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UVC inactivation of MS2-phage in drinking water – Modelling and field testing

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ABSTRACT

UVC disinfection has been recognised by the WHO as an effective disinfection treatment to provide decentralized potable water. Under real conditions there are still unknowns that limit this application including the influence of suspended solids and natural organic matter. This work aims to investigate the influence of two key parameters, suspended solids and natural organic matter, on the efficiency of UVC disinfection of surface water to achieve the drinking water quality requirements established by the WHO for point of use (POU) technologies. Kaolinite (turbidity agent) and humic acids (HA, model of organic matter) were used in a factorial design of experiments (Turbidity from 0 to 5 NTU, and HA from 0 to 3.5 mg/L) to investigate their effect on UVC inactivation of MS2 phage in surface water. A collimated beam (12 W) and a commercial UVC disinfection flow system (16 W) designed to provide drinking water at households were used. The UVC flow system both in the laboratory and in the field was able to achieve the reduction requirements established by WHO (LRV >3.5 for all tested conditions), confirming the good performance of the studied UVC disinfection system. The results found in the lab were used to establish a numerical model that predicts the disinfection rate constant as a function of water turbidity and transmittance at 254 nm (confidence level>95%). The model permitted to elucidate the critical effect of low concentrations of HA in reducing the inactivation rate by 40% for 3.5 mg/L-HA compared with 0, the non-significant detrimental effect of turbidity lower than 5 NTU, and the lack of synergistic effects between both parameters at these levels. The UVC flow system was also tested in the field, in Tzabalho, Chiapas (Mexico), and Antioquia (Colombia), with spiked MS2 into natural surface water. This investigation opens a potential application to monitor the performance of UVC systems with surface water by monitoring transmittance at 254 nm as a tool to control UVC domestic systems to deliver safe drinking water in a household without the need of expensive and laborious biological monitoring tools.

1. Introduction

In 2010, the United Nations (UN) explicitly recognized the human right to water and sanitation, which became the sixth Sustainable Development Goal (SDG) (UN, 2018). Many efforts have been devoted to achieving the SDG6 by 2030, guaranteeing universal access to basic sanitation, including safe and clean drinking water, although current data suggests that efforts are not enough. At present, one in three people do not have access to clean drinking water, exposing them to waterborne diseases (UNICEF-WHO, 2019). Many water technologies have been developed to reach water safety, availability, accessibility and affordability for all. However, there is a general lack of treatment processes specifically designed to operate in low-income countries, where high-technological solutions are not implementable due to large

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operation and maintenance costs, dependence on underdeveloped supply chains for the system components, and required technical end-user preparation. There is the need for technological improvement, adequate resources, capacities and targeted strategies to achieve the set SDG6 (WWAP, 2015), especially in remote and rural developing regions.

For these reasons, point of use (POU) technologies, that consider the needs of the areas of intervention and the end-users, have been developed and applied as a more sustainable and accessible solution providing safe access to clean water to remote areas, empowering local populations and decreasing the risk of waterborne diseases (Sobsey et al., 2008). The World Health Organization (WHO) established a protocol for the efficacy assessment of household water treatment and storage (HWTS) technologies, focusing on the performance against few microbial indicators, and recommended consistent utilization by the users (WHO, 2016). HWTS technologies for disinfection commonly applied include chemical treatment using soluble chlorine-based tablets, filtration typically using ceramic and sand or bio-sand filters (Sobsey et al., 2008), solar treatment using SODIS (McGuigan et al., 2012) and UVC treatment, which is the most promising alternative due to its high efficacy against most waterborne pathogens, its rapid action and minimal operation cost and consumables (only electricity to run the lamps and a pump) (Lui et al., 2016).

UVC disinfection in low-income and remote regions has advantages as a POU system over other approaches because it does not require technical knowledge for operation, does not affect taste and odour of the treated water, does not produce cancerogenic disinfection by-products (DBPs), and no chemical compounds are required (Curtis et al., 2016). However, it may require physical pre-treatment to improve the optical quality of the water, i.e. to reduce its turbidity and organic content, which may be addressed to some extent with the installation of a low-cost filtration system. A power source may not be available in remote and rural areas, which can be resolved with photovoltaic solar panels. The need to replace UV lamps every ~12 months according to the UV lamp lifetime (8 000 to 10 000 hours) maybe a limiting factor in low-income communities even if they are not really costly (eg. ~ 10 $ per 15W-lamp) (Lui et al., 2016). Most of the applications involved the installation of a single low pressure UVC lamp due to its high germicidal efficiency, long lamp lifetime, vast availability on the market and proven POU commercial designs (Bolton and Cotton, 2008). Currently, UV-LED technology is seen as the most promising alternative to UV mercury lamps for their energy efficiency, compactness, robustness, versatility and probable improved inactivation effectiveness by applying multiple wavelengths and adjustable pulsed illumination (Vilhunen et al., 2009; Song et al., 2016). However, it is still a technological solution under development that cannot be considered a sustainable alternative to LP (low pressure) UVC systems in low-income communities, mainly due to its very high cost and low availability on the water-devices market.

Following the development of a standardized method for the determination of the UV dose in collimated beam systems (Bolton and Linden, 2003), numerous studies were conducted on the evaluation of UV disinfection effectiveness and the determination of the effective UV dose necessary for the inactivation of selected pathogens. Hijnen et al., (2005) identified the relationship between waterborne pathogens inactivation and UV dose as follows – from higher to lower sensitivity to UV radiation – bacteria, protozoa cysts and oocysts, bacterial spores and viruses, which are the most resistant pathogens to UV due to the presence of RNA containing uracil instead of thymine, which is the most sensitive base to UV. Moreover, MS2 bacteriophage was confirmed to be an appropriate model organism for both biodosimetry experiments and UV disinfection validation, due to its high UV resistance when compared to all other waterborne pathogens, excluding adenoviruses (Leenheer and Croue, 2003).

