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Modelling of *E. coli* inactivation by chlorine dioxide in irrigation water

Francisco López-Gálvez [a], Imca Sampers [b], María I. Gil [a], Ana Allende [a,*]

[a] Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo, 25, 30100, Murcia, Spain

[b] Laboratory of Food Microbiology and Biotechnology, Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University Campus Kortrijk, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium

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ABSTRACT

Irrigation water has been highlighted as a potential contamination source of fresh produce with foodborne pathogens. Water disinfection treatments can be used to improve its microbiological safety. Growers should ascertain the minimum effective disinfectant doses able to achieve the desired microbiological goals. Furthermore, potential unwanted effects both on crop health and on chemical risks associated with the accumulation of disinfection by-products (DBPs) on the crop must be taken into account. The minimum effective doses vary depending on intrinsic factors (e.g. physicochemical characteristics of the irrigation water, concentration of the target microorganism) and also extrinsic factors (e.g. geographical and weather conditions). In the present study, different types of surface irrigation water were treated with various doses of a stable solution of chlorine dioxide (ClO_2) (0.1–2.5 mg/L) at 21 ± 2 °C for a contact time of 1 min. *Escherichia coli* concentration (before and after treatment) and the residual ClO_2 (after treatment) were analyzed. Several water physicochemical parameters were measured (chemical oxygen demand (COD), turbidity, pH, oxidation-reduction potential (ORP), conductivity and absorbance of filtered water samples at 254 nm (UV254)). Data obtained was used to develop a model for ClO_2 decay and a model for *E. coli* inactivation by linear regression. The ClO_2 decay model (adjusted R^2 = 0.93) included the initial ClO_2 concentration and the UV254 as explanatory variables. The *E. coli* inactivation model (adjusted R^2 = 0.77) included the initial ClO_2 concentration, the initial *E. coli* concentration, and the UV254 as explanatory variables. The development of these models would help growers in making decisions regarding the minimum effective doses they might use to treat their irrigation water when using a stable solution of ClO_2 to reduce microbial risk of fresh produce.

1. Introduction

Fresh produce has been associated with foodborne outbreaks. A number of factors can provoke the contamination of fruits and vegetables with pathogenic microorganisms (FAO/WHO, 2008; EFSA, 2013). Irrigation water is one of the sources of contamination of fresh produce with human pathogenic microorganisms (Pachepsky et al., 2011; Allende and Monaghan, 2015; Uyttendaele et al., 2015). Microbiological criteria for irrigation water present in current guidelines and legislation is normally based on *E. coli* concentration (Real Decreto 1620/2007, 2007; FDA, 2015). *E. coli* is not a perfect indicator, but it is an acceptable indicator of potential contamination of agricultural water with pathogenic microorganisms (Wilkes et al., 2009; López-Gálvez et al., 2016).

It is important that growers have assistance for the assessment of microbiological risks associated with water used in agriculture, and the mitigation options available. One possible intervention strategy is irrigation water disinfection (EFSA, 2013). Treatment of irrigation water with disinfectants could help reduce microbiological risks both for the crop and for human consumers (Suslow, 2010; Raudales et al., 2014). Although there is some scientific information available concerning inactivation of phytopathogenic microorganisms in irrigation water (Cayanan et al., 2009; Fisher et al., 2011; Raudales et al., 2014), information concerning inactivation of human pathogenic microorganisms or their indicators in the same matrix is scarce (Suslow, 2010). Suitable technologies based on their efficiency and potential application in the field should be selected for irrigation water disinfection. One of the options is the use of a stable solution of chlorine dioxide (ClO_2) which possesses higher oxidation capacity and forms less halogenated by-products

* Corresponding author.
E-mail addresses: flopez@cebas.csic.es (F. López-Gálvez), Imca.Sampers@UGent.be (I. Sampers), migil@cebas.csic.es (M.I. Gil), aallende@cebas.csic.es (A. Allende).

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than sodium hypochlorite (López-Gálvez et al., 2010). The studies of Chang (2015) and Killinger et al. (2014) reported good efficacy of ClO_2 for *E. coli* inactivation in irrigation water.

The aim of the present study was the development of mathematical models for determining the *E. coli* inactivation and the residual concentrations of ClO_2 in irrigation water treated with ClO_2. These models would be of assistance for growers as tools to select the suitable disinfectant doses depending on the target residual ClO_2 and on the microbiological goal in different irrigation water sources.

