INTRODUCTION

UV disinfection reactors are an effective and economic means to inactivate oocysts of the protozoan parasite Cryptosporidium in drinking water. Cryptosporidiosis outbreaks can occur as a result of filter breakthrough following increased oocyst loading of treatment processes (Bouchier, 1998). Oocysts are resistant to commonly used chlorine-based disinfectants and therefore filter failure can result in outbreaks of cryptosporidiosis (Korich et al., 1990). Low and medium pressure UV disinfection has been demonstrated to be effective at reducing oocyst infectivity at economic UV doses, and hence UV reactors have become increasingly common as drinking water treatments for inactivating oocysts and other waterborne pathogens (Bukhari et al., 1999; Clancy et al., 1998).

Many countries have published guidance related to UV disinfection reactor operation (DEFRA, 2010; DVGW, 2006; ÖNORM, 2001; ÖNORM, 2003; USEPA, 2006). All guidance requires UV reactors to be dose-validated before installation and to be operated within validated conditions (i.e. at prescribed flow rates, water quality conditions, and lamp conditions). In all existing guidance, dose validation involves biodosimetry, involving a challenge microorganism (e.g. MS2 coliphage) being spiked into the water upstream of the UV reactor and determining the log inactivation of the challenge microorganism across the reactor. The UV dose delivered is determined by reference to standardized bench-scale UV collimated beam experiments in which UV dose can be accurately determined using a calibrated radiometer (Bolton & Linden, 2003). The validation tests generate an envelope of operating conditions under which the delivered UV dose is acceptable for disinfection.

Abstract

This study aimed to isolate waterborne heterotrophic organisms indigenous to water treatment processes with suitable ultraviolet (UV) dose–response profiles for estimation of the UV doses delivered by operational UV disinfection systems. The UV dose–response profiles of two isolated organisms, Flavobacterium succinicans and Sphingopyxis chilensis, were determined. S. chilensis has a UV dose–response with potential for use as an indigenous surrogate for Cryptosporidium inactivation, up to 2.2-logs in the dose range 2–7 mJ/cm² of monochromatic UV light at 253.7 nm. F. succinicans was more sensitive and tailing was observed above 4 mJ/cm² making it unsuitable for UV dose verification above this dose. As a single species in the water samples used in this study, S. chilensis is unlikely to be present in sufficient numbers for routine use as an indigenous surrogate. However, the Sphingomonadaceae family to which it belongs is abundant in various drinking water sources and warrants further investigation.

KEYWORDS

Cryptosporidium, drinking water, Flavobacterium, Sphingopyxis, ultraviolet disinfection

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Dose verification (versus validation) is the routine monitoring of UV dose delivery by an operating UV reactor. The only current means of UV dose verification of installed operational reactors is achieved by logging operating parameters (e.g. flow rate, lamp intensity) to ensure they are within the validated envelope. This operational performance verification relies on calibrated sensors for measuring a range of parameters (e.g. the UV absorbance of the water, the UV irradiance at discrete points within the reactor, and the flow rate through the reactor), because injection of biosimetry organisms is often not possible in operational drinking water treatment works. There are currently direct methods of assessing UV dose delivery that does not either (i) rely on sensor measurements, which in turn relies on sensor calibration and cleaning to avoid sensor errors, or (ii) involve spiking some type of dosimeter reagent (e.g. a biodosimeter or chemical actinometer) into the flow.