The optical properties of the water or, more specifically, its transparency in the UVC range is the key to achieve successful disinfection results, as expected for a UV-driven process. Thus, turbidity and UV-transmittance (UVT) are the most important water parameters in UV disinfection. UVT is determined by the presence of inorganic and organic light absorbing compounds including iron salts, nitrates and more importantly natural (dissolved) organic matter (NOM and DOM), quantified by dissolved organic carbon (DOC) (Leenheer and Croue, 2003). Humic matter was found to be a fundamental component of DOM, having as main constituents fulvic acids and humic acids (HA), which are responsible for UV and visible light absorption and add colour to natural waters (Rodriguez and Nunez, 2011, Filella, 2009). HA has been widely used as a model compound to simulate environmental DOC in a number of studies (Abbt-Braun et al., 2004). Small amounts of HA (below 3.5 mg/L) in water can produce a high attenuation of the UV radiation (UVT (254 nm) < 80%, approx.), due to their high absorption spectra in the short UV range. Water turbidity is defined as the scattered light measured in the visible range, at a 90° angle to the direction of the light source. The scattering particulate matter, organic and inorganic, in the water scatters and or absorbs UV radiation, reducing the UV fluence rate delivered to the microorganisms (EPA, 2006). Micro-particles (1 – 10 μm diameter) were found to shield microorganisms from UV light through shading and encasement mechanisms (Loge et al., 2001). According to the WHO, it is recommended to reduce water turbidity below 1 NTU – and should never exceed 5 NTU – before applying an UV disinfection treatment (WHO, 2014). Common compounds used as turbidity models for inorganic suspended solids (SS) are kaolinite and test dust (WHO, 2014). These accepted models for suspended matter can be added to the water to attain the desired light scattering and turbidity effects regardless their chemical nature (organic or inorganic), as they are non-soluble. The single effect of turbidity was studied for MS2 UV inactivation showing non-significant effect over the dose for turbidity below 5.25 NTU (Templeton et al., 2006); increasing levels of transmittance showed no decrease in MS2 inactivation for applied UV doses between 10-100 mJ/cm^2, when the proper correction factor was applied (Batch et al., 2004).

Very little research has been done on the systematic analysis of turbidity and/or humic acids and their effect over UVC disinfection efficiency. Most of the contributions mention these aspects either making simple comparisons between few values of turbidity or scarcely indicating the recommended UVT required to achieve a desired level of disinfection. Moreover, there is not a lot of information on the separate effects, the possible synergistic effects and the possible interactions between the two critical parameters, turbidity and UVT, simulating real conditions commonly found in the field. The understanding of these effects is key know-how for an efficient application of UVC disinfection to natural surface water sources.

For the first time this contribution presents a systematic research of the role of two water quality parameters -turbidity and dissolved organic matter- and their interference in the efficiency of UV-viral disinfection in surface water. Kaolinite load and HA concentration were selected as the two factors of a complete factorial design of experiments, where the effect of turbidity and dissolved organic matter in UVC disinfection efficiency were evaluated alone and together. MS2 bacteriophage was selected as viral surrogate due to its high resistance to UVC. A collimated beam (12 W) and a commercial UVC (16 W) disinfection flow system, specifically designed to provide drinking water at households, were used for all experiments in the lab and in the field. The results were analysed to determine parametric relationships between both parameters and develop a predictive model for the inactivation rate constant as a function of turbidity and UVT. The gained knowledge has been applied to a real case of UV disinfection for the production of safe drinking water in rural communities of Colombia and Mexico, achieving the WHO drinking water quality requirements (WHO, 2014), that allowed for model validation.

2. Materials and methods

2.1. Experimental design of lab tests

The experimental design (DoE) was constructed to evaluate the effect of DOM and turbidity on UVC disinfection efficiency. The concentration of humic acid (HA) and turbidity were chosen as the DoE factors. The DoE was based on a full-factorial design characterized by two factors with three levels each, identifying nine experimental conditions (Table 1). For each experimental condition, MS2 inactivation experiments were conducted in triplicates, randomly and independently to guarantee statistical significance of results. Inactivation experiments were conducted in the collimated beam system for all 9 conditions, and in a UVC disinfection flow system operating at three flowrates (1.5, 3.0 and 5.2 L/min) only for conditions 1 and 9, namely the best and the worst water quality conditions.

2.2. Synthetic water sample preparation

To evaluate the effect of DOM and turbidity, HA was chosen as model organic compound for its UV radiation absorption characteristics (WHO, 2014), and kaolinite as inorganic suspended solid as turbidity agent. Technical grade HA-sodium salt (H16752, Sigma-Aldrich) and alumina silicate (03584 SIAL, Sigma-Aldrich) were used. The concentration range for HA [0 – 3.5] mg/L and turbidity [0 – 5] NTU were chosen considering: (i) the triple filtration pre-treatment unit (consisting of three commercial pleated filters installed in series of 10, 5 and 1 μm, respectively) installed before the UVC reactor in the system used in the field, (ii) WHO recommendations on turbidity levels for optimal UV disinfection treatment (WHO, 2014), and (iii) literature recommendations on input water quality, as turbidity below 1-2 NTU and light transmission at 254 nm (UVT_{254}) above 75-80% (Abbt-Braun et al., 2004; Cantwell et al., 2008). To quantify the effect of turbidity and UVT_{254} on UVC disinfection performance, the log inactivation values of MS2 were measured as a function of applied UV dose for all water conditions (Table 1). Water matrices were characterized by measuring pH, turbidity, UVT_{254} and absorbance at 254 nm (A_{254}), and total organic carbon (TOC). UVT_{254} and A_{254} were measured with JENWAY 6305 spectrophotometer, calibrated with ultra-purified water in a 1cm-path length quartz cuvette. TOC was measured by TOC (mg/L) – Absorbance (-) reference curve at wavelength of 254 nm defined by plotting TOC and absorbance values of 10 HA solutions (1, 2, 3, 4, 5, 10, 20, 30, 40 and 50 mg/L). Reference TOC measurements were measured in a Shimadzu TOC-5000 Analyzer.

Table 1
Design of experiment factors identifying the three levels (-1, 0, +1) of each factor, being humic acid concentration (HA) and turbidity. Details on the selected values are found in Section 2.2.

Experiment	Flow rate (L/min)	Replicates	Factors HA	Factor levels Turbidity	HA (mg/L)	Turbidity (NTU)
Collimated beam system – batch condition						
1	-	3	-1	-1	0	0
2	-	3	0	-1	1.75	0
3	-	3	1	-1	3.5	0
4	-	3	-1	0	0	2.5
5	-	3	0	0	1.75	2.5
6	-	3	1	0	3.5	2.5
7	-	3	-1	1	0	5
8	-	3	0	1	1.75	5
9	-	3	1	1	3.5	5
UVC Reactor system – flow condition						
1	1.0	3	-1	-1	0	0
	3.5	3				
	5.2	3				
9	1.0	3	1	1	3.5	5
	3.5	3				
	5.2	3				

2.3. MS2 bacteriophage strain and enumeration

MS2 was analysed in triplicate following the double-layer agar method (Adams, 1959). MS2 bacteriophage (ATCC 15597-B1) and its *Escherichia coli* host (ATCC 15597) were obtained from ATCC®. A glycerol stock solution of MS2 kept frozen at -80°C and an overnight culture of *E. coli* host were used. The MS2 stock solution concentration was 10^{13} plaque-forming units per mL (PFU/mL), which was diluted with PBS to obtain 10^6 PFU/mL. The *E. coli* host (from stock solution) was sowing in a Chromocult® Coliform Agar (Merck) plate incubated at 36±2 °C for 21±3 h. A single blue colony from the Chromocult plate was transferred to 15 mL of tryptone soya broth (TSB, Oxoid) falcon tube, that was incubated at 36±2°C for 12 h in a shaker incubator (200 rpm). The suspension was centrifuged at 4,000 rpm for 5 min. The supernatant was suspended with phosphate borate saline (PBS) solution (Oxoid) to obtain an *E. coli* host concentration of 10^{12} CFU/mL.

