

From full-scale biofilters to bioreactors: engineering biological metaldehyde removal

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Abstract

Polar, low molecular weight pesticides such as metaldehyde are challenging and costly to remove from drinking water using conventional treatment methods. Although biological treatments can be effective at treating micropollutants, through biodegradation and sorption processes, only some operational biofilters have shown the ability to remove metaldehyde. As sorption plays a minor role for such polar organic micropollutants, biodegradation is therefore likely to be the main removal pathway. Here, the biodegradation of metaldehyde was monitored, and assessed, in an operational slow sand filter. Long-term data showed that metaldehyde degradation improved when inlet concentrations increased. A comparison of inactive and active sand batch reactors showed that metaldehyde removal happened mainly through biodegradation and that the removal rates were greater after the biofilm was acclimated through exposure to high metaldehyde concentrations. This suggested that metaldehyde removal was reliant on enrichment and that the process could be engineered to decrease treatment times (from days to hours). Through-flow experiments using fluidised bed reactors, showed the same behaviour following metaldehyde acclimation. A 40% increase in metaldehyde removal was observed in acclimated compared with non-acclimated columns. This increase was sustained for more than 40 days, achieving an average of 80% removal and compliance ($< 0.1 \mu\text{L}^{-1}$) for more than 20 days. An initial microbial analysis of the acclimated and non-acclimated biofilm from the same filter materials, showed that the microbial community in acclimated sand was significantly different. This work presents a novel conceptual template for a faster, chemical free, low cost, biological treatment of metaldehyde and other polar pollutants in drinking water. In addition, this is the first study to report kinetics of metaldehyde degradation in an active microbial biofilm at a WTW.

Keywords: metaldehyde, micropollutant removal, acclimation, slow-sand filter, fluidised-bed reactor.

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1. Introduction

In Europe, water treatment works (WTW) are required to meet the European Drinking Water Directive (DWD) standard of $0.1 \mu\text{g L}^{-1}$ for individual pesticides (Council Directive 98/83/EC). These limits include metaldehyde, a widely used molluscicide first identified in drinking water in 2008 (Water UK, 2008). Despite accounting for just 1-2% of all pesticides applied in the UK (FERA, 2013), metaldehyde was responsible for over 90% of the total DWD failures for pesticides in England in 2014 and 2015 (DWI, 2017). Metaldehyde is uncharged, highly soluble and has a low molecular weight, which make it highly mobile in the environment. Although it can be removed from abstracted water using granular activated carbon (GAC), powdered activated carbon (PAC) dosing and advanced oxidation processes (AOPs) (Autin et al., 2013), the binding sites saturation of GAC/PAC for metaldehyde is relatively fast, and hence, these processes are very costly to maintain. On the other hand, biological processes, such as sand filtration, has proven to be an effective treatment for the removal of many micropollutants in drinking water, with reduced regeneration and chemical dosing costs and minimal treatment-by-product formation compared to GAC, PAC and AOPs (Benner et al., 2013; Zearley and Summers, 2012; Hedegaard et al., 2014, Reungoat et al., 2011). Micropollutant removal on slow sand filters (SSF), typically occurs through a combination of biosorption/adsorption onto biofilms growing on the biofilter or directly on the supporting medium and biodegradation by microbial community associated with the biofilm (Hedegaard and Albrechtsen, 2013; Zearley and Summers, 2012). Biodegradation, in particular, occurs for most micropollutants via secondary substrate utilisation or co-metabolism in the presence of primary substrates, often part of the natural organic material (NOM) which are typically present at much higher concentrations (Zearley and Summers, 2012; Ho et al., 2007). However, secondary substrate utilisation can lead to the formation of intermediate products, which might trigger higher toxicity for the degrading microorganisms than the parent compounds and, therefore, limit growth and removal rates (Benner et al., 2013). This is not the case for metaldehyde, whose degradation is energetically favourable and for which the only detectable transformation product is acetaldehyde. Acetaldehyde is a precursor of acetyl-coA that is central to many biochemical pathways, and therefore, is likely to degrade quickly (Bieri, 2003). These characteristics suggest that, after the initial degradation step, achieved only by few microorganisms, metaldehyde can act as a suitable carbon source for supporting microbial growth even at low concentrations (Thomas et al. 2017). SSF biofilms have proven to be able to support slow growing micropollutants'

degraders, which often requires an acclimation phase. Acclimation at high concentrations of the micropollutant stimulate the growth of specialist microorganisms whilst maintaining biofilm function as a treatment process (Vignola et al. 2018). Microbial acclimation within the sand biofilm can be achieved through different mechanisms including selective enrichment, enzymatic metabolic regulation or genetic changes (Stoodley et al. 2002). In the first case, the process usually occurs under periods of carbon scarcity where copiotrophs can survive in a dormant state and increase their uptake of specific micropollutants in periods of high concentration (Wingender and Jaeger 2002). In the second case, the synthesis or activation of specific degradative enzymes responsible for micropollutant removal is regulated by environmental conditions. For example, higher concentrations of the micropollutant, absence of protozoa or inhibitors. In the case of genetic changes, such as mutation, duplication and recombination, the changes in the community are permanent and might not be reproducible (McCarty and Rittmann, 2018). Improved degradation rates of organic pollutants have been reported after acclimation through selective enrichment (Ayra et al. 2016). Buitron et al. (1998) reported an increase of one to two orders of magnitude in the degradation rate of chlorophenol in acclimated activated sludge, due to selection and multiplication of specialised degraders (Wiggins et al., 1988). Understanding the biodegradation kinetics in SSF is essential for improving process resilience and enhancing removal efficiencies of hard-to-treat micropollutants, including metaldehyde. If the benefits of biological SSF could be captured and optimised into more efficient and low-cost technologies, emerging contaminants such as metaldehyde could be treated using existing water treatment assets (Kay and Grayson, 2014; Rolph et al. 2014). Several authors have indicated the importance of operational parameters in micropollutant removal, such as media composition and contact time (Zhang et al. 2017, Zuehlke et al. 2007). In addition, Zhang et al. (2018) highlighted that operational parameters significantly affect the structure of microbial communities in filter media and suggested that water utilities would be able to enhance acclimation and micropollutant removal through the optimisation of the filter's operational parameters. For this reason, we analysed data (7 years) from one of the operational SSF identified by the water company as able to remove metaldehyde from drinking water. This analysis aimed at confirming and explaining the ability of the biofilter to remove metaldehyde. To address this aim, an analysis of the kinetics of metaldehyde degradation was performed using batch bench-scale experiments. This is a novel angle to acclimation as batch studies are not commonly done for biofilters. Finally, the batch acclimation process was reproduced in an up-flow continuous reactor, confirming the

potential for using acclimation in a bioreactor. To our knowledge, this is the first study to report the biodegradation potential and kinetics of metaldehyde in an active microbial biofilm at a WTW.

2. Materials and Methods

2.1. Sampling site and sand treatment

A drinking WTW known to remove metaldehyde was selected for this study. The WTW, situated in the east of England within the Anglian Water region, had a typical flowrate of 10,000 m³.d⁻¹. The flow rate to the SSF was dependent on abstraction rates and was not controlled as part of this study. The typical SSF hydraulic loading rate was 0.1 m/h which equated to an empty bed contact time (EBCT) of 10 hours. The inlet to the WTW was from two reservoirs, the water was treated using rapid gravity filtration in GAC filters followed by SSF. The water was then aerated, chlorinated, and ammonium sulphate and orthophosphoric acid were added prior to distribution. Water samples were collected weekly from 2008–2014 for this WTW, with an intensive sampling campaign undertaken from January 2014–February 2015 (twice a week), as part of this study, to assess metaldehyde biodegradation. In the latter period, partially treated surface water and biologically active sand were sampled for the laboratory work. The sand was collected onsite using a seeding tool to scrape the top 1-2 cm from the filter. Four to five different areas of the filter were sampled where safe to do so without disrupting the filter operation. The samples were then homogenised and used for the lab trials.