2. Materials and methods

2.1. Experimental setup

Three types of surface irrigation water obtained from water reservoirs (reservoir samples), drainage ditches (drainage water) and a river were used for determining residual concentrations of ClO_2 also defined as 'ClO_2 decay' experiments. However, mostly because of the low concentration of *E. coli* in surface water from water reservoirs (1.2 ± 0.4 log cfu/100 mL), only water samples obtained from drainage ditches and the river were used for *E. coli* inactivation experiments. In each experiment, three to five independent samples of each type of water were used. Water samples were characterized according to physicochemical characteristics and *E. coli* concentration. The selection of the range of ClO_2 doses and contact time used in the present study was based on preliminary trials (unpublished data) where the main goal was the reduction of *E. coli* concentrations to levels below recommended (Europe) or mandatory (USA) limits in irrigation water (EU, 2017; FDA, 2015). Additionally, information related to the most probable contact times from the disinfectant injection point to the point of application to the crop in an agricultural irrigation network was provided by commercial growers. Based on these preliminary assays, samples were treated with different concentrations of ClO_2 ranging from 0.1 to 2.5 mg/L for a contact time of 1 min. Obtained data were used for the development of ClO_2 decay and *E. coli* inactivation models by linear regression.

2.2. Water sampling

Water samples were taken from three different locations. Water from a water reservoir was sampled from a commercial setting, property of the company Primaflor S.A.T. which is located in Pozo Higuera (37°44¢ N–1°75¢ W, Almeria, Spain). Drainage water was sampled at drainage ditches surrounding growing fields in the area near Santa Cruz (38°00¢N-1°03¢W, Murcia, Spain). Finally, water samples were obtained from the Segura river in a fresh produce production area of Archena (38°06¢N–1°17¢W, Murcia, Spain). Twelve different samplings were performed at each of the sampling locations from September 2015 until October 2016. In each sampling time, three to five samples of each type of water were taken. Water samples (2.5 L) were taken in aseptic 2.7 L polyethylene bottles (Deltalab, Barcelona, Spain) and transported to the lab under refrigerated conditions. Water was kept at 4 °C and used for experiments within 24 h of sampling.

2.3. Physicochemical analysis

For UV254 measurement, untreated water was filtered through 0.45 mm syringe nylon filters (Fisherbrand-Fisher Scientific, Waltham, USA) and UV absorbance at a wavelength of 254 nm was measured using a UV–vis spectrophotometer (Jasco V-630, Tokyo, Japan) and quartz cuvettes with a 1-cm path length (Hellma, Müllheim, Germany). Chemical oxygen demand (COD) was determined by the standard photometric method (APHA 1998) using a photometer (Spectroquant NOVA 60, Merck). Turbidity was measured by means of the turbidimeter Turbiquant 3000 IR (Merck, Darmstadt, Germany). Oxidation-reduction potential (ORP) and pH were measured using a multimeter pH and Redox 26 (Crison, Barcelona, Spain). Conductivity was assessed by means of a conductivity meter CM35 (Crison, Barcelona, Spain).

2.4. ClO_2 decay and E. coli inactivation experiments

Reagents and instructions for the preparation of a stable chlorine dioxide solution AGRI DIS® (ClO_2) were provided by Servicios Técnicos de Canarias (STC S.L.U., Las Palmas de Gran Canaria, Spain). ClO_2 solution was prepared the day before its use and was kept in an amber glass bottle covered with aluminum foil at 4 °C until the experiment was performed. In this way, a solution with the characteristics of the product that is commercially available for growers was obtained. The ClO_2 concentration in the solution was measured before its use by the DPD method (APHA, 1998) using the Spectroquant photometer (Merck, Darmstadt, Germany). Irrigation water was taken out of the cold room the day of the experiment and experiments were performed when water was at room temperature (21 ± 2 °C). Water was homogenized by shaking the bottles and 250 mL were measured and poured in a sterile 250 mL Schott glass bottle containing a stirring magnet. Potential effect of light in ClO_2 degradation was avoided by covering bottles with aluminum foil. The bottle was placed on a magnetic stirrer and speed was set at 500 rpm. The required volume of ClO_2 was added to obtain the desired initial concentration and the bottle was closed with a cap to avoid disinfectant loss. After 1 min contact time, samples for ClO_2 measurement and *E. coli* concentration were taken. For ClO_2 measurement 10 mL samples were taken and measured by the DPD method. For *E. coli* inactivation, ≈120 mL was poured from the bottle into a sterile plastic beaker containing 1 mL of sodium thiosulfate solution (5 g/L) (Scharlau, Sentmenat, Spain) to quench ClO_2 residuals. Spiral plating, pour plating, and filtration were used to assess *E. coli* counts. In all cases Chromocult coliform agar (Merck, Darmstadt, Germany) was used for *E. coli* enumeration. For the filtration, samples were filtered through sterile 0.45 mm cellulose nitrate filters using a vacuum filtration unit (Sartorius, Goettingen, Germany). Plates were incubated at 37 °C for 24 h before interpretation of results. Dark-blue to violet colonies were identified as *E. coli*.