Previous studies have also sought to identify indigenous microorganisms with dose–response useful for UV reactor validation. Mamane-Gravetz and Linden (2004) investigated aerobic spores from natural waters. They concentrated spores from lake water and determined UV dose–response. Significantly, the UV dose–response of environmental spores were more resistant to UV light than laboratory-prepared spores of Bacillus subtilis. Further work showed that pure cultures of environmental spores grown under lab conditions showed a three-phase UV dose–response relationship (shoulder, linear, and tailing) and demonstrated that the rate constant of the environmentally derived spores’ UV dose–response was often statistically indistinguishable from the tailing region of the curves from pure cultures (Mamane-Gravetz & Linden, 2005). Rochelle et al. (2010) also attempted to identify high UV resistance organisms suitable for use in validating reactors that are designed to deliver the high UV doses required by the USEPA (186 mJ/cm²) for achieving 4-log reduction of adenovirus (USEPA, 2006). That study successfully identified the spore-forming bacteria Bacillus pumilus as a useful challenge organism for virus inactivation. The organisms were isolated in the raw water entering treatment works; it is unclear whether they would exist in treated waters at the stage of application of UV disinfection. They are easily enumerated and slightly more resistant to UV light than laboratory-prepared spores. Rochelle et al. (2010) also attempted to identify high UV resistance organisms suitable for use in validating reactors that are designed to deliver the high UV doses required by the USEPA (186 mJ/cm²) for achieving 4-log reduction of adenovirus (USEPA, 2006). That study successfully identified the spore-forming bacteria Bacillus pumilus as a useful challenge organism for virus inactivation. The organisms were isolated in the raw water entering treatment works; it is unclear whether they would exist in treated waters at the stage of application of UV disinfection. They are easily enumerated and slightly more resistant to UV light than laboratory-prepared spores.

It was decided to screen aerobic heterotrophs for the following reasons: (i) previous studies suggested that they are the most abundant bacterial population in wastewater effluent (Chauret et al., 1999) and they have been cultured in significant numbers in post-filtration samples from drinking water treatment processes previously (Stetler et al., 1992), (ii) as a group, heterotrophic bacteria have similarly shaped UV dose–response profiles to Cryptosporidium, as collated in (Chevrefils et al., 2006), (iii) they can be grown and enumerated using simple culture-based methods.

Although having similarly shaped UV dose–responses to Cryptosporidium, it was expected that the majority of heterotrophs present would be more sensitive to UV light than Cryptosporidium and therefore not useful as a surrogate (Chevrefils et al., 2006). The same successful UV screening method that was used by Rochelle et al. (2010) was adopted to select those heterotrophs that are most resistant to UV treatment. UV screening using a threshold dose facilitates the enrichment of populations for decreased UV sensitivity by increasing the probability of survival of less sensitive species over those that are more sensitive to UV exposure. Therefore, screening can be used as a tool to enrich populations of microorganisms for UV resistance without any a priori link between the screening dose and the sensitivity of any survivors. In the present study, samples were obtained from a UK drinking water treatment works that apply pre-screening, pre-ozonation, coagulation, flocculation, clarification, rapid gravity filtration, post-ozonation, adsorption on a granular activated carbon bed and final disinfection with chlorine before sending into supply. The specific objectives of this work were to: (i) identify representative UV-resistant heterotrophs at the water treatment stages where UV disinfection reactors would typically be installed, (ii) determine the UV dose–response of the isolated organisms, and (iii) assess their suitability for UV reactor performance monitoring considering Cryptosporidium as the target pathogen.

2 | MATERIALS AND METHODS

Samples were obtained from water treatment works in the UK. Four sampling points along the treatment process were selected: raw, post pre-ozone (PPO), post-rapid gravity filters (PRGF), and post-granular activated carbon (PGAC). Water samples were collected in 2 L autoclaved borosilicate bottles and transported the same day in chilled cool bags to the laboratory. Upon arrival in the laboratory, samples were stored at 4°C until processed, with a maximum time to processing of 56 hours.