For MS2 detection and enumeration, 1 mL of sample and 100 μL of *E. coli* host (from the overnight culture) were poured into a Bijou tube with melted Sloppy Agar, which was prepared with TSB and Agar Bacteriological No. 1 (Oxoid). The content of the Bijou tube was poured onto a Tryptone Soya Agar (TSA) plate (Oxoid). Petri dishes were incubated at 36±2°C for 21±3 h and lytic plaques were enumerated as MS2.

2.4. Collimated beam inactivation experiments

The collimated beam system was constructed following standard guidelines (Bolton and Linden, 2003). A LP mercury lamp with nominal power of 12 W and emission at 254 nm (Philips) was used, together with a 100 mL sample vessel with magnetic stirrer (more details in supplementary materials and Figure S1.a). Prior to UVC exposure, the LP lamp was warmed-up for 3 minutes. Water matrix samples (100 mL) were spiked with 100 μL of MS2 bacteriophage - PBS suspension, getting an initial MS2 concentration of 10^6 PFU/ml. The water samples were placed at the centre of the collimated beam on the magnetic stirrer to ensure continuous mixing, and they were exposed to UVC radiation. Water samples of 1 mL were acquired at specific times during the experiment. UVC doses ranged between 60 – 400 mJ/cm^2 (60, 90, 150, 180, 210, 240, 260, 300, 350 and 400 mJ/cm^2) - corresponding to irradiation times between 3.44 – 22.95 min (3.44, 5.16, 8.61, 10.33, 12.05, 13.77, 14.92, 17.22, 20.08, 22.95). For each experimental condition, eight increasing UVC doses were selected within the range to analyse the dose-response correlation. Samples with higher turbidity and UVT_{254} were tested with higher UVC dose values to obtain significant countable results. Water samples were limited to 1 mL to minimize modifications of conditions during experiment execution. Serial ten-fold dilutions of the samples were performed and assessed in triplicates (Section 2.3). MS2 count was performed and expressed as logarithmic reduction value (LRV) with respect to the initial concentration of each experiment.

For each experiment two positive and four negative controls were performed. Positive controls ensured MS2 culture stability during the experiment: MS2 working solution was plated before and after the experiment. Negative controls ensured that there was no virus or host contamination in any of the materials involved.

Sampling times were kept constant for all experiments, and they were determined by Eq. (1).

$$D_{254} = t \cdot E_{254} \quad (1)$$

where D_{254} is the selected UVC dose at 254 nm [mJ/cm^2], t the exposure time [s], and E_{254} [mJ·cm^{-2}·s^{-1}] the lamp's average irradiance at 254 nm incident on the water surface. E_{254} was measured using an optic fibre

Flame S-UV/VIS-ES spectroradiometer (Ocean Optics) to be 0.290 mW/cm^2 and was corrected to calculate the average UVC irradiance (E_m) in the water volume for each experimental condition, as described by Bolton and Linden (2003). E_m was then used to calculate the corrected UVC dose for each condition.

2.5. UVC flow inactivation experiments

The flowrates at which the experiments were performed were chosen for the UVC system's characteristics, the need to meet WHO comprehensive protection reduction requirements (≥3 LRV –log-reduction value– for viruses) (WHO, 2016) and to deliver a sufficient amount of potable water to households (20 – 50 L·person^{-1}·day^{-1} (UN, 2020)). The UVC reactor consists of a commercial UVC sterilizer (UltraRays SDE-016PH model, IWE UV Water Filters) with an annular Philips TUV lamp (16 W, 254 nm) in a quartz case (QT5-360 model) and U-shaped stainless-steel reactor case (304SS model) (more details in Supplementary materials, Figure S1.b and Figure S.3). The UVC fluence of the reactor was determined by chemical actinometry following the iodide-iodate method described by (Rhann, 1997). Prior to experiments, the system was cleaned by pumping a 3%-hydrogen peroxide solution and washing thoroughly. The UVC lamp was warmed-up for at least 3 minutes prior to experiments. Each 5 L water volume was spiked with MS2 and thoroughly mixed. Samples were taken at the inlet to determine initial MS2 concentration and in triplicates at the outlet of the reactor after a time interval equal to three times the hydraulic retention time of the reactor, to ensure the outlet flowrate was stabilized to the selected value (Supplementary material, Table S.1).

2.6. Statistical analysis and numerical model

The inactivation results obtained with the collimated beam were analysed in three phases to identify the correlations between turbidity and UVT_{254}. A linear regression analysis was carried out between the UVC doses and the achieved LRVs to obtain the inactivation rate constant. The linear regression analysis was conducted for all 27 experiments of the DoE, obtaining three values of inactivation rate constant for each experimental condition. These inactivation rate constants were employed in a general linear model (GLM) statistical analysis, performed using the software Minitab (version 2019), to evaluate the effect of UVT_{254} and turbidity on inactivation rate constants.

The complete GLM model was defined considering both single variables and their interaction terms up to the second order (Eq. 2). The UVC inactivation rate constants were the response variables (Y) while turbidity and UVT_{254} were the independent variables (X_1 and X_2).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 {X_1}^2 + \beta_4 {X_2}^2 + \beta_5 X_1 X_2 + \varepsilon \quad (2)$$

where β_j are the regression coefficients and ε the random error. The adequacy of the GLM was assessed by analysis of variance and analysis of residuals, based on a normal probability plot and Shapiro test. The GLM (Eq. 2) was simplified (Eq. 3) considering only single variable and interaction terms up to a first order:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon \quad (3)$$

The adequacy of this GLM was again assessed by analysis of variance and analysis of residuals.

A second linear regression analysis was carried out between the corrected applied UVC dose and measured MS2 LRVs to determine the MS2 inactivation curve.

2.7. Field tests

Field tests were performed by analysing the inactivation of MS2 in different natural water sources at four field locations to assess the efficiency of the UVC water treatment system installed. Two of the natural surface waters, a spring and a waterhole, were collected in the community of Tzabalho, a native and isolated community in Los Altos de Chiapas, south Mexico. The other two water sources were collected from Quebrada La Miel and Quebrada Santa Rita in Medellin, Antioquia Colombia.