2.5. ClO_2 decay and E. coli inactivation modelling

SPSS statistics 23 and Microsoft Excel 2010 were used for the analyses. The stepwise linear regression with backwards elimination was applied to the data.

For ClO_2 decay modelling, ClO_2 measurement data were divided into a calibration set (n = 39, obtained in 17 different experiments) and a validation set (n = 17, obtained in 10 different experiments). The initial set of variables used to explain ClO_2 decay included the linear, quadratic and interaction terms of physicochemical parameters and the initial concentration of ClO_2 (Eq. (1)).

$$C_t = a + b \cdot C_0 + c \cdot X + d \cdot C_0\,2 + e \cdot X^2 + f \cdot C_0 \cdot X \quad (1)$$

Where: C_t is the concentration of ClO_2 after treatment (mg/L), C_0 is the initial ClO_2 concentration (mg/L), and X is the physicochemical parameter.

For *E. coli* inactivation modelling, data on *E. coli* log reduction were divided into a calibration set (n = 68, obtained in 8 different experiments) and a validation set (n = 74, obtained in 4 different experiments). The initial set of variables used to explain the inactivation included the linear, quadratic and interaction terms of the initial *E. coli* concentration, physicochemical parameters, and the

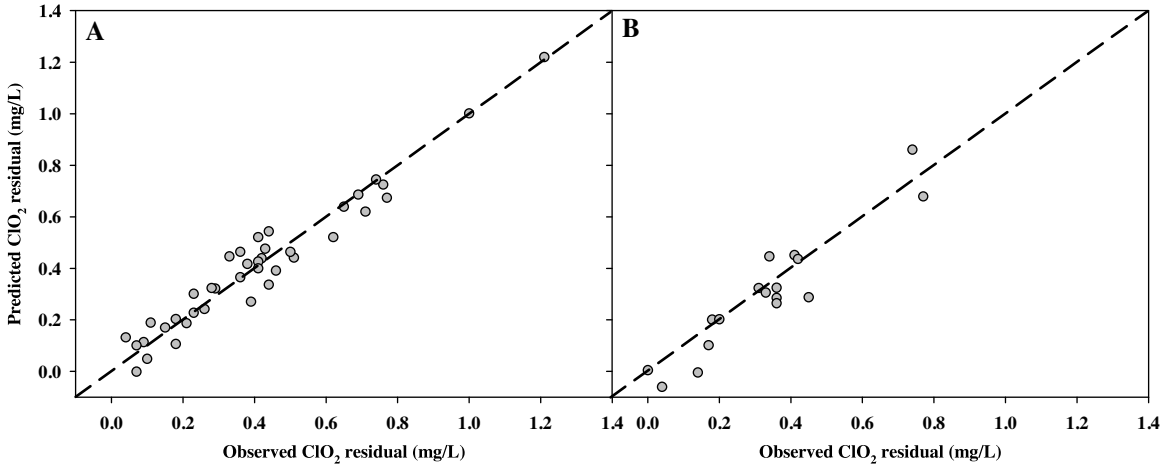

Fig. 1. Scatter plots of observed data used to build the ClO_2 decay model *vs* data predicted by the model (A), and of observed validation data *vs* data predicted by the model (B).

initial concentration of ClO_2.

$$log(\frac{Nt}{N_0}) = a + b \cdot C_0 + c \cdot X + d \cdot N_0 + e \cdot C_0\,2 + f \cdot X^2 + g \cdot N_0\,2 + h \cdot C_0 \cdot X + i \cdot C_0 \cdot N_0 + j \cdot X \cdot N_0 + k \cdot C_0 \cdot X \cdot N_0 \quad (2)$$

Where N_t is the concentration of *E. coli* after treatment (log cfu/100 mL), N_0 is the initial concentration of *E. coli* (log cfu/100 mL), C_0 is the initial ClO_2 concentration (mg/L), and X is the physicochemical parameter.