Sand with a visible biofilm was collected from the top 5 cm of the operational SFF using a seeding tool, as reported in Rolph et al. (2018). At the time of collection, the sand had not been exposed to sustained metaldehyde concentrations above 0.2 µg L⁻¹ for more than a year (n=48) and when used in these experiments it is referred to as ‘non-acclimated’ sand. Prior to use sand and water were stored in the dark at 4°C.

2.2. Chemicals

Metaldehyde was purchased from Fisher Scientific (Loughborough, UK). Metaldehyde-d16 was used as an internal standard and was purchased from QMX Laboratories (Essex, UK). HPLC grade Acetone, Methanol and Dichloromethane were purchased from Rathburn Chemicals (Walkerburn, UK). Stock solutions of metaldehyde was made by dissolving 10 mg of metaldehyde per litre of UPW, this was stirred at 30°C overnight to provide appropriate

conditions for solubility and stability of metaldehyde in solution. A stock metaldehyde-d16 solution was made by dissolving 20 mg of metaldehyde-d16 in 40 mL of methanol. The metaldehyde concentrations of stock solutions were assessed prior to use.

2.3. Metaldehyde analysis

Metaldehyde analysis for water with concentrations above $3 \mu\text{g L}^{-1}$ was performed using liquid chromatography mass spectrometry (LC-MS-MS) as reported in Ramos *et al.* (2018). Analytical standards of metaldehyde which were used as calibration and blanks were run with samples with a typical range of $0\text{-}10 \mu\text{g L}^{-1}$. New calibration curves were generated prior to each sequence, and concentrations were determined using Micromass QuantLynx. The detection limit of the method was $0.3 \mu\text{g L}^{-1}$ with a relative standard deviation $<20\%$ between technical replicates were taken forward for data analysis. A combination of solid phase extraction (SPE) followed by gas chromatography mass spectrometry (GC-MS) was used to quantify metaldehyde at concentrations $<3 \mu\text{g L}^{-1}$, as reported in Rolph *et al.* (2018). The detection limit for the GC-MS + SPE method was $0.05 \mu\text{g L}^{-1}$. Extraction efficiency was assessed through comparison between the observed and expected concentration of metaldehyde-d16. The response values for metaldehyde were corrected based on the metaldehyde-d16 extraction efficiency and was therefore presented as corrected values.

2.4. Metaldehyde Kinetic Experiments

2.4.1. Batch experiments with non-acclimated sand

Assimilable organic carbon (AOC) free glassware was prepared according to the method described in APHA-AWWA-WEF, (2012). To assess the sand sorption capability and the sorption role of the biofilm, control batch reactors, using clean sand and abiotic controls using sand with an inactive biofilm were run in parallel to the non-acclimated reactors. Sand was cleaned using an onsite mechanical cleaning system (sand control) whereas the native biofilm was inactivated by heating the sand at 105°C overnight (inactive sand abiotic control) (APHA-AWWA-WEF, 2012). Qualitative analysis by SEM was undertaken on subsamples using an environmental scanning electron microscope ESEM TMP (XL30, FEI/Phillips, UK) to confirm the presence or absence of a biofilm (Rolph *et al.* 2018). Batch experiments for active sand (non-acclimated), inactive sand (abiotic control) and clean sand (control) were undertaken in triplicate using each media and raw water which was spiked with known final metaldehyde concentrations of 0.5, 1, 2.5, 5, 10, 20, 30, 40 and $50 \mu\text{g L}^{-1}$. 100 g of each media was added to conical flasks with 300 mL of spiked raw water. The samples were

shaken at 150 RPM on a rotary shaker for 72 hours and samples were collected at set time points, filtered using 0.22 µm cellulose filters (Fisher Scientific, UK), refrigerated at 4 °C and analysed within 48 hours. Metaldehyde removal kinetics were calculated using the GC data. For the purposes of this study, the biodegradation of metaldehyde was assumed to follow a single substrate/enzyme Michaelis-Menten model (Cheyins et al., 2010).

$$V_0 = (V_{\max} \times S)/(K_m + S) \dots \dots \dots (1)$$

Where: V_0 is the initial velocity of the enzyme/substrate reaction, V_{\max} is the maximum enzymatic substrate degradation rate, (S) non-limiting substrate. The K_m is the Michaelis-Menten half saturation constant defined as the substrate concentration at half the V_{\max} . The Michaelis-Menten equation (1) was solved using a non-linear least squares method for kinetic parameter estimation (V_{\max} , K_m), whereas the ‘standard error of mean’ and the ‘significance of model fit’ were calculated using a Hessian matrix and t-test respectively (Hassard et al. 2018).

2.4.2. Batch experiments with acclimated sand

Acclimation with metaldehyde and generic carbon source. For acclimation experiments, ‘non acclimated’ sand (as described in 2.1) was incubated at 25 °C and shaken at 150 RPM for one week with 10 µg L⁻¹ of metaldehyde (acclimated sand) or with 25 mg·L⁻¹ of acetic acid (equivalent to 10 mg L⁻¹ of TOC – carbon spiked sand). The pH of these batch experiments was adjusted to its initial value if necessary. Acetic acid was used as an easily assimilable carbon source in order to assess if the increase in metaldehyde removal was a result of a general increase in biomass or an increase in substrate specific degraders. Average concentrations of total nitrogen and total phosphorous in the water were 2.6 (±1.3) mg L⁻¹ N and 0.2 (±0.085) mg L⁻¹ P and 4.6 mg L⁻¹ DOC. Therefore, carbon nitrogen and phosphorous ratios were adjusted to levels ideal for the growth of heterotrophic bacteria 100:10:1 (LeChevallier, 1991) in our case 75:10:1 (double of the existing carbon concentration and to levels reported for similar studies, Li et al., 2012). Following the acclimation period, water was removed by filtration (using 0.22 µm cellulose filters, Fisher Scientific) and the sand was mixed and distributed into clean conical flasks (50g) and 150 mL of metaldehyde solutions (0.5, 1, 2.5, 5, 10, 20 and 50 µg L⁻¹) were added to the sand. Samples were processed as in 2.4.1. The impact of the addition of a generic carbon source was also assessed using lower amount of acetic acid (10, 1 and 0.1 mg L⁻¹ equivalent to 4, 0.4 and 0.04 mg L⁻¹). Following a

week exposure, the sand was used for batch experiments and metaldehyde removal quantified using and initial concentration of $10 \mu\text{g L}^{-1}$ (data reported in supplementary information).

Acclimation with metaldehyde using different spiking concentrations. To assess the concentration of metaldehyde required to promote acclimation, further batch experiments were undertaken by exposing the sand to different spiking concentrations of metaldehyde (5, 10 and $50 \mu\text{g L}^{-1}$) and then exposed to environmental relevant metaldehyde concentrations (0.3 , 0.7 and $1 \mu\text{g L}^{-1}$) for 24 hours. All experiments were run in triplicates and repeated at least once. All equipment was cleaned with acetone after use to prevent metaldehyde contamination.