R2A agar (Oxoid), peptone water (Lab M), tryptone soy agar (Oxoid), tryptone soy broth (Oxoid), and phosphate-buffered saline (Oxoid tablets) were prepared according to supplier instructions. Buffered water (BW) was prepared as per Standard Methods 9050 C (APHA, 2005). All media (except minimal salt media) and diluents were autoclaved at 121°C for 15 min. Liquid L9 minimal media was prepared as previously described (Yim et al., 2010) but without MnCl₂·2H₂O (Leung, 2011). All components were mixed together and filter sterilized using a sterile 0.22 μm vacuum filter unit (Nalgene). Solid L9 media was made by mixing equal volumes of a sterile 2X concentrate of L9 minimal media liquid with autoclaved 3.0% bacteriological agar No.1 (Oxoid). All solid media was prepared according to Standard Methods for spread plating (Method 9215-C). If stored at 4°C the media was warmed to room temperature prior to use.
temperature before plating. For spread plating 0.5 ml samples were spread using a cooled alcohol flamed glass spreader and plates were dried in a class II microbiological cabinet prior to incubation. Plates were counted on a Gallenkamp Colony Counter. Unless noted otherwise, the data reported is from plates with counts between 30 and 300 CFU.

For screening, 40 ml samples were exposed to UV light in bench-scale UV collimated beam experiments. Samples from the water treatment works were placed in 90 mm Petri dishes with a sterile 13 mm x 3 mm PTFE coated stir bar and exposed to a 40 mJ/cm² UV dose, a commonly recommended design UV dose in drinking water treatment (DVGW, 2006; ÖNORM, 2001, 2003). Immediately after the collimated UV beam exposures, samples were placed in a cooled dark box until processed and plated. Samples were treated once and ten-fold serially diluted in 0.1% peptone water. 0.5 ml of each dilution was plated in triplicate on R2A agar. All plates were plated on the day of their treatment, together with untreated controls. After drying, the plates were incubated in the dark and counted at days 3, 5, 7. Counts at 3 and 5 days were recorded for QA/QC purposes to demonstrate maximum recovery, but are not reported. Reported values at day 7 represent the mean of the plated triplicates unless noted otherwise.

To gauge whether the acts of either opening the incubator or counting plates influence repair of UV-damaged DNA an additional set of controls was included in which UV treated samples for the four treatment points and sterile water were incubated in the dark in the incubator, either in a black bag so light could not influence them when the door was opened, or on the shelf (not in a black bag), but not taken out of the incubator to count. These were counted after 10 days incubation. P-values from t-tests on the triplicate plates indicated that there were no DNA repair mechanisms being activated by the act of counting. Two of the isolates were picked from plates in this experiment (39-1 Flavobacterium succinicans and 37-1 Rheinheimera chironomi) following the same isolate picking procedure informed by colony morphology on method colony plates.

UV-exposed isolates were re-streaked and maintained on solid media, and liquid cultures of the isolates were grown under the conditions in Table S1. Liquid cultures were centrifuged at 8000 g for 5 min at 20°C (Sorvall RC-6 Plus Superspeed centrifuge in SS-34 rotor) and pellets were washed twice in BW. During washes, the pellets were re-suspended by vortexing and, if required, repeated aspiration through a sterile pipette tip. After the final wash, the cells were re-suspended in 50–100 ml of BW prior to standardization of UV absorbance, whereby the stock solution of cells was further diluted to an OD 254 of 0.064–0.131 (approximately 10⁶ CFU/ml). Twenty milliliters of this standardized test solution of cells were then irradiated using a collimated beam over a range of UV doses. Irradiated samples were then chilled in the dark after exposure prior to being 10-fold serially diluted in BW and dilutions plated in triplicate; reported values are the mean of triplicates for each UV dose.

The inactivation data points at each UV dose were analysed using GLnAFit (Geeraerd et al., 2005) and model fits compared to determine the best fits. The best fit overall model for the dose–response data from the isolates in this study was a biphasic plus shoulder model (Geeraerd et al., 2005). The Cryptosporidium dose–response curve was taken from the United States Environmental Protection Agency Ultraviolet Disinfection Guidance Manual (USEPA, 2006) and fitted to a Double Weibull model, as the GLnAFit software indicated that the data was unlikely to have a shoulder.