In both cases, the selection of the most suitable model was based on the adjusted coefficient of determination (R^2) and the ratio of performance to deviation (RPD) values. RPD expresses the increase of prediction accuracy compared to using the mean log reduction value to predict the result of all disinfection trials.

In addition to the validation using data obtained in the lab, tests were performed to check the applicability of the models in a commercial irrigation water network located in Pozo de la Higuera (Almería, Spain). The water used at the agricultural fields was surface water from the Negratín basin (Granada, Spain) stored close to the growing fields in a water reservoir. AGRI DIS® solution was prepared at the irrigation head following manufacturer instructions. The initial ClO_2 solution (≈6000 mg/L) was diluted using irrigation water in an opaque plastic tank (1000 L). The diluted ClO_2 solution was added to the irrigation water using a programmable Venturi system suction unit. Measurements of ClO_2 in water taken from the sprinklers during irrigation were carried out by chronoamperometry analysis (Palintest, Gateshead, UK). For microbiological analyses, in each test, water samples (2.5 L) from the sprinklers were taken into aseptic polyethylene bottles (2.7 L) (Deltalab, Barcelona, Spain), sodium thiosulfate (Sigma-Aldrich, Darmstadt, Germany) was added to the bottles to quench ClO_2 residuals, and samples were transported to the lab in refrigerated conditions. Water was analysed for the concentration of culturable *E. coli* as explained in paragraph 2.4. Between the point of disinfectant application and the sampling point at the sprinkler the irrigation network comprised 50 m of Polyvinyl chloride (PVC) pipe with a diameter of 125 mm, and 54 m of Polyethylene pipe with a diameter of 50 mm. Contact time was approximately 1 min.

3. Results and discussion

3.1. ClO_2 decay modelling

In preliminary tests, contact times from 0.5 to 5 min were tested. These contact times represent different residence times of irrigation water from the disinfectant injection point to the point of application to the crop in an agricultural irrigation network. Also during the preliminary tests, several physicochemical parameters were measured in water (COD, turbidity, pH, ORP, conductivity and UV254). In order to select the most relevant physicochemical parameters, the relationship between the different parameters and the initial ClO_2 demand of water was tested by linear regression. Initial ClO_2 demand was calculated based on Eq. (3) (Falsanisi et al., 2006).

$$C_t = (C_0 - D) \cdot e^{-k_0 \cdot t} \quad (3)$$

Where: C_t is the concentration of ClO_2 at time = t (mg/L), C_0 is the initial ClO_2 concentration (mg/L), D is the initial disinfectant demand, k_0 is a constant, and t is the contact time.

It was observed that beyond 1 min of contact time the concentration of ClO_2 did not change significantly. As a consequence, for the model development, only data obtained with a contact time of 1 min were used. Regarding physicochemical parameters, UV254 showed the strongest relationship with ClO_2 demand of water (R^2 = 0.63), followed by conductivity (R^2 = 0.45). As a consequence, UV254 was the physicochemical parameter selected for ClO_2 decay modelling and was measured in all the tests performed. UV254 is a parameter that provides information on the amount of some organic compounds (lignin, tannin, humic substances, aromatic compounds) present in different types of water samples (APHA, 2012). UV254 ranged between 0.02 and 0.11 (cm^{-1}) in the water samples used for ClO_2 decay tests. UV254 levels measured in the present study are commonly found in surface water. Ghoochani et al. (2013) measured UV254 levels ranging between 0.09 and 0.17 (cm^{-1}) in river water in Iran. Nuckols et al. (2001) reported UV254 levels of 0.03–0.07 (cm^{-1}) in water from the Ohio river and the Eagle Reservoir (USA). A. Yavich, 2017 reported UV254 levels of 0.01–0.2 cm^{-1} in water from Lake Michigan (USA). Temperature can affect concentration of disinfectants in water. The ClO_2 decay model obtained in our study was developed with data obtained at temperatures around 20 °C. Applicability at other temperatures should be assessed.

Eq. (4) shows the ClO_2 decay model obtained by stepwise linear regression. None of the terms initially included were removed by the regression procedure. The model had an adjusted R^2 of 0.93 and a RPD of 4.06.

$$C_t = 0.33 + 0.79 \cdot C_0 - 15.70 \cdot UV254 + 0.19 \cdot C_0\,2 + 107.99 \cdot UV254^2 - 5.00 \cdot C_0 \cdot UV254 \quad (4)$$

Where: C_t is the concentration of ClO_2 after treatment (mg/L), C_0 is the initial ClO_2 concentration (mg/L), and UV254 is the UV absorbance of filtered irrigation water measured at 254 nm (cm^{-1}).