For Mexico, the two natural water sources considered (Fig. 1) are used daily by local families for drinking, cooking and personal hygiene without any treatment. Water batches (15 L) of each source were collected in clean lid-closed containers and stored at ambient temperature in the dark for few hours (<24 h), for particle sedimentation and reduction of turbidity. As MS2 is not naturally present in the environment, water batches (15 L) were spiked with a stock solution of MS2. Initial concentration was of 10^6 PFU/mL. The UVC disinfection system (Evans®, Mexico) had a 16 W LP-lamp (emission 254 nm). Due to high turbidity (>100 NTU waterhole), the water was pre-treated with a filtration unit consisting of three (2.5"x10") spun filters of 10, 5 and 1 μm (Hydronix Water Tech., USA) installed in series. The surface water with MS2 was pumped through the system at 3.3 L/min using a pressure pump (Seaflo, SFDP1-012-03521, China). Water samples were taken before and after treatment (at inlet and outlet of the system) for analysis and MS2 enumeration. Turbidity, A_{254} and UVT_{254} were measured with a turbidimeter (Hana instruments, HI-98703) and UV/VIS spectrophotometer (Pharmacia LKB-ultrisoecIII, 80-2097-62). TOC was estimated by absorbance-TOC calibration curve (section 2.2) due to field limitations.

In Colombia, water batches of 10 L for each source were collected in clean lid-closed containers and stored in the dark at ambient temperature for preliminary particle sedimentation. Water batches (10 L each) were spiked with stock solution of MS2 to get an initial concentration of 10^6 PFU/mL. The water treatment system installed was similar to the one in Mexico, but saw a filtration unit consisting of two compact polypropylene filters of 5 μm and 1 μm installed in series and a water flowrate of 4.3 L/min. Water samples were taken before and after treatment (at inlet and outlet of the system) for analysis and MS2 enumeration. Turbidity, A_{254} and UVT_{254} were measured by portable turbidimeter (HACH 2100Q) and UV/VIS spectrophotometer (Genesys ThermoFisher). Other water quality parameters measured before treatment were pH, dissolved oxygen, electrical conductivity with a multiparameter analyzer (HACH40d) and TOC with a TOC analyzer (Shimadzu 5264 TOC-VCPH).

3. Results and discussion

3.1. MS2 UVC dose response (collimated beam) – effect of kaolinite and HA

The results of all MS2 inactivation experiments for all 9 water conditions were evaluated with the linear least-square fitting method, estimating inactivation rate constant and intercept with the corresponding correlation coefficient R^2 (Fig. 2; Supporting material Figure S.6).

Linear regression analysis was applied to determine the inactivation curves for the 9 conditions of the experimental plan, obtaining an average value of MS2 inactivation rate constant for each experimental condition (Table 2). The inactivation rate constant values ranged between 0.012 – 0.020 cm^2/mJ. These were plotted separately against UVT_{254} and turbidity (Fig. 3) to have a clear visual representation of results.

The relation between LRVs and applied UVC doses followed a linear trend (Fig. 2) as described by Chick and Watson inactivation model (Watson, 1908), showing neither shouldering nor tailing effect, as previous studies (Hinjen et al., 2005; Batch et al., 2004). Table 2 shows that both UVT_{254} and turbidity affected UVC inactivation rate constant to a different extent. The results obtained in presence of HA (tests 2, 3, 5, 6, 8, 9) resulted in decreased inactivation rate constants for the same applied UVC dose in comparison to those without HA (tests 1, 4, 7,

Fig. 1. Sources sampled in the indigenous community of Tzabalho (Chiapas, Mexico), spring (a) and waterhole (b).

Fig. 3.a). Turbidity showed a very small effect on the inactivation rate constants when comparing experiments with different turbidity levels and equal concentrations of HA (Fig. 3.b). In the absence of HA, the inactivation rate constant was 0.019 (±0.002) cm^2/mJ for 0 NTU, 0.020 (±0.002) cm^2/mJ for 2.5 NTU, and 0.018 (±0.003) cm^2/mJ for 5 NTU. A similar trend was observed in the presence of 1.75 mg/L-HA, with a lower kinetic constant of 0.013 – 0.014 (±0.001) cm^2/mJ, and for the case 3.5 mg/L-HA, with a very similar kinetic constant of 0.012 – 0.013 (±0.001) cm^2/mJ. The observed effect of turbidity is in agreement with previous results by Batch et al. (2004) and Hijnen et al. (2006), which showed no decrease in inactivation rate constant for turbidity <0.3 NTU and <5.25 NTU respectively.

Thus, the HA concentration which contributes directly to UVT_{254}, greatly affects the inactivation rate constant, whereas turbidity does not have a significant effect (Table 2, Fig. 3). This can be attributed to radiation absorption by HA, which negatively influences the efficiency of advanced oxidation treatment processes (UV, UV/H_2O_2, Photo-Fenton, Photocatalysis) by an increase in light radiation absorbance reducing the water matrix UVT_{254} (Cantwell et al., 2008; Templeton et al., 2006). Cantwell et al. (2008) showed a 50% decrease in LRV of bacteria for water samples containing 120 mg/L of HA with respect to those containing no HA, due to UV radiation absorbance of HA and HA coating of bacteria cells that reduced their sensitivity to UV radiation. Templeton et al. (2006) found similar results when studying UV inactivation of bacteriophages in samples containing HA up to 150 mg/L.

To confirm the insignificant effect of turbidity on the inactivation rate constant (within the range studies in this work) the optical properties of kaolinite suspensions were experimentally determined, showing that kaolinite particles only scatter light without absorbing it (Li et al., 2018). The absorption coefficient and the scattering coefficient were found equal to 0 [cm^2/mg] and 5.86 [cm^2/mg] respectively. Further investigations on the optical properties of kaolinite and their role on UV disinfection will be carried out as a result of these findings. It can be concluded that the optical behaviour of kaolinite particles does not have a detrimental effect on viral UVC inactivation, while the UVC light attenuation due to UV radiation absorption by HA strongly reduces the viral inactivation . The results were also statistically analysed to determine potential interactions between both factors (section 3.2).

