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Elucidating bacterial regrowth: Effect of disinfection conditions in dark storage of solar treated secondary effluent

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ABSTRACT

In this study, we systematically investigate solar disinfection of synthetic secondary wastewater, with the effort to decrypt the effects disinfection conditions have on post-irradiation bacterial regrowth in the dark. A full factorial design of 240 experiments was employed to investigate the effects of (i) exposure time (1, 2, 3 and 4 h), (ii) treatment temperature (20, 30, 40, 50 and 60 °C), (iii) initial bacterial concentration (10^3, 10^4, 10^5 and 10^6 CFU/mL) and (iv) sunlight intensity (0, 800 and 1200 W/m^2) on *Escherichia coli* survival for a subsequent 48-h dark control period. The decisive implications treatment temperature inflicted in regrowth were monitored and interpreted within two temperature ranges, from 20 to 40 °C and 40 to 60 °C. In dark tests, bacterial populations presented initial moderate growths at 20–40 °C range, followed by intense regrowth. At 40–60 °C range, acute thermal inactivation without long-term regrowth predominated at 50 °C and was total at 60 °C, within the 4-h treatment period. Introduction of light resulted in higher removal rates or permanent inactivation for 800 and/or 1200 W/m^2, respectively. No post-treatment regrowth in the dark was observed after 24 and 48 h, in completely inactivated samples, and its demonstration, when observed, was well correlated to the bacterial numbers at the end of the disinfection period. Statistical observations on the transferred bacterial populations from day to day are also discussed in this paper.

1. Introduction

The greatest disadvantage of UV disinfection of wastewater, regardless of the source, i.e. either UV-C lamps or solar UV disinfection, is its point efficiency, which lacks residual effect [41]. In any UV disinfection unit, the effluent of the process will include inactive (completely decayed microorganisms), injured (not lethally damaged, potentially dangerous if healed) and a fraction of microorganisms that escaped the process. The absence of the residual disinfecting factor could possibly allow the reactivation of injured microorganisms, if favorable downstream conditions are presented [13,12]. The remaining bacteria could increase their numbers while being in the treated effluent, due to a variety of reasons; for example, the existence of nutrients and related chemicals in wastewater could provide an abundant food source for the bacteria, allowing them to metabolize and reproduce [18]. Hence, the main two factors that are responsible for bacterial regrowth are [11]: (i) the growth of injured microorganisms, (ii) the reactivation and regrowth of the reactivated microorganisms.

Long after regrowth as a phenomenon was observed, the "viable but non-cultivable" (VNC) hypothesis was developed to explain the repopulation of a sample, although appearing microorganism-free at the end of the treatment; this statement provided explanations to similar findings and was adopted by various researchers [42,35]. This hypothesis suggests that not all the bacteria are destroyed by the action of light, but there is a significant number that is alive, but unable to reproduce.

DNA is one of the main targets of both direct and indirect actions of UV light, through the direct dimerization of thymines or indirect attacks by reactive oxygen species, (ROS) [25]. The generated ROS have a well-explained action mode, especially hydroxyl radicals;

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they interact with the intracellular components of the microorganism. Bacteria possess the ability to repair a number of their DNA damages through two main mechanisms: light-dependent ones, namely photoreactivation, and light-independent (dark repair), which help them recover from during photo-exposure.

Photoreactivation is completed by a two-step mechanism. First, there is the formation of a complex between a photoreactivation enzyme (PRE) and the dimer to be repaired [23] and afterwards, release of PRE and repaired DNA. The restoration of the dimer to its original monomerized form is absolutely dependent upon light energy intensity [23]; the energy needed to repair the damage is provided by visible light (310–480 nm) [13,11].

The dark repair methods are regulated by the expression of recA, a critical gene in the bacterial cell, with well-known properties [38,14]. The nucleotide and base excision repair, includes numerous molecular steps, including identification of the damage, assimilation of a repair complex, incision and removal of the damaged strand and filling with DNA polymerase, finalized by attaching the replaced DNA with the rest of the strand with a ligase [4,1,37].

There is extensive literature on the genetic interpretation of regrowth, as well as experimental findings on the factors that affect this process; among the most common factors affecting regrowth are the effects of temperature [5,37], the salt and nutrient contents of the treated water [22,30], the effect of UV dosage and light intensities [16,23], the pre-illumination with non-coherent visible and infrared wavelengths [15], the initial bacterial population [6,10] and the type of bacterial strain [31]. However, most of the works either focus on photoreactivation, employ artificial UVC irradiation, focus on drinking water or treat regrowth exclusively as added value on the evaluation of a treatment method. This occurs due to the fact that dark repair tests offer a good evaluation of the durability of a process, namely the ability to handle post-treatment events.