2.4.3. *Up-flow columns in through-flow with acclimated sand*

Acclimation experiments were undertaken using a 2.5cm x 50cm econo-columns (Bio-Rad Laboratories, Hemel Hempstead UK) filled with 5 cm of gravel and 200g of active sand which reached a non-fluidised height of 30 cm. This resulted in a media volume of $1.5 \times 10^{-4} \text{ m}^3$. For through-flow experiments, the column was fed with raw water from 10 L containers to a 300 mL recycle reservoir, which was twice the volume of the bed. This was allowed to stabilise for one week prior to the commencement of spiking experiments. Columns were covered to reduce algae growth. The raw water containers and all tubing (Tygon, Fisher scientific, UK) were sterilised frequently and the inlet water was changed at least weekly to prevent degradation of metaldehyde in the containers or tubing. Fluidisation was achieved at a rate of 10 L h^{-1} resulting in an EBCT of 0.8 minutes. ‘Non acclimated’ sand (as described in 2.1) was exposed to high concentrations of metaldehyde ($50 \mu\text{g L}^{-1}$, identified as the best concentration for acclimation) or acetic acid (25 mg L^{-1}) for five days and then removed and replaced with a spike of $0.5 \mu\text{g L}^{-1}$ to represent an environmentally relevant metaldehyde influent. The column was run with a contact times of 828 minutes. Samples were taken regularly and analysed by GC-MS for metaldehyde.

2.5. Statistical analysis

Statistical analyses were performed using Primer v7 with PERMANOVA+ add on as reported in Hassard et al. (2017).

3. Results and Discussion

3.1. Biodegradation of metaldehyde in full scale sand filters

Metaldehyde concentration in two surface reservoirs ranged between $< 0.05 \mu\text{g L}^{-1}$ and $2.1 \mu\text{g L}^{-1}$ between March 2008 and March 2015, with high peaks usually occurring between October and February (Figure 1), consistent with peaks application of slug pellets to farmland and high rainfalls (Kay and Grayson, 2014).

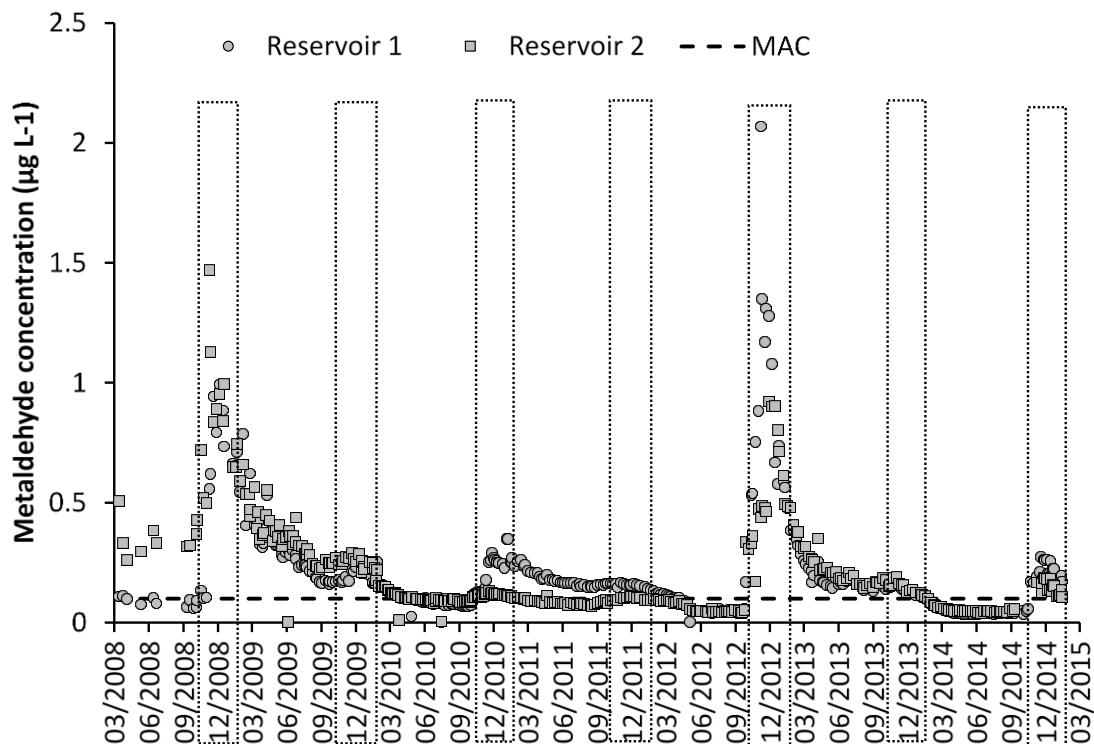


Figure 1 Metaldehyde concentrations from the reservoirs feeding the WTW (2008-2015).

Dry winters in the area produced smaller metaldehyde peaks (2011 and 2013). The SSF removed metaldehyde consistently between 2008 and 2015 (Figure 2A). During this period, the average metaldehyde concentration of the primary filtrate was $0.16 \mu\text{g L}^{-1}$, whilst the metaldehyde concentration in the SSF filtrate was $0.06 \mu\text{g L}^{-1}$, representing 63% removal (Figure 2A). From December 2012 to May 2013, the inlet metaldehyde rose to $0.4 \mu\text{g L}^{-1}$ which resulted in improved metaldehyde removal up to 93% across the SSF and a metaldehyde residual of $0.03 \mu\text{g L}^{-1}$. The SSF responded well to fluctuations in the source

water quality in terms of metaldehyde. The 50th percentile was 0.16 $\mu\text{g L}^{-1}$ at reservoir 1, which decreased to 0.06 $\mu\text{g L}^{-1}$ after the SSF (Figure 2B).

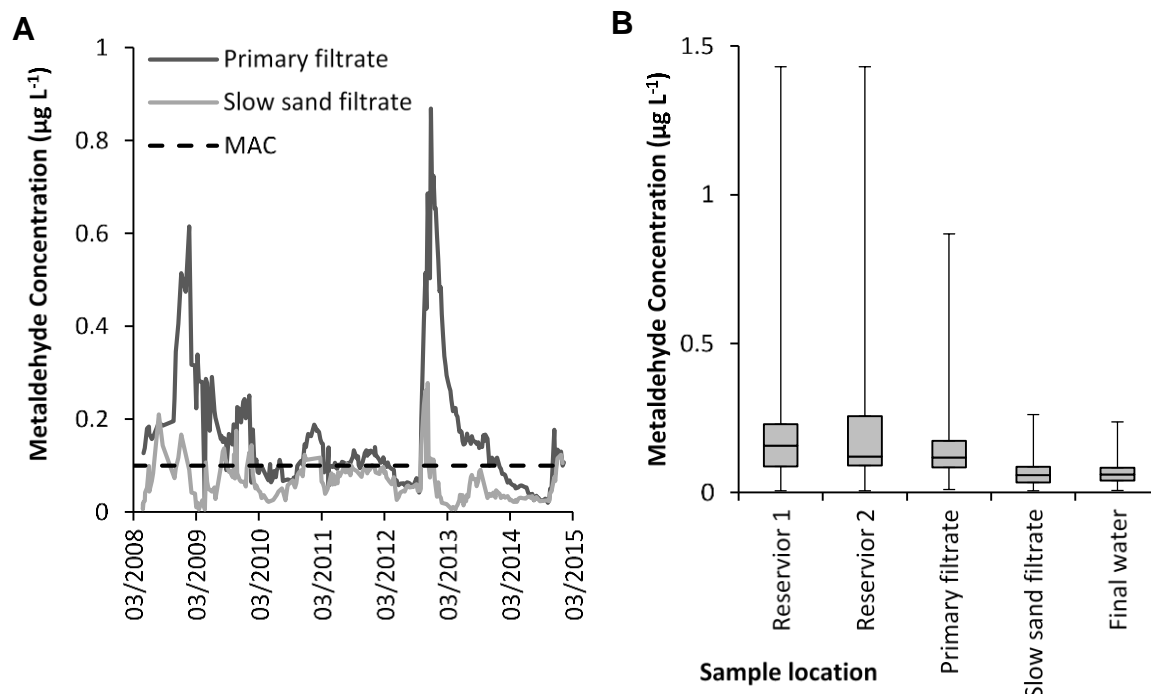


Figure 2 (A) Metaldehyde levels pre and post SSF and (B) metaldehyde concentrations through treatment ($n = 234-390$). Boxes represent 25-75 percentiles and 50th percentile value (middle black line). Whiskers represent the minimum and maximum data.

