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Short Communication

Temperature-dependent change of light dose effects on *E. coli* inactivation during simulated solar treatment of secondary effluent

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HIGHLIGHTS

- We investigated the effects of dose during photolytic inactivation of *E. coli*.
- Dose effects change according to the temperatures under solar treatment.
- Reciprocity law failed to apply in the majority of cases.
- Irradiance and dose are not the exclusive driving forces in photolysis.

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ABSTRACT

In this study, simulation of solar disinfection of secondary effluents was performed, to assess the dose effects, as instructed by the reciprocity law. A full factorial experimental design on the operational parameters of the process was performed (time, temperature, bacterial load, light intensity) and three response variables were estimated (disinfection efficiency, regrowth after 24, and 48 h). In the 240 disinfection experiments, an erratic behavior was observed in all responses to light exposure, attributed to the combination of both irradiation intensity and temperature during treatment. As a result, the validity of the reciprocity law between light dose and irradiation intensity is challenged. The majority of the cases failed to comply with it, indicating the dependence on temperature conditions, as well as the applied intensity. Dose affected the bacterial regrowth potential after 24 and 48 h in a more conventional way. It appears that in order to attain a valid projection of the outcome of solar disinfection in secondary effluent, intensity and dose are not the only parameters to be considered, with temperature also having to be taken under consideration.

1. Introduction

In the majority of medical and biological applications, a consensus existed on the response of light dose, the so-called reciprocity law. Its formulation is stating that the total dose applied to a system will cause the same effects, independently of light intensity and exposure time (Zetterberg, 1964). Like every rule, this law has been proven false in a number of applications (Martin et al., 2003), mostly because of the irradiation fluxes used in the experiments; either very high or very low intensities can cause deviations from the expected behavior. A major application of photochemistry/photobiology, which has been extensively studied and recently reviewed (McGuigan et al., 2012) is the solar disinfection of water. This practice has drawn interest among other disinfection methods in developing countries, because of its simplicity and high acceptance rates (Ubomba-Jaswa et al., 2009; McGuigan et al., 2012). Following its introduction, more sophisticated aspects were gradually studied, such as the responses of different microorganisms and solar light delivery methods (continuous-intermittent) (Rincon and Pulgarin, 2003; Sichel et al., 2007).

Since it is natural to expect abnormalities in different species and irradiation patterns around the world, a need for experiment standardization was developed. It was soon discovered that dose can mask the reality or provide a false image on the conditions of microorganisms after irradiation. The reciprocity law in solar disinfection (SODIS) could provide an answer to that problem. Rincon and Pulgarin (2004a) stated that for the same dose, the high irradiation/low time combination provided better results for

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solar treatment of water, indicating a non-linear dependence between light intensity and deactivation of bacteria. Sichel et al. (2007) found that increasing the dose did not significantly improve disinfection in fungi, if the intensity was kept constant, while Bosshard et al. (2009) and Berney et al. (2006) directly challenged reciprocity law in SODIS; Bosshard et al. (2009) found some deviations of *Sh. flexneri* and *S. typhimurium* from this law, while Berney et al. (2006) verified the law for *E. coli* in temperatures between 40–52 °C. Peak and Peak (1982) verified the law in irradiance values only above 750 W/m^2. Furthermore, a notion of the post-irradiation regrowth was present in some of the previous works, as well, searching for the necessary dose (Ubomba-Jaswa et al., 2009; McGuigan et al., 2012) or exposure time, according to the respective irradiation intensities (Rincon and Pulgarin, 2004b).

To our knowledge, so far there has not been an analysis on the reciprocity law in solar wastewater treatment. Although some works evaluated the photo-inactivation potential in real wastewater (Maïga et al., 2009; Igoud et al., 2014), the phenomena involved are very complex and assumptions concerning the tested parameters need to be made. Here, the reciprocity law is tested in disinfection of simulated secondary effluent, a different matrix, which supports bacterial growth, rather than their plain survival (Marugan et al., 2010). We assume that the turbidity levels required for disinfection are achieved (NTU < 1) (Safari et al., 2013), (theoretically) in applied pre-treatment of municipal wastewater treatment, so light action mode is not disrupted and the reported associated problems are neglected (Haider et al., 2014).

In our work, a multilevel, full factorial experimental design was applied to estimate how a set of parameters (treatment time, temperature, initial bacterial concentration and irradiation intensity) lead to deviation from reciprocity in both disinfection efficiency and subsequent regrowth. Short term (24 h) and long term (48 h) effects were studied.