The present study focuses clearly on bacterial dark repair of previously solar irradiated of secondary effluent. After the extensive works for drinking water in developing regions [20,17,45], there is an interest in introducing low-cost treatment methods in developing countries, in order to efficiently help controlling contagious diseases [21]; solar disinfection of wastewater could offer a solution, under certain conditions. A system that could treat the effluent, for instance a series of shallow ponds, and could drastically reduce microbial load, would be of great interest in these areas, where the number of sunny days per year is an order of hundreds [3]. In that manner, there would be an extra source of water, maybe not for direct consumption, but potentially able to enrich local availability, intended for secondary use [8]. Such a practice would be of equal interest in both developed and developing countries, since a considerable amount of water could be recovered.

Considering the application point of view, a preliminary approach has been done [9], in terms of complexity of factors involved, but there are few statistical findings and experimental processes verifying the effect of basic parameters of treatment, for instance, treatment time [26] and temperature conditions with regard to the dark repair potential of the target bacterial population. Bacterial regrowth has been observed to occur in both in water [31,36] and wastewater samples [43]. Wastewater is a rich in nutrients matrix which could support bacterial growth, and given the time treated water could spend in the dark, due to the storage times potentially required to further use, regrowth is rendered as a primary problem. Since the goal is to increase the water supplies of a specific region, regrowth of bacteria in the natural environment could possibly mean a re-contamination of downstream water supplies. In both cases of aquifers used for drinking water, or, water reuse for irrigation, the limits set by the World Health Organization could be exceeded a posteriori [44]; either result in dangerous conditions for the end-users.

Table 1
Synthetic wastewater composition.

Chemical composition of the synthetic municipal wastewater before dilution	
Chemicals	Concentration (mg/L)
Peptone	160
Meat extract	110
Urea	30
K_2HPO_4	28
NaCl	7
$CaCl_2{\cdot}2H_2O$	4
$MgSO_4{\cdot}7H_2O$	2

Therefore, in this study we recreate the conditions of solar treatment of secondary effluent and perform a multilevel, full factorial design of experiments (DOE), in order to fully investigate the effects of the treatment conditions, during solar disinfection, on bacterial regrowth. With the application of an experimental design valuable information can be acquired that are not evident due to interaction of the parameters [46]; the factorial experimental design has been proven an efficient method in bacterial inactivation studies [34,9]. The parameters under investigation are (i) exposure time, (ii) temperature, (iii) initial population and (iv) intensity of the solar simulated light, on *E. coli*-spiked synthetic wastewater, as a model microorganism. After the measurements of the process efficiency, post-treatment control in the dark was made, to estimate the bacterial regrowth/survival capabilities of the treated samples.

2. Materials and methods

2.1. Preparation of the synthetic secondary effluent

The pre-experimental processes involved with the preparation of the synthetic wastewater included two significant parts, the preparation of the *E. coli* solution and the actual wastewater, as follows.

2.1.1. Bacterial culture preparation

E. coli K12 (MG 1655) was acquired from "Deutsche Sammlung von Mikroorganismen und Zellkulturen". A colony was loop-inoculated in pre-sterilized 5 mL Luria-Bertani broth; for each L of sterile distilled water, 10 g Bacto™ tryptone, 5 g yeast extract and 10 g NaCl were added. 25 mL sterile plastic falcons, containing the spiked LB, were incubated for 8 h and another 1/100 dilution to LB solution (2.5 mL sample into 250 mL LB) was incubated for another 15 h. Bacterial cells were then centrifuged (5000 rpm for 15 min) and washed 3 times with sterilized saline solution (8 g/L NaCl and 0.8 g/L KCl). The bacterial pellet was dispersed in fresh, sterilized saline solution, forming a solution with 10^9 CFU/mL initial population.

2.1.2. Synthetic wastewater composition

The employed wastewater was a 1/10 dilution of the presented in Table 1, instructed by [24]. 1 mL of the prepared (10^9) bacterial solution was added per liter to obtain a bacterial concentration of 10^6 CFU/mL. In order to obtain 10^3, 10^4 and 10^5 CFU/mL, dilution of the same proportion (wastewater/distilled water = 1/10) were done.

2.2. Suntest solar simulator

The artificial solar simulator employed in our experiments employed was a Suntest, acquired from Hanau. It bears a 1500 W