Importantly, the primary filters did not effectively remove metaldehyde, with 41% of samples being equal to or less than the European MAC of 0.1 $\mu\text{g L}^{-1}$ between 2008 and 2015. In contrast, 90% of samples post SSF being below this threshold. Therefore, the SSF was responsible for most of the metaldehyde removal at this WTW. This indicates that the slow sand filter is the primary metaldehyde removal process at this WTW. Previous studies have demonstrated the ability of drinking water filters to remove micropollutants, including 2-methylisoborneol (MIB), geosmin, microcystins, endocrine disrupting compounds (EDCs) and pharmaceuticals (Ho et al 2006). The biological removal of the herbicide mecoprop (MCP) was also demonstrated at full scale from an initial concentration of 0.037 $\mu\text{g L}^{-1}$ to below the detection limit with an EBCT of 63 minutes (Hedegaard et al., 2014). Notwithstanding these works, data of full scale SSF performance on pesticides removal is still very limited. This study includes a useful dataset, which will help to improve our understanding on biological micropollutant removal at scale.

3.2. Metaldehyde removal kinetics in batch experiments with non-acclimated sand

Metaldehyde degradation at UK environmentally relevant concentrations, $0.5\text{--}5\ \mu\text{g L}^{-1}$, using non-acclimated sand, was analysed over a 72 hour study period (Figure 3). At $0.5\ \mu\text{g L}^{-1}$ initial concentration, only 34.5% of the metaldehyde was removed. All the other concentrations (1, 2.5 and $5\ \mu\text{g L}^{-1}$) metaldehyde was reduced between 58 and 72%. Negligible removal of metaldehyde was observed over the 72 hours using inactive sand, heated at 105°C to act as an abiotic control, or clean sand (control).

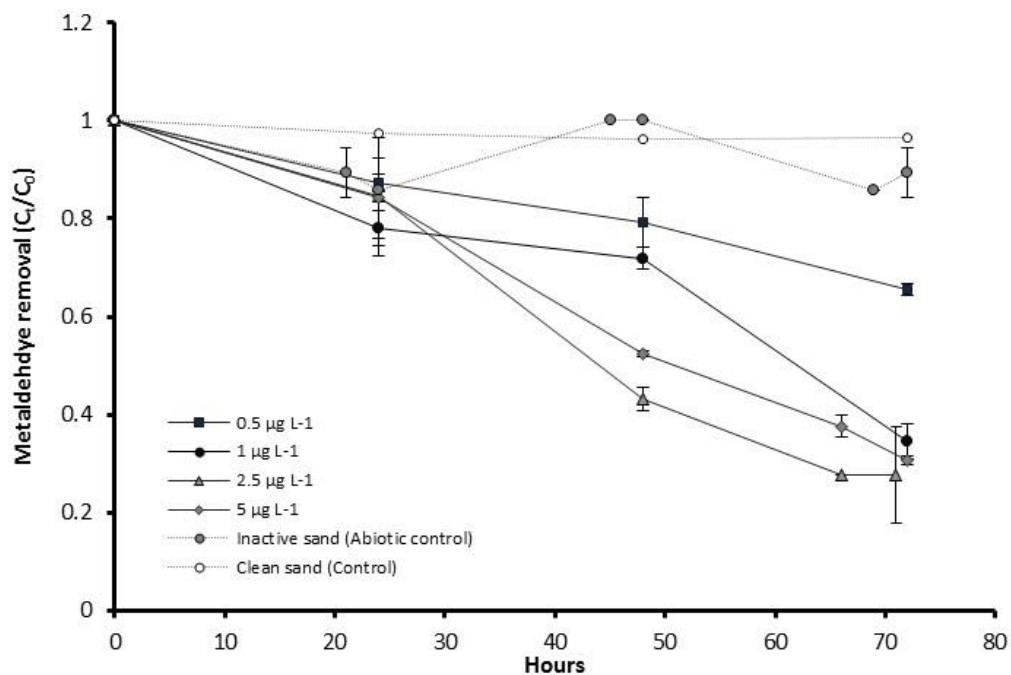


Figure 3 ‘Non-acclimated’ sand kinetics: degradation of different concentrations of metaldehyde ($0.5\text{--}5\ \mu\text{g L}^{-1}$) in batch test containing non-acclimated sand with biofilm. Clean sand (control) and inactive sand (abiotic control) were tested with a metaldehyde concentration of $1\ \mu\text{g L}^{-1}$.

This indicates that degradation is due to the biofilm activity and removal is not occurring in the water phase or through adsorption to the sand/inactive biofilm media. Previous work looking at the removal of different pesticides in rapid sand filters determined that biodegradation was the primary removal mechanism and that, similarly to our systems (Rolph et al., 2018), biosorption was negligible for the removal of those pesticides (Hedegaard and Albrechtsen, 2013).