Fig. 1 shows a good fit of calibration data used to develop the model with the data predicted by the model (1A), and also a good fit of validation data to model predictions (1B). Validation had an adjusted R^2 of 0.94 and a RPD of 2.87. The RPD >2 in calibration and validation indicates good fit and good predictions of the model (Sinnaeve et al., 2001).

UV254 of water used in field tests ranged between 0.02 and 0.03 cm^{-1}. The initial ClO_2 concentration applied in field tests varied between 0.09 and 1.22 mg/L. Residual ClO_2 in treated water from field tests ranged between 0.00 and 0.75 mg/L. When data from field tests (n = 11) were compared with the predictions obtained using the model, a systematic bias was observed (Supplementary data 1, S1; adjusted R^2 = 0.43). Concentrations of disinfectant predicted by the model were always higher than those measured in the field. Disinfectant decay in water distribution networks occurs due to reactions in the bulk liquid phase, but also due to reactions of the disinfectant with pipe walls, deposits, biofilms, etc. (Arevalo, 2007). The model developed in the present study could predict decay due to reactions in the liquid phase, but the other causes of ClO_2 decay were not included in the model. This would explain the systematic overprediction of ClO_2 residuals by the model.

3.2. E. coli *inactivation modelling*

Water samples used for *E. coli* inactivation tests were characterized by a UV254 level between 0.01 and 0.09 (cm^{-1}). *E. coli* concentration did not change when contact time was prolonged beyond 1 min, and therefore only data obtained using 1 min contact time were used for model development. Initial *E. coli* concentration ranged between 2.4 and 4.2 log cfu/100 mL, and between 2.8 and 5.3 log cfu/100 mL for water obtained from the river and drainage ditches, respectively. The limit of detection for *E. coli* concentration in water was 0 log cfu/100 mL (1 cfu/100 mL). Reductions in water obtained from the river ranged between 0.0 to 2.7 log cfu/100 mL, while they ranged between 0.0 and 3.6 log cfu/100 mL in drainage water.

The most suitable model for *E. coli* inactivation in irrigation water had the following expression (Eq. (5)):

$$log(\frac{Nt}{N_0}) = 0.69 - 0.82 \cdot C_0 - 54.87 \cdot UV254 + 0.63 \cdot C_0\,2 - 29.81 \cdot UV254 \cdot C_0 - 0.24 \cdot N_0\,2 + 25.27 \cdot N_0 \cdot UV254 \quad (5)$$

Where Nt is the concentration of *E. coli* after treatment (log cfu/100 mL), N_0 is the initial concentration of *E. coli* (log cfu/100 mL), C_0 is the initial ClO_2 concentration (mg/L), and UV254 is the UV absorbance of filtered irrigation water measured at 254 nm (cm^{-1}).

The adjusted R^2 value of the model was 0.77, while RPD was 1.92. Adjusted R^2 values of the intermediate equations can be found in the Supplementary data 2 (S2). Fig. 2 shows the fitting of the coupling of predicted and observed data to the line of best-fit. It can be observed that in some cases, too large reductions are predicted by the model when low reductions (<0.5 log) are observed. These cases correspond to specific trials in which low concentrations of disinfectant were used in water with high UV254 (drainage water). This trend was confirmed by additional data obtained by experiments in which low disinfectant concentrations (0.1 mg/L) were used to treat drainage water (Supplementary data 3, S3). Fitting of validation data to model predictions (Fig. 3) gave a R^2 value of 0.55 and an RPD of 1.75. The RPD value in calibration and validation (2 > RPD > 1.5) indicates fair fit and fair predictions of the model (Van Haute et al., 2013).

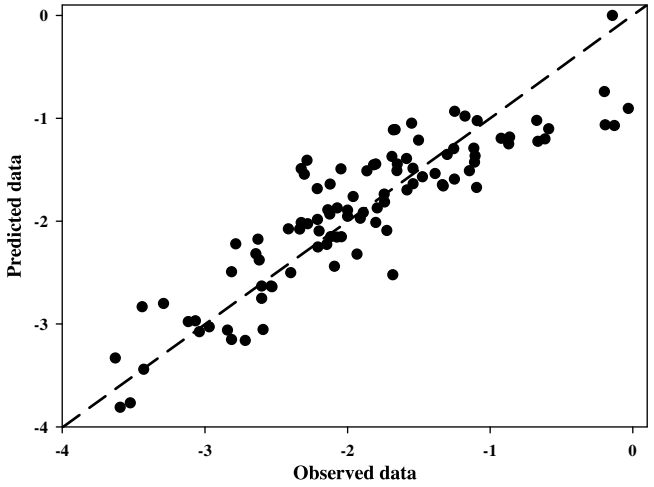

Fig. 2. Scatter plot of observed *E. coli* reductions data (log cfu/100 mL) used to build the inactivation model *vs* reduction data predicted by the model.