3.2. Statistical analysis and modelling

To investigate the interaction between the inactivation rate (k), turbidity (symbolised as T in the following equations) and UVT_{254}, a statistical analysis was conducted for two models of decreasing complexity: a complete GLM with Equation 4 (GLM_1) and a simplified GLM with Equation 5 (GLM_2).

$$k = -7\cdot10^{-6}\cdot UVT_{254}^{\ 2} + 6\cdot10^{-6}\cdot T^2 + 0.0017\cdot UVT_{254} + 0.0055\cdot T - 6.2\cdot10^{-5}\cdot UVT_{254}\cdot T - 0.08 \quad (4)$$

$$k = 4.4\cdot10^{-4}\cdot UVT_{254} + 1.84\cdot10^{-3}\cdot T - 1.7\cdot10^{-5}\cdot UVT_{254}\cdot T - 0.02 \quad (5)$$

where k (cm^2/mJ) is the inactivation rate constant, UVT_{254} (%) is the measured transmittance at wavelength 254 nm and T is the measured

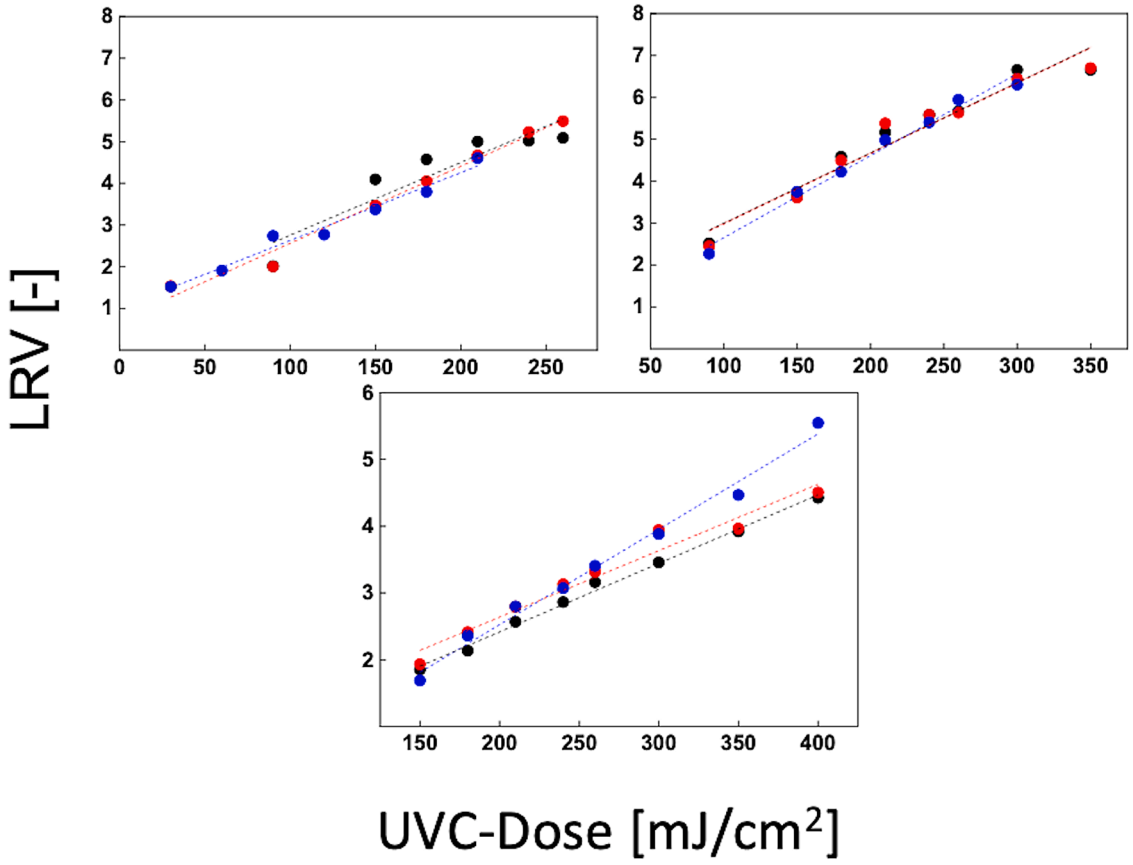

Fig. 2. UVC dose response of MS2 in three conditions, #1 (0 NTU, HA=0 mg/L), #4 (2.5 NTU, HA=0 mg/L), and #9 (5 NTU, HA=3.5 mg/L), the triplicates for each one (dots in three colours), and the corresponding linear least-square fittings (lines).

turbidity level (NTU). The two models were statistically analysed resulting in a R^2 of 93.8% and 86.7% for GLM_1 and GLM_2 respectively, determining a goodness-of-fit of both models. GLM_2 was selected for the study for its mathematical simplicity compared to GLM_1, while guaranteeing goodness-of-fit. The analysis of variance (confidence interval 95%) for GLM_2 determined that the only significant term was the UVT_{254} term since the p-value was 0.006 (less than the significance level of 0.05). The other terms (turbidity and the interaction term) resulted having a p-value greater than the significance level, respectively of 0.566 and 0.648. The model was further simplified obtaining a simple linear model (LM) with k as a function of UVT_{254} (Equation 6):

$$k = 3.8 \cdot 10^{-4} \cdot UVT_{254} - 0.018 \quad (6)$$

The statistical analysis resulted in a R^2 value of 84.6%, confirming the results of GLM_2 and showing that the applied simplification only results in a 2% decrease of R^2. GLM_2 and LM adequacy was also assessed through the analysis of residuals, showing that all assumptions at the base of the analysis (residuals normally distributed, random and independent distribution of residuals, constant variance of residuals) were met. Thus, both GLM_2 and LM adequately describe the inactivation rate constant, k. From the statistical analysis, it can be concluded that, for the ranges considered in the present work, UVC disinfection efficiency is affected significantly by UVT_{254}, yet insignificantly by turbidity. Similarly, Batch et al. (2004) conducted a t-statistics which indicated no decrease in MS2 inactivation with increasing turbidity, confirming the statistical analysis results.

The proposed model (Equation 6) was used to estimate the viral inactivation rate constant for each experimental condition based on the samples' UVT_{254} measurements. The model was validated by comparing the estimated viral inactivation rate constant values with the experimental ones determined through linear regression (Table S.2). The results show that the proposed model is able to predict the viral inactivation rate constant quite accurately. Almost every estimated inactivation rate constant value falls within the 95% CI of the experimentally determined inactivation rate constants, with exception of experiment #2 and #9 which distance 0.0007 and 0.0002 from the upper bound and lower bound of their respective CI. The goodness of the model can also be observed from the percent error which is <10% for all estimated inactivation rate constant values, except #2 (Fig. 4).

3.3. MS2 dose-response curve analysis

The following analysis was carried out to compare literature and expands the understanding of the MS2 dose response. A linear regression analysis was conducted on the inactivation experiment results considering the corrected applied UVC dose, for the determination of the effective MS2 inactivation curve. The inactivation curve (Fig. 5.a) was defined by Equation 7:

$$LRV = 0.0261(\pm 0.0007) \cdot \text{UVC dose} + 0.64(\pm 0.08) \quad (7)$$

where the UVC dose is expressed in mJ/cm^2 and LRV is dimensionless.