The collimated UV beam exposures followed the protocol presented in Bolton and Linden (2003). Briefly, a collimated beam apparatus (Figure S1) was used to expose samples and cultures to UV doses as indicated. A 230 V low-pressure high output mercury lamp (Trojan Technologies) powered by a ballast from a UVMax-C reactor was collimated using a dark coloured 140 mm internal diameter PVC tube to generate a quasi-parallel monochromatic UV beam. Prior to performing collimated beam experiments the UV lamp was turned on and allowed to stabilise for a minimum of 20 min. A magnetic stirrer was centred within the UV beam and the UV irradiance across two horizontal orthogonal axes of the beam was measured at 5 mm intervals using an IL1700 radiometer with a SED240 sensor. The time required to deliver the indicated UV doses was calculated using spreadsheets obtained from Bolton Photosciences and accounted for the UV absorbance of the matrix (measured using a UV-VIS spectrophotometer at 254 nm [Perkin Elmer Lambda 3], reflection from the matrix surface, the divergence of the collimated beam, and variation of irradiance over the surface of the matrix). All exposure samples were contained in 90 mm diameter polystyrene Petri dishes and a sterile PTFE-coated 13 mm × 3 mm stir bar was set to mix the samples during exposure. The stirring speed was adjusted to avoid the formation of a vortex. Once the stirring speed had been set, the sample was exposed for the required time to achieve the desired UV dose.

Isolates were identified by sequencing part of the 16S rRNA gene. Genomic DNA from isolates grown on their respective maintenance media was prepared by re-suspending cells in 1 ml lysis buffer from a PowerWater DNA Isolation Kit (MoBio Inc. CA, USA) and lysing cells in the bead beating tubes provided for 5 minutes on a vortexer. The remainder of the extraction was performed as per the supplier instructions except that elution was in 30 μl Milli-Q water. DNA concentration was determined spectrophotometrically using a Thermo Scientific NanoDrop 1000 by absorbance at 260 nm. An approximately 1.47 kilobase fragment of the 16S rRNA gene sequence was amplified by polymerase chain reaction (PCR) between the 27F and 1492R degenerate primers. Lymphophilised primers (MWG Eurofins) were re-suspended in Milli-Q water to a concentration of 100 ng/μl and each pair (27FC/27F-A; 1492RC/1492F-A) mixed in equal volumes to give a degenerate primer mix for the forward primer 27F and reverse primer 1492R. Primer sequences were 27F-C 5’-AGAGTTTTGATCCTGGCTCAG-3’, 27F-A 5’-AGAGTTTTGATCCTGGCTCAG-3’, 1492R-C 5’-TACGGCTACTTGTTAGGCT-3’, 1492R-T 5’-TACGGTTACCTGTGACTT-3’ (Lane, 1991). PCR reactions were carried out using a G-STORM GS482 PCR machine, in 25 μl using a Hi-Fidelity DNA polymerase master mix (Accuzyme, Bioline, UK). 12.5 μl of PCR mix was mixed with 10 μl of a...
template (1 ng/μl) and 1 μl of each degenerate primer mix, and 0.5 μl of Milli-Q water. The PCR reaction programme was 94°C 5 min, 30 cycles of 94°C 1 min, 55°C 1 min, 68°C 2.5 min, followed by a final extension at 68°C for 5 min. One microlitre of PCR reactions were checked on a 1% agarose gel (Bioline) and DNA was stained for visualization with GelRed (2 μl in 40 ml) and visualized under UV light on a BioRad Gel documentation station. The remaining PCR reaction, to be used for sequencing was run out by electrophoresis in a 1% agarose gel and the PCR fragment purified by gel extraction where DNA was stained with SYBER-SAFE (4 μl in 40 ml) and visualized under non-UV light using a Visi-Blue UV Transilluminator (UVP). Finally, PCR fragments were sequenced from the degenerate primers by dye terminator sequencing (MWGEurofins). To determine the isolate identities 16S rRNA gene sequences were imported into MEGA5 (Jogler et al., 2011). Sequences were trimmed according to recommendations from the sequencing reports. Each pair of sequences from the PCR products was aligned using ClustalW and base pair mismatches confirmed using the primary sequence trace data. The identification of phylogenetic neighbours was initially carried out by the BLAST (Altschul et al., 1997) and megaBLAST (Zhang et al., 2000) programs against the database of type strains with validly published prokaryotic names (Kim et al., 2012). The top thirty sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon-e server http://eztaxon-e.ezbio cloud.net/ (Kim et al., 2012). The threshold for identity in the 16S rRNA gene comparison for identification to species level was 97% identity to type strains (Stackebrandt & Goebel, 1994).

