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Monitoring the post-irradiation *E. coli* survival patterns in environmental water matrices: Implications in handling solar disinfected wastewater

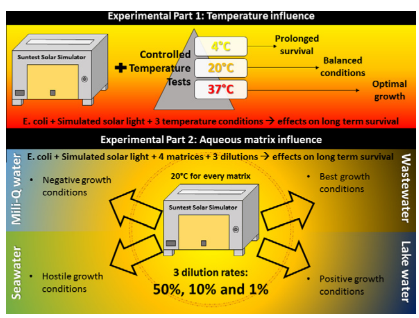
Stefanos Giannakis [a,b,c], Ana Isabel Merino Gamo [c], Efthymios Darakas [a], Antoni Escalas-Cañellas [b,d], César Pulgarin [c,*]

[a] *Laboratory of Environmental Engineering and Planning, Department of Civil Engineering, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece*
[b] *Laboratory of Control of Environmental Contamination, Institute of Textile Research and Industrial Cooperation of Terrassa (INTEXTER), Universitat Politècnica de Catalunya, Colom 15, 08222 Terrassa, Catalonia, Spain*
[c] *Swiss Federal Institute of Technology, Lausanne, Institute of Chemical Sciences and Engineering, 1015 Lausanne, Switzerland*
[d] *Department of Chemical Engineering & Terrassa School of Engineering, Universitat Politècnica de Catalunya, Colom 1, 08222 Terrassa, Catalonia, Spain*

HIGHLIGHTS

- We investigated the post-illumination fate of *E. coli* in various water matrices.
- Storage temperature, receiving medium and dilution ratio were investigated.
- *E. coli* kinetics was correlated with temperature, light dose, dilution and type of receiving matrix.
- Post-treatment survival was increasing in: Seawater < Mili-Q < lake water < wastewater.
- The expected survival patterns can influence the design of solar treatment systems.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, simulated solar disinfection of secondary effluent was followed by dark storage at different temperatures or different receiving water matrices. *Escherichia coli* illumination was followed by 3-day monitoring of the bacterial population and its adaptation in different temperature conditions in the dark. The subsequent survival was linked to the dose received during exposure to light, and results were obtained on the environmentally induced prolongation of survival, maintenance of population or excessive growth, at 4 °C, 20 °C and 37 °C, respectively. An additional set of experiments at 20 °C was subjected to dilution in *E. coli*-free synthetic wastewater, water from Lake Leman, (synthetic) seawater and Mili-Q water. Post-irradiation monitoring was also conducted, studying 50%, 10% and 1% dilution rates, and the results were attributed to the two parameters of dilution medium and dilution ratio. However, different responses were found based on the acquired dose during pre-treatment. This indicates the importance of the illumination prior to storage, and the preference of bacteria in some matrices over the others. Survival was linked to initial population, dose, dilution and medium; shorter illumination times are to be considered according to the receiving water matrix.

* Corresponding author. Tel.: +41 216934720; fax: +41 216936161.
E-mail address: cesar.pulgarin@epfl.ch (C. Pulgarin).

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1. Introduction

Modern wastewater treatment plants use either automated control systems [1], or specially trained personnel to handle the operation and management of their facilities. For this reason, many works have shown concern for their high operating and maintenance costs [2]. Other studies, to provide an effective alternative, suggest the simplicity of natural systems [3,4]. These systems are simpler and cost-effective, while ensuring no compromise is made in terms of efficiency. As a result, a variety of solutions is offered to the developed countries. However, one must consider the available solutions in the cases that neither of the key factors in applying modernized wastewater treatment systems can be acquired. Rural or isolated communities or even developing countries are unable to support technologically advanced solutions. Therefore, research interest should be directed to more natural-based approaches, such as waste stabilization ponds [5,6] or constructed wetlands [7].

However, the presence of the developing countries around earth's Equator, leads to the reception of a vast number of sunny days per year [8]. The combination of this supply with the unfeasibility of technologically demanding solutions, makes solar treatment systems a sustainable solution, which could improve the poor sanitation conditions often encountered. For more than 30 years [9,10] and many more] priority has been given to water disinfection, since people suffered from waterborne diseases [11,12]. These applications were focused in applying solar disinfection techniques for the production of safe drinking water, with remarkable results wherever it was applied [13,14]. Nevertheless, it can be assumed that waterborne diseases, which were connected to microorganisms of gastro-intestinal track, were result of fecal pollution due to poor sanitation conditions. Therefore, the improvement of these conditions will improve the quality of water supplies. Literature suggests the recommended features that the candidate water must fulfill [15], the required dose for a variety of microorganisms [16] and studies about the evolution of water disinfection experiments after the end of treatment [17].