It can be seen that the correction suggested by Bolton and Linden (2003) was able to adequately correct the applied UVC dose obtaining a uniform linear distribution of the data points (Fig. 5.a). It should be noted that not all data points perfectly follow the linear regression curve (Fig. 5.a). This can be attributed to the applied correction (Bolton and Linden, 2003), which does not consider: (i) sample depths greater than 1 cm for which an appropriate divergence factor should be calculated, and (ii) effects of turbidity as light scattering that might increase the UVC fluence rate in the reactor, increasing the delivered UVC dose.

It is noticeable that the inactivation rate constant (k) of the

Table 2
Main properties of the water matrices (Turbidity, TOC, UVT_{254}) in all the experiments. Inactivation rate constant k of MS2, intercept and R^2-coefficient obtained via linear regression.

Experiment	Turbidity [NTU]	TOC [mg/L]	UVT_{254} [%]	k (± 95% CI) [cm^2/mJ]	Intercept [-]	R^2 [-]
1	0.00	0	100.0	0.019 (0.002)	0.43	0.96
2	0.00	0.36 ± 0.03	89.2 ± 0.3	0.014 (0.001)	0.15	0.96
3	0.00	0.77 ± 0.01	80.5 ± 0.3	0.013 (0.001)	0.25	0.97
4	2.68 ± 0.66	0.08 ± 0.01	95.5 ± 0.2	0.020 (0.002)	0.52	0.97
5	2.45 ± 0.32	0.51 ± 0.03	85.5 ± 0.6	0.014 (0.001)	0.12	0.97
6	2.55 ± 0.53	1.06 ± 0.05	80.1 ± 0.4	0.012 (0.001)	0.08	0.96
7	4.85 ± 0.17	0.45 ± 0.38	91.4 ± 7.3	0.018 (0.003)	0.82	0.91
8	5.40 ± 0.57	0.71 ± 0.01	81.5 ± 0.2	0.013 (0.001)	0.17	0.97
9	5.31 ± 0.62	0.95 ± 0.17	77.0 ± 3.3	0.013 (0.001)	0.04	0.96

Notes: Values were calculated as the average of five single measurements and error is the standard deviation. The pH value of all samples ranged between 7.0 and 7.5 ± 0.1, with an average value of 7.3 ± 0.2. CI is the confidence interval.

inactivation curve defined by Equation 7 is slightly lower than the ones reported in literature (Table S.3) (Loge et al., 2001; Cantwell et al., 2008; Beck et al., 2015, 2016). This could be due to the higher corrected UVC dose values considered for the inactivation experiments, which ranged from 12 – 267 mJ/cm^2 compared to those used in past studies (EPA, 2006; Park et al., 2011). The inactivation curve could be better represented by two linear models (Fig. 5.b): the first for low UVC doses ranging from 10 – 90 mJ/cm^2, and the second for high UVC doses ranging from 90 – 250 mJ/cm^2. The inactivation rate for the first curve is similar to the one reported in literature, which was also determined for a low UVC dose range from 5 – 139 mJ/cm^2 (Hijnen et al., 2006); while the inactivation rate (k) for the second curve resulted equal to that of the inactivation curve defined by Equation 7 (Table S.3). These results are in good agreement with the ones of Beck et al. (2015), showing that the dose-response curve has curvature and is not completely linear. For this reason, a two linear regression model for the dose-response curve would have great application (i) as a tool in estimating the viral inactivation of a LP UVC system in a rapid and precise way, and (ii) in UVC flow reactor modelling for which it is a fundamental parameter requiring high accuracy.

3.4. UVC inactivation in flow systems

The aim of these tests is to show the efficacy of the UVC flow system to provide clean and safe water at a household. The flow inactivation experiments were conducted for experimental condition #1 utilizing pure water (experiment 1 of DoE) and experimental condition #9, with 3.5 mg/L of HA and turbidity of 5 NTU (experiment 9 of DoE). The results are summarised in Table 3 and confronted with LRV requirements for HWT systems established by WHO, which state that for viruses a minimum of 3 log-reduction and a maximum of 5 log-reduction are needed to achieve a 'protective' or a 'highly protective' level protection for viral waterborne pathogens (WHO, 2016). The LRVs for condition #1 were higher than those for condition #9 due to the lower turbidity and UVT_{254} of #1. For #1, over a 5 LRV was obtained with a flowrate of 1.50 L/min, and over a 3 LRV with flowrates of 3.00 and 5.20 L/min. For #9, over 3 LRV was achieved only with flowrate of 1.50 L/min; while it was not able to meet a 3 LRV with the greater flowrates. Therefore, with water characterized by significant content of organic matter (few mg/L of HA or similar), the operating flowrate should be set lower than 1.50

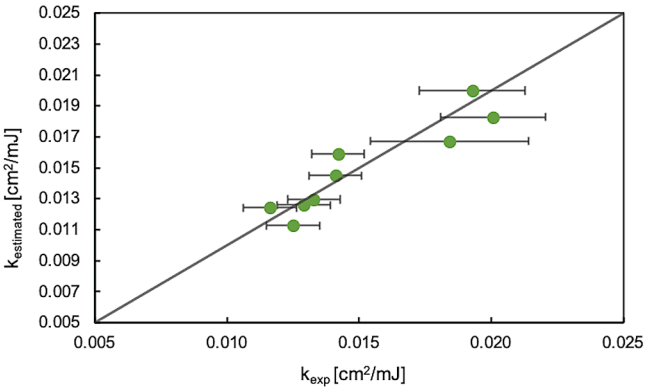

Fig. 4. Inactivation rate constant results of the simplified proposed LM model ($k_{estimated}$) compared with the inactivation rate constant values determined experimentally (k_{exp}). The 95% CI of k_{exp} is also shown.

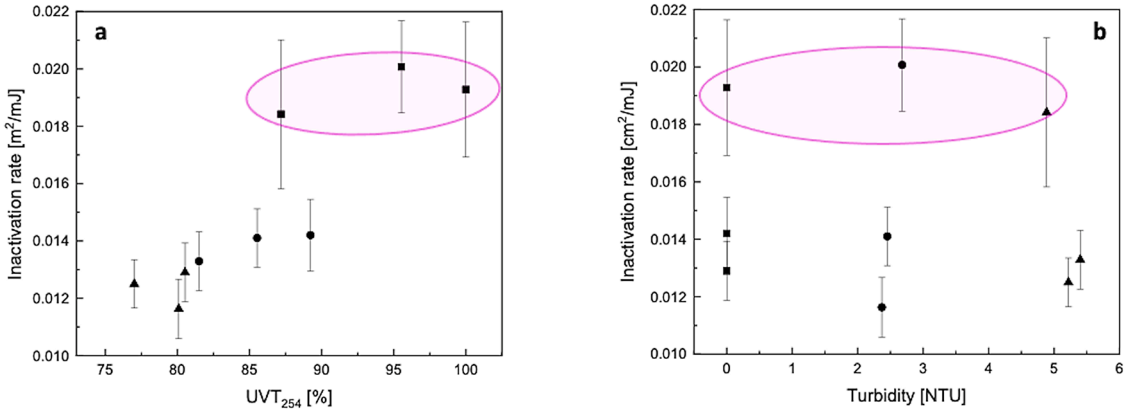

Fig. 3. Inactivation rate constants of the nine experimental conditions (with the 95% CI) versus UVT_{254} (a) -where square, dot and triangle points correspond to HA concentration values of 0, 1.75 and 3.5 mg/L, respectively and Turbidity (b) -where square, dot and triangle points correspond to turbidity values of 0, 2.5 and 5 NTU, respectively. The points in the pink circle are the experiments without HA.

