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Impact of different light intermittence regimes on bacteria during simulated solar treatment of secondary effluent: Implications of the inserted dark periods

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Abstract

In this study, the effect of light intermittence on solar disinfection of secondary treated wastewater was investigated. Synthetic secondary effluent was spiked with *Escherichia coli* and submitted to 3 different light intermittence regimes by circulating the effluent between a dark storage tank and three in-series illuminated reactors. The relative influence of the recirculation rate on bacterial inactivation was studied, in short (3–7 min) light regimes and a dark-to-light ratio of 2.04. Lower recirculation rates resulted in poorer disinfection results, showing the detrimental effect of longer dark storage periods on the removal efficiency. Also, longer time intervals were employed in batch tests, to investigate the effect of 1, 2 and 3-h dark intervals, during recreated solar disinfection conditions; fourteen different scenarios were tested. Three hours of continuous or cumulative illumination were proven enough to provide the necessary dose to damage bacteria irreparably, while interruption during these hours favored bacterial resistance. Finally, absence of regrowth was observed in all cases that derived from samples with null bacterial counts. However, when a fraction of viable bacteria was present at the end of the solar treatment, survival was favored.

Keywords: Solar disinfection; Synthetic wastewater; Light intervals; Intermittent illumination; *E. coli*

1. Introduction

Sunlight is able to inactivate micro-organisms due to the synergistic effect of the UV and heating of water. The first ones to study the germicidal activity of sunlight were Downes and Blunt (1877), followed by others (Gameson, 1975; Mitchell, 1978; Acra et al., 1980). UV wavelengths which reach the earth's surface, along with the visible region, are classified as UV-A (320–400 nm) and UV-B (290–320 nm) (Rincón and Pulgarin, 2004).

Solar disinfection of water is based on the bacteriostatic effect of the UV-A solar radiation as well as on the presence of dissolved oxygen; in presence of natural photosensitizers highly reactive forms of oxygen, the reactive oxygen species (ROS), are produced, which have bactericidal effect (Gelover et al., 2006). UV-B radiation on the other hand, can cause direct DNA damage by inducing the formation

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of DNA photoproducts, of which the cyclobutane pyrimidine dimer (CPD), the pyrimidine (6–4), pyrimidinone (6–4PP) and Dewar valence isomers are the most common (Douki, 2013). The accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription (Harm, 1980; Britt, 1996; Rincón and Pulgarin, 2004).

The mechanism of inactivation, apart from the direct UV-B attacks against the bacterial DNA, is described briefly as a partial decomposition of the outer membrane, followed by disordering of the cytoplasmic membrane, resulting in cell death (Sunada et al., 2003). Vital cellular functions like the transcription and translation apparatus, transport systems, amino acid synthesis and degradation, respiration, ATP synthesis, glycolysis, the TCA cycle, chaperone functions and catalase are targeted by UVA irradiation (Bosshard et al., 2010).

When it comes to field-scale real-water or wastewater disinfection applications, two of the most crucial factors are the temperature of the treated water and the availability of light (Fabriccino and d' Antonio, 2011). In some cases, areas with poor water supplies are provided with a large number of sunny days per year (more than 3000 h) (Meichtry et al., 2005), but for instance, solar-UV power is dependent on the clarity of the sky and the absence of clouds. Hence, studies have been made to assess the potential impact that this process has on disinfection (Rincón and Pulgarin, 2003) and the effects of intermittence in light supply on the solar disinfection process (Misstear et al., 2013). Even in the sunniest areas in the world, there is no guarantee that the UV supply will be continuous; therefore, there is a need to further investigate the mechanisms and possible implications of intermittence in the overall efficiency of the process.

Although some works have demonstrated indifference of the effect of light intermittence on some matrices (Lanao et al. 2012), this is not the rule for all microorganisms and all light waves (Velez-Colmenares et al., 2011). However, there is a general consensus that the intermittent process deviates from the behavior expected in a normal test; hence, this could be attributed to the disinfection installations as well. A very common method of solar disinfection is the compound parabolic collector (CPC) reactors (Malato Rodrıguez et al., 2004), which recirculate the sample around an illuminated surface and a dark storage tank. Therefore, technical aspects can affect the process, causing intermittence, such as the storage of water in the dark tank (Moncayo-Lasso et al., 2009; Fernández et al., 2005; Fernández-Ibáñez et al., 2009; Rincón and Pulgarin, 2007). Sciacca et al. (2011) with a minimum dark storage volume (83% illuminated volume), reported different results while performing solar CPC intermittent tests, compared to the equivalent batch tests, stating that there are actions that intervene (such as shear forces or oxygenation of the sample) and modify the final outcome. Finally, Ubomba-Jaswa et al. (2009), concluded that the continuous manner of irradiation has greater inactivation potential, compared to the interrupted manner of solar UV light supply.

The revision of the said situations reveals that there is a significant gap in scientific literature on photolytic processes for wastewater treatment – particularly on the effect of light intermittence – compared to the amount of work devoted to drinking water treatment. The present work contributes directly to the limited resources of this knowledge area, in order to assess the potential applications of solar treatment in water reclamation in sunny areas or areas with poor water quality, which can greatly influence the amounts of water supply (Gamage and Zhang, 2010).

In this study, the solar disinfection of secondary treated wastewater under intermittent illumination was simulated in a lab-scale plant, using a synthetic secondary effluent and controlled laboratory conditions, namely, predefined light supply, wastewater composition, and microorganisms (*Escherichia coli*). The microbial response to different light and dark phases was evaluated. Specifically, this study focuses on:

1. High-frequency intermittence (3–6 cycles per hour) by recirculating wastewater between a dark storage tank and an illuminated area. The recirculation in this setup imitates a compound parabolic collector reactor (CPC), a typical solar-disinfection configuration.
2. Low-frequency intermittence, by inserting 1-h, 2-h or 3-h dark phases into 6-h batch disinfection tests. These tests simulate the breaks in high-intensity light caused by temporal clouding in solar batch-reactor applications.
3. The results were evaluated through process efficiency, in terms of viable plate counts throughout the tests. Also, dark repair (DR) of the bacterial population was studied on the disinfected samples.

2. Materials and methods

2.1. Synthetic secondary effluent preparation

2.1.1. Microbial methods

The *E. coli* strain K12 (MG1655) used for the experiments was supplied by the "Deutsche Sammlung von Mikroorganismen und Zellkulturen". Luria–Bertani (LB) broth (10 g Bacto™ Tryptone, 5 g Yeast extract, 10 g NaCl, per liter) was prepared for each experimental series by suspension in Milli-Q water and heat-sterilization by autoclaving. One colony was picked from the pre-cultures and loop-inoculated into a 50 mL sterile falcon, containing 5 mL of LB broth. The flask was then incubated at 37 °C and 180 rpm in a shaker incubator. Further dilution was made after 8 h in 1% v/v and the new solution was incubated under the same conditions for 15 more hours.

Cells were harvested during the stationary phase by centrifugation from batch culture for 15 min at 5000 rpm and at 4 °C, in a universal centrifuge. After centrifugation the