The application of solar treatment systems requires two main axis of caution: disinfection and post-irradiation events. Over the years, solid knowledge concerning the exploitation of solar power to reduce microorganisms in water has been accumulated, which we could extrapolate to wastewater [13,18–19]. These results include works water disinfection in various treatment methods and media, such as PET bottles [20], or CPC reactors [21]. For wastewater, the most feasible processes in the context of developing countries could be superficial flow constructed wetlands [22] or waste stabilization ponds [23].

The second axis deals with the fate of microorganisms, once disinfection is over. There are a number of works concerning the occurrence of microorganisms in natural waters, such as rivers [24], lakes [25], estuarine [26,27] or brackish water [28] and seawater [29,30]. However, only few studies deal with the microbial post-irradiation fate of microorganisms when treated wastewaters are discharged in these water matrices (for instance [31,5]). What is mostly discussed is the fate of microorganisms occurring in different water types, while others include the simultaneous application of solar irradiation; Yukselen et al. [32] have studied the inactivation of bacteria in seawater, Jenkins et al. [33] the die-off in pond waters etc., thus providing information on the concurrent action of adaptation and illumination.

Considering the parameters that affect microorganism survival and regrowth in natural context, reactivation of bacteria is possible when the treated effluent is released in natural water bodies, although it depends on many factors. Chan and Killick [31] performed a series of experiments that proved the correlation between temperature and regrowth potential. These findings were verified and extended by Shang et al. [34], who stated the elasticity of the regrowth dynamics response between 10 °C and 35 °C, observing the minimization of the potential under 10 °C. The experiments of Munshi et al. [35] proved that bacteria recover within 24 h when the proper conditions are given. Within the same framework, it was shown [34] that the supply of nutrients in the matrix demonstrated higher final numbers. Rincon and Pulgarin [17] observed that after solar illumination and reproduction of the same experiments in the laboratory, almost all samples that were conducted with natural water, which contained even lower concentrations of dissolved organic carbon, presented regrowth in dark conditions. This was unexpected, since low organic matter concentration should have limited the regrowth, as Tassoula [36] showed, because low COD values lower survival dynamics. Finally, natural water bodies as a receiving medium are interesting, because it is believed that enteric bacteria may survive in natural waters but are not able to multiply themselves [37]. In natural aquatic environments carbon availability and temperature are much lower and, therefore, the expected specific growth rates of enteric bacteria are lower [38].

In this study, we investigate the bacterial pathway from the moment they are introduced into the disinfection system, the effect of solar dose and the introduction into several water matrices. While keeping the solar dose at same levels, treated secondary effluent is first kept in a range of temperature (4 °C, 20 °C and 37 °C), so as to test regrowth dynamics as a function of temperature. In another context, previously illuminated samples are lead into (i) untreated wastewater (*E. coli*-free synthetic secondary effluent), (ii) lake water, (iii) sea water and (iv) distilled water, in order to assess the effect of the receiving water body on the regrowth potential, the survival and the mortality rate. The same assessment was carried out in a series of dilutions (50%, 10% and 1%), with the aim to systematically investigate the effects of different osmotic conditions and nutrient content. We aim to locate the ability of bacteria to adapt in the environment while being pre-stressed by solar light and the variation of the environmental conditions on the required dose for safe handling of the treated samples. The examination of their post-irradiation period will provide information on the constraints that limit discharge to the natural environment and consequently, on the risk of downstream contamination in a similar application.

2. Materials and methods

2.1. Water matrices

2.1.1. Synthetic wastewater preparation

Analytical preparation of the synthetic secondary effluent and the specifications concerning the microorganism strain used in this research, are described in detail elsewhere [39]. In summary, the synthetic wastewater composition 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 $Mg_2SO_4{\cdot}7H_2O$, with 250 mg/L COD. The chemicals were used as received. A 10% dilution of the said wastewater was inoculated with *E. coli*, to form a suspension containing 10^6 CFU/mL and bear organic carbon values closer to the desired ones [59].

2.1.2. Lake Leman water, synthetic seawater and wastewater, and Mili-Q water specifications

The physicochemical characteristics of Lake Leman water and the composition of synthetic seawater are presented in Table 1 [40]. Mili-Q water is characterized by 18.2 MΩ·cm resistivity at 25 °C. Lake water was heat-sterilized to avoid the effects of indigenous microorganisms and focus only on the physicochemical