2. Materials and methods

2.1. Synthetic secondary effluent preparation: microbial methods and wastewater composition

The *E. coli* strain used was supplied by “Deutsche Sammlung von Mikroorganismen und Zellkulturen”. The bacterial preparation, have been described analytically elsewhere (Giannakis et al., 2013), as instructed by OECD (2001). The composition of wastewater was 160 mg/L peptone, 110 mg/L meat extract, 30 mg/L urea, 28 mg/L K_2HPO_4, 7 mg/L NaCl, 4 mg/L $CaCl_2 \cdot 2H_2O$ and 2 mg/L $MgSO_4 \cdot 7H_2O$, with 250 mg/L initial COD and turbidity around 1 NTU. Then, a 1/10 dilution of the effluent was performed, in order to simulate 90% BOD removal, a typical value in secondary treatment of municipal wastewater. Contrary to actual secondary effluent samples, this synthetic wastewater contained only small amounts of suspended solids. This preparation procedure for synthetic secondary effluent has been used in other published works (Velez-Colmenares et al., 2011; Giannakis et al., 2014a,b). Finally, bacteria were added until 10^3 to 10^6 CFU/mL were achieved, as instructed by the experimental design.

2.2. Experimental details: light source, bacterial enumeration and design of experiments

Light was provided by a Suntest solar simulator, emitting 0.5% of photons in 290–300 nm range (UVC cut-off at 290 nm), 7% among 300 and 400 nm and follows the solar spectrum above that value until IR range (IR was cut-off by filtering). The batch reactors were glass, double-wall cylindrical reactors (height: 9 cm, outer diameter 7.5 cm, inner diameter 6.5 cm, effective exposed surface 20.41 cm^2), which were temperature controlled by a thermostat, as presented in Fig. 1.

Experiments (and sampling) were done under mild stirring, while sampling was performed in hourly intervals. At each time point, 2.0 mL were drawn and pour-plated in PCA agar for the determination of the viable bacterial count. Diluted aliquots were done, when necessary, to achieve measurable counts on the Petri dishes. An hourly drawn sample was kept in sterile plastic flasks in the dark, at room temperature (~25 °C), for post-irradiation measurements of survival/regrowth after 24 and 48 h. Experiments were performed twice, while sampling (and afterwards, plating) was done in duplicates, from 2–3 consequent dilutions.

Table 1 summarizes the design of experiments (DOE) employed in this study. Experiments were performed in three discreet intensity levels, with different initial population and controlled temperature. The choice of irradiation values represent the range of intensities reaching the earth's crust and temperature values correspond to temperatures reached in ponds around the Equator and/or in technical applications (Giannakis et al. 2014a). Finally, the runs with null intensity were not used in dose calculations, as they reflect no dose impact and act as blank thermal treatment experiments.

3. Results and discussion

3.1. Challenging reciprocity law in temperature-controlled disinfection experiments

An extensive analysis of the disinfection kinetics and the regrowth potential of the photo-treated secondary effluent was published before by the authors (Giannakis et al., 2014a; 2014b). In summary, reverse results were observed for the process efficiency, when the DOE was analyzed; for the same dose, efficiency dropped when treatment temperature was between 20–40 °C and increased for temperatures between 50–60 °C. Literature suggests an existing

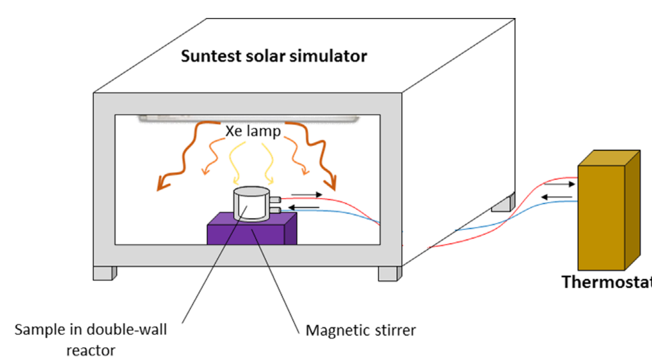

Fig. 1. Schematic representation of the experimental set-up.

Table 1
Summary of the DOE.

Parameters	Levels	Units
Time	**4** (1, 2, 3, 4)	h
Initial population	**4** (10^3, 10^4, 10^5, 10^6)	CFU/mL
Temperature	**5** (20, 30, 40, 50, 60)	°C
Light intensity	**3** (0, 800, 1200)	W/m^2