3 | RESULTS

The individual CFU/ml counts, with and without a 40 mJ/cm² UV dose after 7 days of incubation, for the four treatment plant sample points, are shown in Table 1. As CFU counts per plate were below 30 in the post-rapid gravity filter (PRGF) and post-granular activated carbon (PGAC) samples, controls were also included to demonstrate that UV-surviving colonies are unlikely to have originated from the experimental methods.

Table 2 shows data related to the effectiveness of the treatment process in reducing HPC levels cumulatively along the treatment process. In addition, it shows the log inactivation of HPCs achievable at each treatment stage by a 40 mJ/cm² UV dose. The treatment process achieved a cumulative mean log reduction in HPCs of 0.86-log reduction after rapid gravity filtration and 1.58-log prior to chlorine disinfection (Table 2). The mean CFU/ml counts at these sampling points were 4257 and 802, respectively.

The PRGF and PGAC sample locations represent the typical points in a treatment process to install a UV reactor. Isolates were selected from PRGF plates treated with UV light at 40 mJ/cm² and initially streaked on R2A and TSA agar plates and grown at 30°C. Isolates 9-1 and 56-1 failed to grow on TSA agar and were subsequently restreaked on R2A agar for maintenance. Isolate 39-1

### Table 1

| Expt. | Incubation Time (days) | Total heterotrophs CFU/ml | Post rapid gravity filter | Post granular activated carbon | Mean @ 7 day |
|-------|------------------------|---------------------------|---------------------------|-------------------------------|-------------|
| 1     | 7                      | 102 000                   | 205                        | 36 247                        | 48 045      |
| 2     | 7                      | 102 000                   | 205                        | 36 247                        | 48 045      |
| 3     | 7                      | 102 000                   | 205                        | 36 247                        | 48 045      |
| 4     | 7                      | 102 000                   | 205                        | 36 247                        | 48 045      |
|       | ±SD                    | ±43 817                   | ±137                       | ±20 514                       | ±137        |

Note: Values in italics include counts from plates with fewer than 30 CFU; values in parentheses are standard deviations of the replicates. +UV indicates a UV dose of 40 mJ/cm².
showed no growth at 30°C and was streaked and grown successfully on R2A and TSA agar incubated at 22–25°C. Table S1 shows the media used for the culture and maintenance of the isolates.

Isolates 9-1 and 56-1 shared the following colony characteristics: shiny bright yellow, circular, pulvinate colonies with an entire margin. Isolates 55-2 and 37-1 were both off-white to dark cream, circular, convex colonies with entire to irregular margins. These isolates when grown on TSA agar produced a brown pigment that stained the colonies and media after 5–7 days at 30°C. On R2A agar pigment was less noticeable. Isolate 39-1 produced yellow, round, spreading umbonate colonies with entire edges. Colonies from either R2A or TSA agar were identified by 16S ribosomal subunit partial DNA sequencing. The genus level identity of the isolates is shown in Table 1 and was determined by sequence comparison of the partial 16S rRNA DNA sequences against the EzTaxon-e database (Kim et al., 2012). Significantly, isolates with similar colony characteristics isolated from independent experiments returned sequences giving the same identification and similar percentage identity to type species.

To determine if these isolates had the potential as indigenous surrogates for the inactivation of Cryptosporidium by UV disinfection, UV dose–response profiles for the isolates were generated by using the collimated beam to deliver a range of measured UV doses to suspensions of isolate cells grown in pure liquid cultures. Figures 1 and 2 shows the UV dose–response curves for three of the isolates.