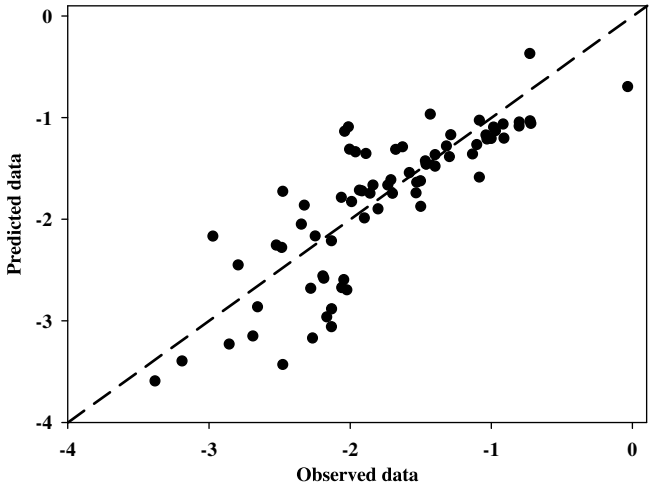

Fig. 3. Scatter plot of observed validation data *vs E. coli* inactivation model predictions (log cfu/100 mL *E. coli* reductions).

UV254 of water and initial ClO_2 concentration in field tests was shown in paragraph 3.1. Initial *E. coli* level in water used in field tests ranged between 0.38 and 3.21 log cfu/100 mL *E. coli* reduction in water samples after treatment varied between 0.07 and 1.95 log cfu/100 mL. When data from field tests (n = 11) were compared with the predictions obtained using the model, a value of 0.52 was obtained for the adjusted R^2 (Supplementary data 4, S4). For the set of data used for this validation obtained in a commercial field, the performance of the model was probably affected by the low initial concentration of *E. coli* in the treated samples. Only two out of the eleven field water samples had an initial *E. coli* level within the range used for model development (2.4-4.2 log cfu/100 mL).

Temperature is another parameter that affects the efficacy of disinfectants in irrigation water (Raudales et al., 2014). Irrigation water temperature varies between locations, between seasons, and even during the day in the same location, affecting microbial inactivation. The inactivation model obtained in our study was developed at temperatures around 21 °C. Applicability of this model at other temperatures should be assessed. The predictive model for *E. coli* inactivation could help growers in the selection of appropriate doses to comply with microbiological criteria established in guide-

lines and legislation (Real Decreto 1620/2007, 2007; FDA, 2015). For example, Spanish legislation on reuse of reclaimed water states a maximum concentration of 10^2-10^3 cfu *E. coli* per 100 mL in irrigation water (number of sample units (n) = 10, threshold value for the number of *E. coli* (m) = 100 cfu/100 mL, maximum value for the number of *E. coli* (M) = 1000 cfu/100 mL, number of sample units where the *E. coli* count may be between m and M (c) = 3) when there is direct contact of the water with produce that is going to be consumed raw (Real Decreto 1620/2007, 2007). On the other hand, the Food Safety Modernization Act (FDA, 2015) establishes a criteria of geometric mean <126 CFU *E. coli*/100 mL and statistical threshold <410 CFU *E. coli*/100 mL for agricultural water directly applied to growing produce.

4. Conclusions

Absorbance of irrigation water at 254 nm was shown to be a suitable physicochemical parameter for the prediction of ClO_2 consumption in the bulk liquid phase and *E. coli* inactivation in different types of irrigation water (reservoir, river, drainage ditches) at pilot plant scale. Concordance between model predictions and observations in real agricultural field applications could have been better if the characteristics of water used to develop the models had matched better the characteristics of water used in field experiments. A number of factors (water temperature, irrigation network design, piping materials, presence of biofilms) can affect dynamics of ClO_2 decay and *E. coli* inactivation in real growing field applications. It would be necessary to include some of these factors to obtain applicable models. Suitable models on ClO_2 decay and *E. coli* inactivation could be implemented in an on-line tool that could be used by growers to select appropriate ClO_2 doses depending on their goals and on the microbiological and physicochemical quality of their irrigation water.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agwat.2017.07.001.

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