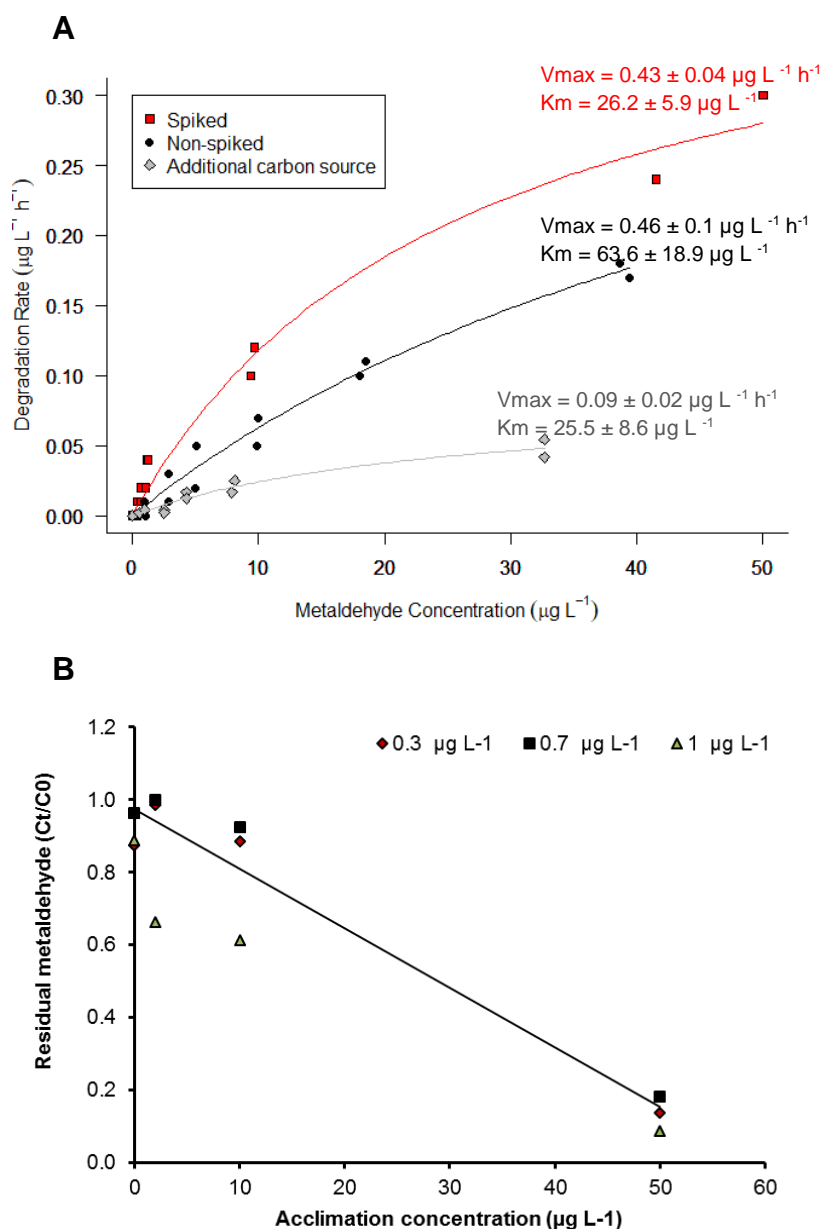


Figure 4 A. Biodegradation kinetic parameters of metaldehyde assessed using the Michaelis-Menten model. Comparison of acclimated at $2 \mu\text{g L}^{-1}$ (red, $p < 0.001$), non-acclimated (black $p < 0.001$) and additional carbon source (grey $p < 0.05$) degradation rates for metaldehyde removal ($0.5, 1, 2, 5, 10, 20, 40$ and $50 \mu\text{g L}^{-1}$). The solid line represents Michaelis-Menten modelled data. **B.** Metaldehyde removal after acclimation to different concentrations of metaldehyde followed by exposure to low metaldehyde concentrations for 24 hours ($p < 0.01$). Line represents linear regression of % removal against metaldehyde acclimation concentration; p value represents significance of model fit to observed values. Data represent average of 6 independent measurements.

The experimental data did not differ significantly from the Michaelis-Menten model for all V_{max} and K_m treatments (t-test between observed and expected, $p < 0.05$) which suggested this model was suitable for our data (Okpokwasili and Nweke, 2006). The maximum number

of iterations to convergence for the EEA models was < 1 in all cases. The achieved convergence tolerance was $< 5 \times 10^{-6}$, which is below the accepted upper limit of 1×10^{-4} , suggesting low error accumulation and therefore model accuracy to achieve convergence (Hassard et al., 2018). The maximum reaction rate (V_{\max}) and the half velocity constant (K_m) were calculated as $0.46 \mu\text{g L}^{-1} \text{h}^{-1}$ and $63.59 \mu\text{g L}^{-1}$ (Figure 4A).

To our knowledge, no data on degradation kinetics have previously been reported for metaldehyde from sand filters during drinking water treatment and very limited information is available on the biodegradation and sorption kinetics of metaldehyde in biological processes. A DEFRA study reported a 6% metaldehyde removal over 28 days in sewage sludge (DEFRA, 1996) whereas Kay and Grayson (2014) reported half-lives in soil ranging between 3 and 223 days. Therefore, even at low concentrations of metaldehyde these sand filter microbiome are able to remove untreated metaldehyde faster than previously reported in other biological media.

3.3. Acclimation for enhanced metaldehyde removal rate in batch systems

Data from the full-scale filter (Figure 2B) and from our batch systems (Figure 3) showed that metaldehyde removal rates change depending on its concentration in the medium. These findings presents an opportunity to improve the removal of metaldehyde through microbial acclimation by enrichment with metaldehyde, possibly through controlled substrate dosing in side-stream reactors to change the function of the microbiome (Hellings et al. 1998; Vignola et al. 2018). To confirm whether this acclimation approach could be achieved in our conditions, the SSF media was exposed to elevated metaldehyde levels (2 Figure 4 A and $10 \mu\text{g L}^{-1}$ Figure 1S, supplementary material) for one week to promote acclimation. Following this, the sand was exposed to different concentrations of metaldehyde, $0.5\text{-}50 \mu\text{g L}^{-1}$, and the removal rates measured for the different conditions. Sand was also exposed to a spike of a generic carbon source, acetic acid ($25 \text{ mg L}^{-1} = 5 \text{ mg L}^{-1} \text{ TOC}$), for one week in order to assess whether the increase in removal was a result of a general increase in biomass or substrate specific degraders. The acclimated sand achieved higher degradation rates than non-acclimated sand at all concentrations. The relationship between metaldehyde concentration and metaldehyde removal rate was linear between $0.5\text{-}50 \mu\text{g L}^{-1}$ ($p < 0.001$) and removal rates increased in proportion to metaldehyde concentration up to $0.30 \mu\text{g L}^{-1} \text{h}^{-1}$ at $50 \mu\text{g L}^{-1}$ (Figure 4A). This was faster than with the non-acclimated sand where the highest

metaldehyde removal rate was $0.17 \mu\text{g L}^{-1} \text{h}^{-1}$. For acclimated sand, at low concentrations, first order rate constants over 72 hours for concentrations ranging from 0.5 to $5 \mu\text{g L}^{-1}$ were calculated as 0.01 - 0.02h^{-1} resulting in a metaldehyde half-life between 34.7 and 69.3hr . The estimated V_{max} for metaldehyde acclimated and carbon spiked sand were $0.43 \mu\text{g L}^{-1} \text{h}^{-1}$ (± 0.04) and $0.09 \mu\text{g L}^{-1} \text{h}^{-1}$ (± 0.02) respectively, with a 4.7-fold increase in maximum specific metaldehyde degradation rate for acclimated sand, suggesting that metaldehyde degrading activity was not stimulated in the presence of a readily biodegradable carbon source (Figure 4A). Sand was also exposed to lower amount of generic carbon source (10 , 1 and 0.1 acetic acid equivalent to 4 , 0.4 and 0.04mg L^{-1} TOC) to provide equivalent levels of carbon to the metaldehyde acclimated experiments.

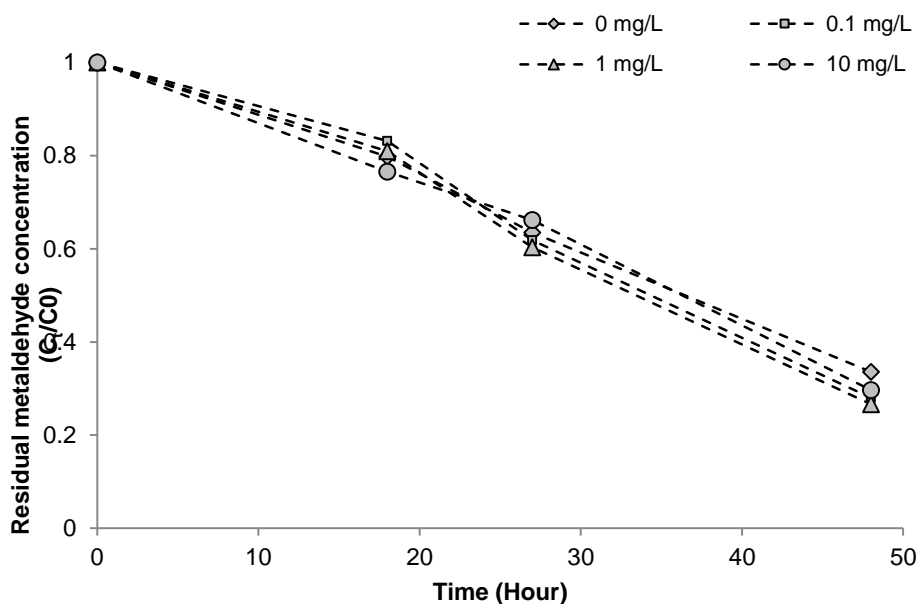


Figure 5. Degradation of metaldehyde ($10 \mu\text{g L}^{-1}$) using sand exposed to different concentrations of acetic acid (0.1 , 1 and 10mg L^{-1} equivalent to 0.04 , 0.4 and 4mg L^{-1} carbon).