The UV dose–response profile for R. chironomi grown in TSB was determined. However, the isolate demonstrated reductions in viability over time when stored on TSA media. Dose–response experiments for R. chironomi grown in TSB and plated on TSA showed considerable variation between experiments and this, taken together with the reductions in viability during storage on TSA, made these results unreliable to report.

All the isolates indicated some degree of tailing at higher UV doses (Figures 1 and 2). The two isolates of S. chilensis demonstrated similar UV dose–response relationships and exhibited a biphasic response (Figure 1a,b), an aggregate dose–response curve for S. chilensis based on the data from both S. chilensis isolates is shown in Figure 1c. The aggregate dose–response data indicates that between 2 and 7 mJ/cm² S. chilensis is more resistant to 253.7 nm UV light than Cryptosporidium and therefore that this species might be suitable as a surrogate for UV dose verification for Cryptosporidium inactivation in this UV dose range. These values correspond to maximum Cryptosporidium inactivation of 2.2 to 2.5-log (USEPA, 2006). Above 7 mJ/cm² the S. chilensis isolates were more sensitive to UV than Cryptosporidium and therefore not suitable for dose verification in that dose range.

The F. succinicans isolate was more sensitive to UV than Cryptosporidium between 2 and 12 mJ/cm² (Figure 2). The shoulder region for F. succinicans runs from 4 mJ/cm² making it unsuitable as a UV dose verification measure above this dose.

### 4 | DISCUSSION

In this study, we have sought to address the current inability to directly verify by biodosimetry the dose delivery of operational UV disinfection reactors in drinking water treatment. We have used a UV screening method, similar to Rochelle et al (2010), to enrich for heterotrophic organisms with higher UV resistance than the general population of heterotrophs from pre-disinfection water samples. We identified and determined the UV dose–response of two independent isolates of Sphingopyxis chilensis and an isolate of F. succinicans. Sphingopyxis spp. are known to inhabit diverse environments including, but not limited to, soil, natural mineral water, sea water, wastewater treatment plants (Sharma et al., 2021). S. chilensis and other members of this genus have been isolated from environments with high UV exposure and/or been found to have higher UV tolerance than other heterotrophs (Baraniecki et al., 2002; Joux et al., 1999). Sphingopyxis alaskensis, a marine ultramicrobacterium demonstrated significant cyclobutane pyrimidine dimer (CPD) repair compared to other marine bacteria (Joux et al., 1999). Our study demonstrates a shoulder in the UV dose–response of both S. chilensis isolates. The shoulder of S. chilensis
appears greater than that of *F. succinicans* and may be indicative of a higher CPD repair rate, similar to that reported for *S. alaskensis* (Joux et al., 1999).

*Flavobacterium* spp. are common in both soil and freshwater (Bernardet & Bowman, 2006). The genus *Flavobacterium* has a number of members that cause pathologies in fish (Bernardet et al., 1996; Joux et al., 1999).
Good et al., 2015; Groff & Lapatra, 2000). The UV dose–response of *F. succinicans* has not previously been reported. In contrast to *S. chilensis* there was no distinct shoulder in the *F. succinicans* UV dose–response, suggestive that DNA repair is slower compared to *S. chilensis*, and consistent with the overall higher sensitivity of *F. succinicans* to UV irradiation, although other potential reasons for increased sensitivity cannot be ruled out, e.g. lower cytosine-guanine content or physical reasons related to growth habit. The data from the present study also suggest that UV treatment might be a highly effective treatment for this group of fish pathogens. *F. succinicans* is more sensitive to UV than *Cryptosporidium* throughout the relevant UV doses applied in this study and therefore cannot, in isolation, be considered a useful indigenous biodosimeter for *Cryptosporidium* inactivation.

