Microbiol. 45, 428–435.

Stosiek, C., Scholz, G., Schroeder, S.L.M., Kemnitz, E., 2010. Structure and properties of noncrystalline aluminum oxide-hydroxide fluorides. Chem. Mater. 22, 2347–2356.

Sviridov, A.V., Shushkova, T.V., Zelenkova, N.F., Vinokurova, N.G., Morgunov, I.G., Ermakova, I.T., Leontievsky, A.A., 2012. Distribution of glyphosate and methylphosphonate catabolism systems in soil bacteria *Ochrobactrum anthropi* and *Achromobacter* sp. Appl. Microbiol. Biotechnol. 93, 787–796.

Sviridov, A.V., Shushkova, T.V., Ermakova, I.T., Ivanova, E.V., Epiktetov, D.O., Leontievsky, A.A., 2015. Microbial degradation of glyphosate herbicides (review). Appl. Biochem. Microbiol. 51, 188–195.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis. Mol. Biol. Evol. 30, 2725–2729.

Thomas, J.C., Helgason, T., Sinclair, C.J., Moir, J.W.B., 2017. Isolation and characterization of metaldehyde-degrading bacteria from domestic soils. J. Microbial. Biotechnol. 10, 1824–1829.

Vishniac, W., Santer, M., 1957. The Thiobacilli. Bacteriological Reviews 21, 195–213.

Wingender, J., Jaeger, K.E., 2003. Extracellular enzymes in biofilms. Encyclopedia of environmental microbiology.

Yao, Y., Lu, Z., Min, H., Gao, H., Zhu, F., 2012. The effect of tetrahydrofuran on the enzymatic activity and microbial community in activated sludge from a sequencing batch reactor. Ecotoxicology 21, 56–65.

Yuan, J., Fox, F., Crowe, G., Mortazavian, S., Passeport, E., Hofmann, R., 2022. Is in-service granular activated carbon biologically active? An evaluation of alternative experimental methods to distinguish adsorption and biodegradation in GAC. Environ. Sci. Tech. 56, 16125–16133.

Zhan, H., Feng, Y., Fan, X., Chen, S., 2018. Recent advances in glyphosate biodegradation. Appl. Microbiol. Biotechnol. 102, 5033–5043.



https://dspace.org/

Biodegradation of (Aminomethyl)phosphonic acid (AMPA) by isolated microbial consortia extracted from biological filters at drinking water treatment plants

Pickering, Laura; Folkes, Miles; Holden, Barrie; Jarvis, Peter; Campo Moreno, Pablo ...

2024
Elsevier
https://doi.org/10.1016/j.crbiot.2024.100248
Pickering L, Folkes M, Holden B, et al., (2024) Biodegradation of (Aminomethyl)phosphonic acid (AMPA) by isolated microbial consortia extracted from biological filters at drinking water treatment plants. Current Research in Biotechnology, Volume 8, 2024, Article number 100248

Downloaded from a DSpace repository. https://dspace.org/