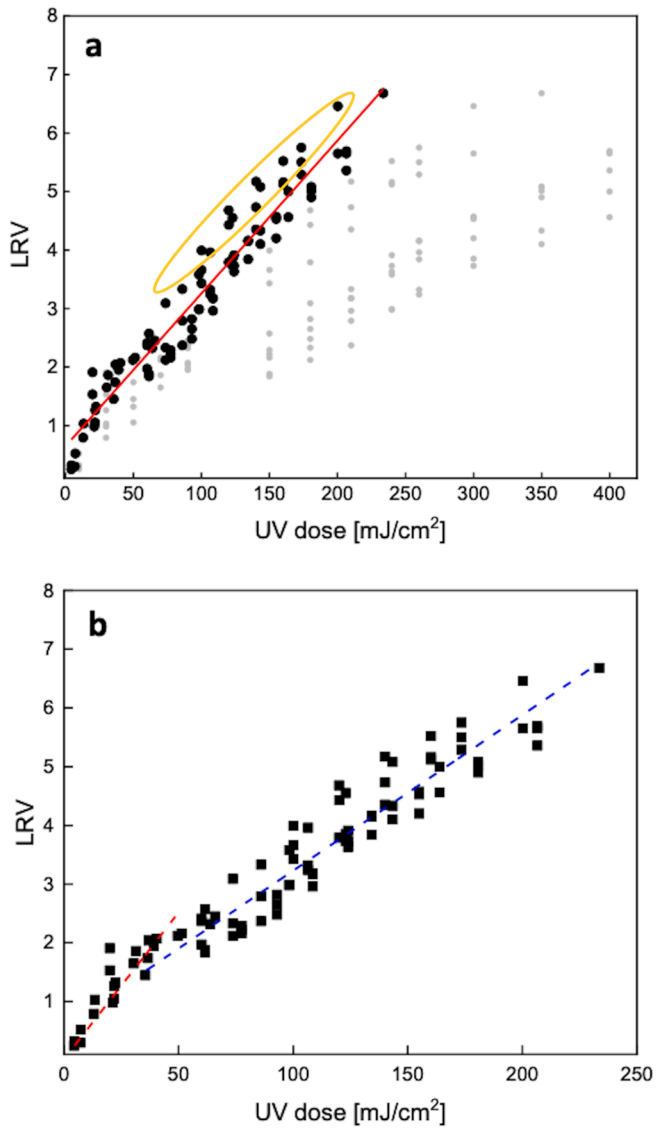

Fig. 5. (a) LRVs plotted against corrected UVC dose (black dots) and not corrected UVC dose (grey dots) respectively, with identification of MS2 bacteriophage inactivation curve (red line). The dots in the yellow area are the dots that are furthest from the linear regression curve. (b) Inactivation curve described by two fitting lines: one for low UVC dose range (red) and the other for higher UVC doses range (blue).

L/min to meet the criteria for a 'highly protective' HWT system.

These results are in agreement with the ones shown by Brownell et al. (2008), who assessed the performance of a similar UVC technology determining that the system was able to achieve 4.1 – 4.5 LRV of MS2, when operated at a flowrate of 5 L/min characterized by very low absorbance (0.002 - 0.01 cm^{-1} at wavelength 254nm). Younis et al. (2019) also showed the ability of a UVC technology equipped with a swirl at reactor inlet to achieve a 7.1 and 5.5 viral LRV for flowrates of 9.46 and 12.9 L/min, respectively both with water UVT_{254} of 95%. Although these results are in accordance with those obtained in the collimated beam inactivation experiments (Section 3.1), showing the negative effect of high HA concentration in the water on viral UVC disinfection efficiency, they should be further analysed coupled with reactor hydraulics and UVC fluence distribution in the reactor chamber through reactor modelling.

3.5. Field tests results

Results from the field are summarized in Table 3. Chemical actinometry determined a lamp intensity of 5.2 mW/cm^2. In Mexico, the water quality of the spring source was better than the one of the waterhole, where turbidity was above 100 NTU. With settling and filtration pre-treatment, turbidity was reduced to 12.1 NTU and 5.00 NTU, respectively. Organic matter (OM) decreased 20.3% and UVT_{254} increased from 59.3% to 69.0%. Initial MS2 concentration was of 1.3 × 10^6 PFU/mL. Spring water presented turbidity and OM levels below 1 NTU and 1 mg/L, respectively. With filtration, turbidity was reduced to 87.0% and OM decreased 6.5%. UVT_{254} slightly increased from 83.4% to 84.3%, due to the retention of small amounts of dissolved organic and suspended matter in the filters. Initial MS2 concentration was of 3.2 × 10^6 PFU/mL.

With regard to surface waters from Colombia, the water quality of the two water sources resulted similar. Initial turbidity for the source La Miel ranged between 3.72 and 2.10 NTU, while the one for Santa Rita ranged between 8.13 and 2.19 NTU. After filtration, turbidity decreased by 20% to 33% for both sources and UVT_{254} increased by 2.07% for La Miel and between 1.35% - 5.43% for Santa Rita. Initial MS2 concentration was in the order of 10^6 PFU/mL for both sources.

Field results are in agreement with laboratory results (Figure S.7) showing that the considered UVC disinfection system is able to meet the WHO reduction requirements not only in the laboratory but also in field. Even in the worst water quality condition (M-Borehole water), UVT_{254} of 69% and turbidity of 5 NTU, the system was able to achieve a 'protective' level of 3.5 viral LRV.

One of the main problems with UVC disinfection in rural developing regions, is the need to monitor the system's operation to guarantee disinfection efficiency without the frequent microbiological testing. Lab

Table 3
LRVs obtained for specific flowrates conducted with the UVC flow reactor.