Data exposed to the generic carbon source showed no difference in removal rates (Figure 5), suggesting that a generic increase in biomass would not produce an increase in metaldehyde removal. V_{max} of acclimated sand was very similar to non-acclimated sand ($0.46 \mu\text{g L}^{-1} \text{h}^{-1} \pm 0.1$). As the metaldehyde concentrations were very low compared to the half saturation constant, the degradation is assumed to be first order (Plósz et al., 2009). The K_m were estimated as $26.2 \mu\text{g L}^{-1}$ (± 5.9) and $25.5 \mu\text{g L}^{-1}$ (± 8.6) for metaldehyde acclimated and carbon

spiked sand respectively. Both values are significantly smaller than the non-acclimated sand one ($63.6 \mu\text{g L}^{-1} \pm 18.9$) indicating that the maximum rate of reaction will be achieved at lower concentrations, e.g. at the concentrations expected at the water treatment plant.

To confirm the above findings and to evaluate the impact of different levels of acclimating metaldehyde, sand was acclimated using different concentrations of metaldehyde (0, 5, 10 and $50 \mu\text{g L}^{-1}$) for one week. The acclimated sands (5, 10 and 50) and the non-acclimated sand (control) were used in batch tests with raw water containing metaldehyde at environmentally relevant concentrations (0.3, 0.7 and $1 \mu\text{g L}^{-1}$) and removal was monitored for 72 hours. Metaldehyde removal was achieved in all batch tests, even in control samples containing background metaldehyde levels of $0.097 \mu\text{g L}^{-1}$. In line with other results, the most significant metaldehyde removal was observed following the acclimation period with $50 \mu\text{g L}^{-1}$ where between 82 and 91% of the initial concentration (0.3, 0.7, and $1 \mu\text{g L}^{-1}$) was removed in the first 24 hours (Figure 4B).

This suggests that (1) acclimated biofilm removes more pesticide, (2) this removal can be stimulated in non-acclimated biofilm (3) there is less lag-time in removal efficiency when biofilm is acclimated. Previous studies have demonstrated that repeated exposure to a substrate could result in enhanced removal (Spain and Van Veld, 1983; Kanissery and Sims, 2011). Vischetti et al. (2008) reported a reduction in the half-life of the fungicide metalaxyl from 37 days to 4 days after the first and third application respectively. This phenomenon was also observed by Ho et al. (2007) who found that rate constants almost doubled upon re-exposure of the biofilm to taste and odour compounds. In addition, Wiggins and Alexander (1988) reported that the lag time of bacteria to *p*-nitrophenol (PNP) was less at higher PNP acclimation concentration due to growth of a small number of functional degraders within the microbiome. In biofilms a similar trend was observed where the linuron removal efficiency was ~80% at between $100 - 1000 \mu\text{g L}^{-1}$ but 35% at $10 \mu\text{g L}^{-1}$ of linuron (Horemans et al., 2014). Models by Rittmann (2002) predicted that a quick loss of activity could follow these improvements. Despite this, as bacteria become adapted to oligotrophic conditions good removal (> 85%) of trace organics was observed for up to a year. This has implications for the removal of trace organics such as metaldehyde in biological drinking water treatments.

In our study, relatively slow degradation of metaldehyde was observed at the start of the experiments, however if a rate as high as $0.43 \mu\text{g L}^{-1} \text{ h}^{-1}$ could be maintained, effective treatment could be achieved in approximately 20 minutes for an average influent concentration of $0.16 \mu\text{g L}^{-1}$. Therefore, future research effort is required to explore the

potential of a rapid filter for this process, particularly identifying how long it would take for a biofilm to acclimate to metaldehyde. Studies have shown that biofilms can be quite resilient and, despite not being exposed to high concentrations of a pollutant for several months, they might be able to continue degrading that pollutant due to legacy of degraders within the biofilm and long-term redundancy for biocenosis by facultative degraders. Zearley and Summers (2015) reported that biofilters adapted to MIB and 2,4-D retained their ability to remove these pollutants after non-exposure periods of up to 5 months. This shows there may be potential to use a biological technology seasonally for metaldehyde removal.

3.4. Acclimation for enhanced metaldehyde removal rate in upflow columns in through-flow

The hypotheses were postulated for the increased removal observed in the batch tests: (1) increased growth of non-specialised biomass; (2) increased growth of non-specialised biomass acclimated to use metaldehyde as carbon source; and (3) growth of specialised degraders. The first hypothesis was tested in a through-flow experiment by adjusting carbon, nitrogen and phosphorous ratios to levels ideal for the growth of heterotrophic bacteria (100:10:1) (LeChevallier, 1991). Whereas, the second and third one were tested through biomass acclimation, although further specialised molecular work will be necessary to differentiate the two.

To verify our hypotheses and to scale-up our findings in continuous systems, fluidised-bed through-flow column were established with freshly collected sand. The impact of exposure to increased carbon or metaldehyde concentrations was evaluated using two columns run with the same inlet water. The columns were fed with either 50 $\mu\text{g L}^{-1}$ of metaldehyde, proved to be the most effective concentration for acclimation (Figure 4B) or 25 mg L^{-1} of acetic acid, equivalent to 10 mg L^{-1} C, double of the existing carbon concentration and to levels reported for similar studies (Li et al., 2012). Following the acclimation period the inlet was returned to raw water containing 0.5 $\mu\text{g L}^{-1}$ of metaldehyde. The columns run with a contact time of 828 minute as described in material and methods. The results are reported in Figure 6.

The non-acclimated columns achieved steady removal of 52.7 (± 4.2) % from an inlet concentration of 0.48 (± 0.02) $\mu\text{g L}^{-1}$ was achieved under this configuration with effluent concentrations ranging from 0.20 - 0.26 $\mu\text{g L}^{-1}$. This set up achieved good removal but not compliant water ($>0.1 \mu\text{g L}^{-1}$). An increased removal of metaldehyde was observed in the metaldehyde-spiked column, which produced compliant water for more than 20 days with an

average outlet concentration of $0.08 (\pm 0.015) \mu\text{g L}^{-1}$. Whereas the column fed with the additional carbon source showed a decrease in removal rates with an average removal of 55% from day 6 to 13 and 38% from day 22 to 58.

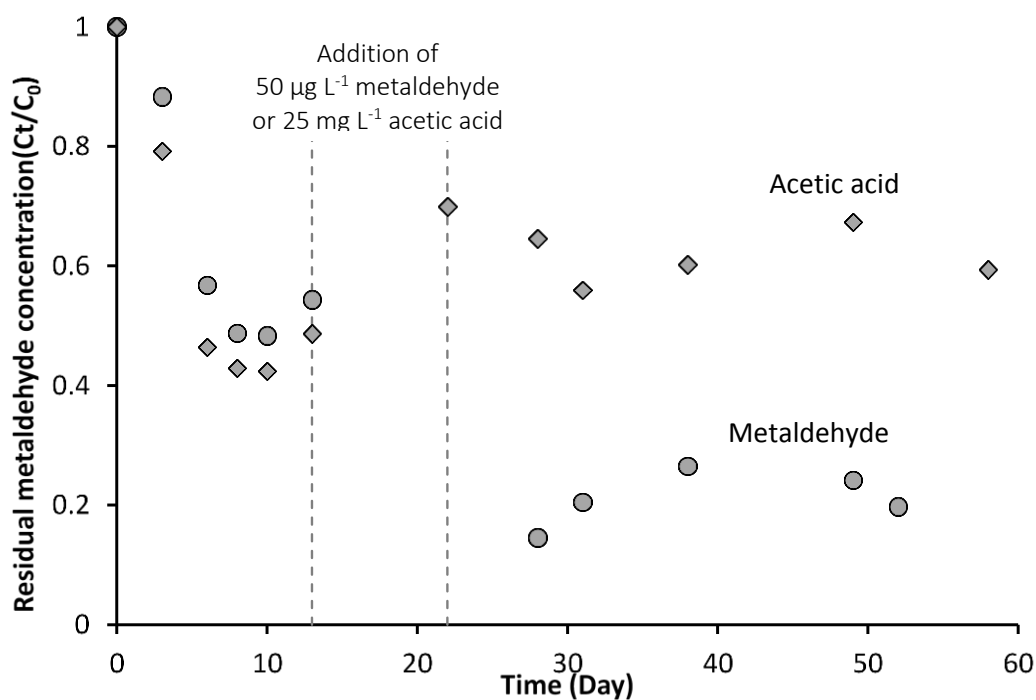


Figure 6 Impact of carbon addition and metaldehyde acclimation on column performance (contact time = 828 minutes) on metaldehyde removal at initial environmental concentration of $0.5 \mu\text{g L}^{-1}$.