- *S. chilensis* was more UV-resistant than *F. succinicans*, and generally more similar to the dose–response of *Cryptosporidium*. Significantly, between doses of 2 and 7 mJ/cm², *S. chilensis* was more resistant than *Cryptosporidium* and therefore may be useful up to UV doses of 7 mJ/cm². As such it may be of use for dose-verifying UV reactors that aim to provide at most 2.2-log inactivation of *Cryptosporidium*. Additionally, high sensitivity organisms find application in approaches in which two organisms are used to validate reactors, one with a lower and one with a higher sensitivity than the target organism. Such validations remove the bias introduced by differences in sensitivity between challenge and target organisms. The data suggests the slow growing, oligotrophic Sphingomonadaceae are able to grow in the treatment process and further into the distribution system. As they are common members of biofilm populations the large surface areas of filter substrates in the treatment process and extensive biofilms built up within distribution infrastructure provide biofilms habitats in which they can grow and compete effectively in the low nutrient conditions of partially and fully treated water (de Vries et al., 2019). The Vaz-Moreira et al. (2011) study supports our observation that *S. chilensis* is less tolerant than many of the family as *S. chilensis* was recovered by them only on R2A or *Pseudomonas* Isolation Agar (PIA).

Vaz-Moreira et al. (2011) did not sample pre-chlorination water at the WTP, so the likely densities available prior to chlorination, where UV reactors would typically be installed, can only be estimated. There is evidence that the Sphingomonadaceae family exhibit a range of resistances to chlorination with CT values from (0.03 to 32 mg min/L) for a 4-log reduction (Furuhata et al., 2007). The lower value is similar to the value for a 2-log reduction of *E. coli* at 5°C and pH 6–7 (Clark & Regli, 1993). Combining data from Vaz-Moreira et al. (2011), a process that provides 2-log reduction in *E. coli* may have at most 105 CFU/ml of Sphingomonadaceae. Thus it is unlikely that sufficient densities of *S. chilensis*, a single species within the group, would be present at the pre-chlorination step of the process for routine UV reactor performance monitoring.

Enumeration of the Sphingomonadaceae is complicated by their slow growth rate relative to other heterotrophs. The specific growth rate of *Sphingopyxis alaskensis* of 0.2/h, even under optimum growth conditions (Cavicchioli et al., 2003; Fegatella & Cavicchioli, 2000; Fegatella et al., 1998), is significantly lower than other heterotrophs. As such, in the absence of a means of selection, the slower growing Sphingomonadaceae would be out competed by faster-growing heterotrophs. Slow growth rates combined with a low abundance of potential indigenous surrogates could complicate counting in mixed cultures. If concentration on filters is required to obtain sufficient numbers of organisms for counting, faster-growing members of the population would effectively out-compete the biodosimeters, preventing effective enumeration. As such, competition is likely a confounding issue when enumerating Sphingomonadaceae in natural mixed populations.
To address concerns regarding competition affecting CFU counts, a desirable feature of a potential surrogate would be an inherent selective opportunity to enable growth and counting to be undertaken easily, and enable concentration of surrogates in waters with low abundance. Yim et al. (2010) have published a selective media and PCR identification method for Sphingomonas spp.

5 CONCLUSIONS

1. S. chilensis was identified as a UV dose delivery surrogate for Cryptosporidium inactivation up to 2.2-log inactivation, in the dose range 2–7 mJ/cm² of monochromatic UV light as 253.7 nm.

2. While dose–responses are promising for low dose verification or protocols involving to challenge microorganisms, numbers counted in this study and those estimated from the literature suggest abundances of this single species are unlikely to be sufficient for routine use as an indigenous surrogate. However, the Sphingomonadaceae as a family may warrant further investigation as a group to identify other potential UV dose surrogates.

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DATA AVAILABILITY STATEMENT

The data that support the findings will be available in Mendeley Data at https://data.mendeley.com/datasets/2ds8nn3rhk/draft?view_type=chart&data=ref&num_rows=1000&ref_id=10.17632/2ds8nn3rhk.1 following publication.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.