Water sample	Q [L/min]	Turbidity [NTU]		UV_{T254} [%]		MS2-initial [PFU/mL]	MS2-LRV [-]	WHO category [5]*
Lab tests		-		-		*Before UVC*	*After UVC*	
#1 (Table 1)	1.50	0.0 ± 0.1		**100.0 ± 0.5**		(1.20 ± 0.32)·10^6	**5.82 ± 0.28**	**Highly protective**
	3.00	0.0 ± 0.1		100.0 ± 0.5		(1.35 ± 0.25)·10^6	4.49 ± 0.19	Protective
	5.20	0.0 ± 0.1		100.0 ± 0.5		(1.60 ± 0.60)·10^6	3.87 ± 0.28	Protective
#9 (Table 1)	**1.50**	5.31 ± 0.62		77.0 ± 3.3		(1.50 ± 0.82)·10^6	**4.90 ± 0.12**	**~ Highly protective**
	3.00	5.31 ± 0.62		77.0 ± 3.3		(2.10 ± 0.40)·10^6	2.67 ± 0.28	Limited protection
	5.20	5.31 ± 0.62		77.0 ± 3.3		(1.36 ± 0.23)·10^6	2.18 ± 0.15	Limited protection
Field tests		*Before filtration*	*After filtration*	*Before filtration*	*After filtration*	*Before UVC*	*After UVC*	
Mexico-Spring water	3.3	0.64 ± 0.05	0.09 ± 0.05	83.4 ± 0.5	84.3 ± 0.5	(1.30 ± 0.40)·10^6	4.1 ± 0.2	Protective
Mexico-Bore hole	3.3	12.1 ± 0.1	5.00 ± 0.05	59.3 ± 0.5	69.0 ± 0.5	(3.20 ± 0.14)·10^6	3.5 ± 0.3	Protective
Colombia-La Miel 1	4.3	3.5 ± 0.4	0.72 ± 0.04	**94.2 ± 0.3**	**95.7 ± 0.6**	(1.11 ± 0.13)·10^6	**4.63 ± 0.56**	**~ Highly protective**
Colombia-La Miel 2	4.3	2.9 ± 0.5	1.0 ± 0.4	**93.7 ± 3.4**	**95.7 ± 3.0**	(4.84 ± 1.93)·10^6	**5.08 ± 0.35**	**Highly protective**
Colombia-S. Rita 1	4.3	6.8 ± 1.6	1.7 ± 0.4	66.9 ± 2.1	69.2 ± 1.7	(1.06 ± 0.07)·10^6	3.51 ± 0.28	Protective
Colombia-S. Rita 2	4.3	3.5 ± 0.9	1.2 ± 0.5	87.2 ± 0.7	88.4 ± 2.5	(1.50 ± 0.16)·10^6	3.47 ± 0.61	Protective
Colombia-S. Rita 3	4.3	2.7 ± 0.5	0.89 ± 0.13	86.8 ± 3.9	91.5 ± 3.7	(5.26 ± 1.13)·10^6	4.35 ± 0.39	Protective

Note: *Highly protective (LRV≥5), Protective (LRV≥3), Limited protection (LRV<3).*

and field inactivation experiments were utilized to test a simple model for flowrate estimation, given a target viral LRV (Supporting material, section S.1.4). The simple model underestimated the LRVs by 64% to 88%, as it does not account for the hydrodynamic behaviour or radiation distribution in the UVC flow system. There is need for more research and a deeper understanding of the phenomena occurring in the reactor chamber as reactor hydraulics, distribution of the UVC dose and the role on light scattering phenomena, which might be key in mass-photonic transfer inside the reactor. These factors are of fundamental importance for the precise modelling of the reactor behaviour to accurately predict the LRV achieved by the system treating a flowrate with known water quality parameters (Bolton and Cotton, 2008; Hijnen et al., 2005).

4. Conclusions

This study presented the assessment of UVC disinfection performance as a function of water quality parameters namely UVT_{254} and turbidity. We first established the critical role of DOM and the non-significant effect of turbidity on UVC disinfection efficiency within the experimental range studied (from 77 to 100 % of UVT254 and from 0 to 5.35 NTU of turbidity). This permitted the definition of a linear model to estimate the MS2 inactivation rate constant only as a function of UVT_{254} and therefore, the UV dose required to achieve a set reduction of MS2.

The collimated beam inactivation results along with the corrected applied UVC dose were utilized to determine the UV-dose response curve for MS2, which was best described by two linear fittings: for low UVC doses (10 – 90 mJ/cm^2) with an inactivation rate constant of 0.050 cm^2/mJ, and for high UVC doses (>90 mJ/cm^2) with 0.026 cm^2/mJ. It was noticed that the correction applied to the UVC dose enabled to obtain an overall uniform linear distribution highlighting some discrepancies. In fact, the conditions characterized by only turbidity resulted having greater residuals 0.28 – 0.88, compared to the other conditions. These could be attributed to the lack of a light scattering correction factor to account for the phenomena associated to the presence of inorganic particulate matter, that may have an important effect on the accuracy of the estimation of the applied UV dose.

The flow inactivation experiments in the laboratory and in the field, confirmed the good performance of the studied UVC disinfection system which was able to meet the reduction requirements established by WHO. The lab tests confirmed LRV of 5.8, 4.5 and 3.9 for flowrates of 1.5, 3.0 and 5.2 L/min, respectively for clean water (UVT254=100%, turbidity=0 NTU), and >3 LRV for 1.5 L/min for dirty water characterized by UVT254 of 77% and turbidity of 5.35 NTU. Whereas, in field testing with spring water and water hole water it achieved a 5.0 and 3.5 LRV for UVT254 of 93.9% and 66.9% respectively.

This investigation opens a potential application to monitor the performance of UVC systems with surface water by monitoring UVT_{254} as a tool to control UVC domestic systems to deliver safe drinking water in a household without the need for frequent microbiological analysis which may not be easily available. There is a need for more work on UVC reactor modelling to consider fluid dynamics and radiation distribution within commercial UVC systems to correlate to the (Curtis, 2016) dose response determined with collimated beam experiments.

Statement

Authors hereby declare previous originality check, no conflict of interest and open access to the repository of data used in this paper for scientific purposes.

Authors' contributions

VB (experimental research, modelling, data analysis, manuscript writing), HL (experimental work, manuscript revision), NP (experimental work, manuscript revision), AT (modelling, manuscript revision), Manuela Antonelli (funding, manuscript revision), MHP (field research, manuscript revision), LB (field research, manuscript revision), FR (field research, manuscript revision), AG (field research, manuscript revision), JAB (funding, manuscript approval), PFI (conceptualization, funding, interpretation of data, manuscript writing and submission).

Declaration of competing interest

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

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Supplementary materials

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