Similarly to what obtained in batch systems the additional carbon source did not support metaldehyde degradation while acclimation produced complaint water. Indeed, the increase in non-specialised biomass had a negative impact on metaldehyde removal, negating hypothesis (1). This behaviour has been observed in the degradation of other pollutants. Liu *et al.* (2017) reported a decrease in terephthalic acid and para-toluic degradation when glucose was used as additional carbon source in an up-flow anaerobic sludge blanket (UASB). On the contrary, addition of molasses resulted in an improvement of para-toluic acid removal. However, both substrates produced a decrease in syntrophs and methanogens and an increase in carbohydrate-fermenting bacteria. In the column spiked with metaldehyde the biomass had either ‘learned’ to use the xenobiotic compound (Spain and Van Veld, 1983)

or developed new metabolic pathways under conditions of low carbon (Egli, 2010). Thomas et al. (2017) reported the isolation of specialised microorganisms able to use metaldehyde as a primary carbon source. In this situation, increasing the general quantity of biomass would not have resulted in increased metaldehyde removal. However, exposure to repeated or high concentrations of metaldehyde could have enhanced the action of degrading microorganisms, specialist or not.

Due to the presence of dissolved organic matter, it is assumed that the degradation of metaldehyde at trace concentrations would occur in the biofilm mainly through co-metabolism or by secondary substrate utilisation (Zearley and Summers, 2012; Ho et al., 2007). However, due to the nature of the transformation product of metaldehyde, acetaldehyde, it is also possible that metabolism of the degradation products are undertaken by similar consortia. Acetaldehyde, being a potential precursor of acetyl-coA, can be central to many high-energy metabolic pathways, and potentially delivering per each molecule double the amount of acetyl-coA than glucose (four vs two). Indeed, the inhibition of metaldehyde removal following exposure to a carbon source and when in competition with high levels of DOC indicate that removal is most effective when metaldehyde is being utilised as a primary carbon source (Figure 6). This has also been observed with the degradation of PNP where the addition of carbon decreased the rate of degradation despite increased cell growth (Qiu et al., 2007). Horemans et al. (2014) observed that when a carbon source was fed to a biofilm alongside the pesticide linuron, the biofilm increased but there was no increase in linuron removal, which remained at 30%. Our results therefore seem to support either hypothesis (2) or (3) of an increase in slow-growth microorganisms able to use metaldehyde directly as a primary carbon source when acclimatised to higher concentration ($50 \mu\text{g L}^{-1}$). It is possible that only a small group of specialised microorganisms (hypothesis 3) is responsible for metaldehyde uptake and small increases in total biomass would not produce significant changes in its removal. Arya et al. (2016) reported that microbial biomass of biological reactors could be acclimatised to a mixture of pharmaceuticals and produced a steady removal of these compounds. Similarly, other authors have also shown that continuous exposure of different micropollutants could enhance their degradation rate by supporting slow-growth microorganisms or the production of enzymes responsible for their degradation (Clara et al., 2005; Majewsky et al., 2011). In our case, metaldehyde is known to undergo hydrolysis in the presence of acid and it may be possible that enzymes are produced that can rapidly degrade metaldehyde into acetaldehyde. In order to substantiate

both hypotheses (2 and 3), and provide seeding data for further research, sequencing was undertaken on sand samples from the SSF before acclimation and acclimated sand samples (ACC) from a lab scale column experiment, which effectively removed metaldehyde for several months. The results showed a shift in the microbial community and a non-parametric comparison of samples indicated that the difference between the acclimated and SSF samples were statistically significant with a p value of 0.03 (Figures 2S and 3S supplementary material). Within the SSF and acclimated samples there was good repeatability, PCA demonstrated the acclimated and slow sand samples cluster differently (Figures 2S, supplementary material). More detailed microbiological work should give a greater insight into the factors controlling metaldehyde degradation and could lead to the isolation of specific metaldehyde degraders to seed biofilters.

4. Conclusions

The operational data demonstrated that metaldehyde removal rates increased following exposure to higher concentration of this micropollutant. Batch experiments with the same sand, showed that the removal was mainly biological and that this process could be replicated in the lab *via* acclimation. Therefore, this is the first study to offer an explanation on why some biofilters can degrade metaldehyde for drinking water treatment whilst other do not.

The acclimation process was also translated in continuous up-flow fluidised bed reactors achieving around 80% removal for over 40 days and compliance ($< 0.1 \mu\text{L}^{-1}$) for more than 20 days.

These findings are of interest to water treatment practitioners as they present an opportunity for existing water treatment assets to be commissioned, utilised or upgraded for biological metaldehyde treatment to improve drinking water quality and treat this hard-to-remove pesticide. This work presents a novel conceptual template for chemical free, low cost, biological treatment of metaldehyde in drinking water and broadly, the role of microbial communities for the treatment of micropollutants.

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7. Electronic Supplementary Information

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8. Competing interests

The authors have no competing interests to declare.

9. References

- APHA-AWWA-WEF. (2012). *Standard Methods for the Examination of Water and Wastewater*, 22nd Ed. Washington.
- Arya, V., Philip, L. and Murty Bhallamudi, S. (2016). Performance of suspended and attached growth bioreactors for the removal of cationic and anionic pharmaceuticals. *Chemical Engineering Journal*. 284. pp. 1295–1307.
- Autin, O., Hart, J., Jarvis, P., Macadam, J., Parsons, S. and Jefferson, B. (2013). The impact of background organic matter and alkalinity on the degradation of the pesticide metaldehyde by two advanced oxidation processes: UV/H₂O₂ and UV/TiO₂. *Water Research*. 47 (6). pp. 2041–9.
- 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., Walravens, E. and Boon, N. (2013). Is Biological Treatment a Viable Alternative for Micropollutant Removal in Drinking Water Treatment Processes? *Water Research*. 47 (16). pp. 5955–5976.
- Bieri, M. (2003). *The environmental profile of metaldehyde*. In: World Agriculture, British Crop Protection Council, Symposium Proceedings 80. 2003, pp. 255–260.
- Buitron, G., Gonzalez, A. and Lopez-Marin, L.M. (1998). Biodegradation of phenolic compounds by an acclimated activated sludge and isolated bacteria. *Water Science and Technology*. 37 (4-5). pp. 371–378.
- Cheyns, K., Mertens, J., Diels, J., Smolders, E. and Springael, D. (2010). Monod kinetics rather than a first-order degradation model explains atrazine fate in soil mini-columns: implications for pesticide fate modelling. *Environmental Pollution*. 158 (5). pp. 1405–11.
- Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N. and Kroiss, H. (2005). Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Research*. 39 (19). pp. 4797–807.
- Council Directive 98/83/EC (1998) *On the quality of water intended for human consumption*, Official Journal of the European Communities L 330, pp. 32–54
- DEFRA (1996). *Evaluation on: Metaldehyde*. York: DEFRA.
- Drinking Water Inspectorate - DWI (2017). *Annual Report Drinking Water 2016*, Drinking Water Inspectorate, London. Available at: <http://www.dwi.gov.uk/about/annual-report/>
- Egli, T.(2010) How to live at very low substrate concentration, *Water Research*. 44 (17), pp 4826-4837.

- FERA (2013). *Pesticide Usage Statistics*. [Online] Available from: <https://secure.fera.defra.gov.uk/pusstats/>. (Accessed: 11 October 2018).
- Hassard, F., Biddle, J., Harnett, R., Stephenson, T., (2018). Microbial extracellular enzyme activity affects performance in a full-scale modified activated sludge process. *Science of the Total Environment*. 625, pp. 1527–1534.
- Hassard, F., Andrews, A., Jones, D.L., Parsons, L., Jones, V., Cox, B.A., Daldorph, P., Brett, H., McDonald, J.E., Malham, S.K., (2017). Physicochemical Factors Influence the Abundance and Culturability of Human Enteric Pathogens and Fecal Indicator Organisms in Estuarine Water and Sediment. *Frontiers in Microbiology*. 8, pp1996.
- Hedegaard, M.J. and Albrechtsen, H.J. (2013). Microbial pesticide removal in rapid sand filters for drinking water treatment - Potential and kinetics. *Water Research*. 48 (1), pp. 71–81.
- Hedegaard, M.J., Arvin, E., Corfitzen, C.B. and Albrechtsen, H.-J. (2014). Mecoprop (MCP) removal in full-scale rapid sand filters at a groundwater-based waterworks. *Science of the Total Environment*. 499. pp. 257–64.
- Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M., Heijnen, J.J., 1998. The sharon process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology*. 37 (9), 135–142.
- Ho, L., Hoefel, D., Bock, F., Saint, C.P. and Newcombe, G. (2007). Biodegradation rates of 2-methylisoborneol (MIB) and geosmin through sand filters and in bioreactors. *Chemosphere*. 66 (11). pp. 2210–8.
- Horemans, B., Hofkens, J., Smolders, E. and 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 Microbiology Ecology*. 88. pp. 184–194.
- Kanissery, R.G. and Sims, G.K. (2011). Biostimulation of the enhanced degradation of herbicides in soil. *Applied and Environmental Soil Science*. 2011, Article ID 843450. pp.1-10.
- Kay, P. and Grayson, R. (2014). Using water industry data to assess the metaldehyde pollution problem. *Water and Environment Journal*. 28 (3). pp. 410–417.
- LeChevallier, M.W. (1991). Bacterial Nutrients in Drinking Water. *Applied and Environmental Microbiology*. 57 (3). pp. 857–862.
- Li, Z., Dvorak, B. and Li, X. (2012). Removing 17 β -estradiol from drinking water in a biologically active carbon (BAC) reactor modified from a granular activated carbon (GAC) reactor. *Water Research*. 46 (9). pp. 2828–36.
- Liu, M., Wang, S., Nobu, M.K., Bocher, B.T.W., Kaley, S.A., Liu, W. (2017) Impacts of biostimulation and bioaugmentation on the performance and microbial ecology in methanogenic reactors treating purified terephthalic acid wastewater. *Water Research*. 122. pp. 308-316.
- Majewsky, M., Gallé, T., Yargeau, V. and Fischer, K. (2011). Active heterotrophic biomass and sludge retention time (SRT) as determining factors for biodegradation kinetics of pharmaceuticals in activated sludge. *Bioresource Technology*. 102 (16). pp. 7415–7421.
- McCarty, P.E., Rittmann, B.E., (2018). *Environmental Biotechnology: principles and applications*. International Ed. McGraw-Hill. ISBN: 1260440591, 9781260440591.
- Okpokwasili, G.C., Nweke, C.O. (2006) Microbial growth and substrate utilization kinetics *African Journal of Biotechnology*. 5. pp. 305-317.
- Plósz, B.G., Leknes, H., Thomas, K. V, (2009). Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in

- municipal wastewater treatment. *Environmental Science and Technology*. 44, pp.734–742.
- Qiu, X., Zhong, Q., Li, M., Bai, W. and Li, B. (2007). Biodegradation of p-nitrophenol by methyl parathion-degrading *Ochrobactrum* sp. B2. *International Biodeterioration and Biodegradation*. 59 (4). pp. 297–301.
- Ramos, A. M., Whelan, M. J., Cosgrove, S., Villa, R., Jefferson, B., Campo, P., Jarvis, P. and Guymer, I. (2017), A multi-component method to determine pesticides in surface water by liquid-chromatography tandem quadrupole mass spectrometry. *Water and Environment Journal*, 31. pp 380–387.
- Reungoat, J., Escher, B.I.I., Macova, M. and Keller, J. (2011). Biofiltration of wastewater treatment plant effluent: effective removal of pharmaceuticals and personal care products and reduction of toxicity. *Water Research*. 45 (9). pp. 2751-2762.
- Rolph, C.A., Jefferson, B., Villa, R., (2014). Switching on pesticide degraders in biological filters used in drinking water production. *Progress in slow sand and alternative biofiltration*. 195.
- Rolph, C.A., Jefferson, B., Hassard, F., Villa, R., (2018).Metaldehyde removal from drinking water by adsorption onto filtration media: mechanisms and optimisation. *Environmental Science: Water Research & Technology*. 4. pp. 1543-1552.
- Rittmann, B.E., Stilwell, D. and Ohashi, A., (2002). The transient-state, multiple-species biofilm model for biofiltration processes. *Water Research*. 36. pp. 2342-2356
- Spain, J.C. and Van Veld P.A. (1983). Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure time, inoculum and chemical structure. *Applied Environmental Microbiology*. 45(2) pp. 428-435
- Stoodley, P., Sauer, K., Davies, D.G., Costerton, J.W., (2002) Biofilms as complex differentiated communities. *Annual Reviews in Microbiology*. 56. pp. 187-209.
- Thomas, J., Helgason, T., Sinclair, C.J., Moir, J.W.B., (2017). Isolation and characterisation of metaldehyde-degrading bacteria from domestic soils. *Microbial Biotechnology* 10(6). pp.1824-1829.
- Vignola, M., Werner, D., Wade, M.J., Meynet, P., Davenport, R.J., (2018). Medium shapes the microbial community of water filters with implications for effluent quality. *Water Research*. 129, pp. 499–508.
- Vischetti, C., Monaci, E., Cardinali, A., Casucci, C. and Perucci, P. (2008). The effect of initial concentration, co-application and repeated applications on pesticide degradation in a biobed mixture. *Chemosphere*. 72 (11). pp. 1739–43.
- Water UK (2008). Metaldehyde - Water UK Briefing note. London: Water UK.
- Wingender, J. and Jaeger K.-E. (2002) Extracellular enzymes in biofilms. in G. Bitton (Ed.), *Encyclopaedia of Environmental Microbiology*, 3, pp. 1207-1222
- Wiggins, B.A. and Alexander, M. (1988). Role of Chemical Concentration and Second Carbon Sources in Acclimation of Microbial Communities for Biodegradation. *Applied and Environmental Microbiology*. 54 (11). pp. 2803–2807.
- Zearley, T.L. and Summers, R.S. (2012). Removal of Trace Organic Micropollutants by Drinking Water Biological Filters. *Environmental Science and Technology*. 46. pp. 9412–9419.
- Zearley, T.L. and Summers, R.S. (2015). MIB and 2, 4-D Removal by Biofilters During Episodic Loading. *Journal AWWA*. 107 (12). pp. 666–673.