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New kinetic model for predicting the photoreactivation of bacteria with sunlight

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ABSTRACT

Exposure to ultraviolet radiation is a disinfection process that is used worldwide for the treatment of wastewater in order to minimize microbial contamination caused by wastewater discharge to natural waters. Once organisms have been exposed to ultraviolet radiation, they are able to repair the damage through two processes – dark repair and photoreactivation. In the work described here, the photoreactivation process after ultraviolet disinfection has been studied in pure culture of *Escherichia coli* ATCC 11229, ATCC 15597 and in real wastewater, using both a laboratory plant and a pilot plant. A new kinetic model is proposed that is a modification of the model proposed by Kashimada et al. [15] including a first order decay phase. This model was applied to the photoreactivation process with sunlight. The new model incorporates a decay rate constant (M_s) for solar reactivation in order to explain correctly the decay phase detected in the experimental data for photoreactivation with sunlight. The new model fits the data obtained in reactivation experiments, thus allowing the interpretation of the kinetic parameters S_m, $S_m - S_o$, k_s, and M_s and their relationship with UV dose.

1. Introduction

UV irradiation has become one of the most important alternatives to chlorination for wastewater disinfection throughout the world [31]. The reuse of reclaimed wastewater for agricultural purposes and golf course irrigation is increasing, and the advantages of applying UV disinfection to enable wastewater to be reused are widely recognized [15].

Light at wavelengths between 250 nm and 270 nm shows the maximum effectiveness for inactivating microorganisms, a situation that is consistent with the maximum absorbance of nucleotide bases of the genome including thymine, cytosine and uracil [11,24]. For this reason, UV-C radiation at 254 nm causes damage in DNA and RNA with inhibition of cell transcription and replication. The alteration of DNA is caused by the induction of the formation of photoproducts such as thymine–thymine cis-syn cyclobutane pyrimidine dimer (CPD), thymine–thymine pyrimidine (6-4) pyrimidone photoproducts [TT (6-4) photoproducts] [3] and pyrimidine photohydrates, whose significance in biological inactivation is low. CPDs have biological consequences in the inactivation of cells and the photoproducts play an important role in UV-induced cytotoxic damage [8]. Some microorganisms, particularly bacteria, are known to be capable of repairing their damaged DNA in the presence or absence of visible light by dark repair or photoreactivation mechanisms [28].

Dark repair or nucleotide excision repair is a multi-enzyme repair process that involves the excision of dimers [38] and requires the coordination of over a dozen proteins to excise and repair the damaged DNA segment [6]. In contrast to dark repair, photoreactivation requires light to activate the repair mechanism. Light between 330 and 480 nm (UV-A) activates repair enzymes that split the pyrimidine dimers to recover the damaged DNA [36,16,22]. Photolyase is the light enzyme responsible for photoreactivation processes [31]. This enzyme contains two co-factors: 5,10-methenyltetrahydrofolate (MTHF) absorbs about 90% of visible light, while the double-electron reduced form of Flavin Adenine Dinucleotide ($FADH^-$) catalytically reverses DNA damage [10].

Photoreactivation is considered to be the most important of the two mechanisms and it follows a two-step reaction scheme [11]:

Step 1: Formation of a complex between a photoreactivation enzyme (PRE) and the dimer to be repaired. This step does not require light, but is dependent on temperature, pH and ionic strength [17].

Step 2: Release of PRE and repaired DNA. The restoration of the dimer to its original monomeric form is absolutely dependent on the intensity of light energy [27]. The PRE-dimer complex is formed in the process. An extended period of exposure to photoreactivating light would enable the release of PRE and this would then be available to form new complexes (Step 1) [20].

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Wastewaters after UV disinfection are often exposed to visible light and the ability of microorganisms to be reactivated must therefore be considered to ensure conditions for hygienically safe water disinfection [30].

Reported exposure times that result in maximum photoreactivation have ranged from minutes [11,27,20,10], to hours [21,37,12,5,39,9] to days [19]. The differences in these time scales, although not fully understood, may be attributable to several factors, including the particular experimental set up, the initial number of pyrimidine dimers formed, the number of photoreactivating enzymes present in the organism, the temperature during PRE-dimer complexing, and the dose rate of photoreactivating radiation.

According to Lindenauer and Darby [17], the extent of photoreactivation is dependent upon the number of PRE-pyrimidine dimer complexes formed. The number of complexes formed is limited by the number and availability of the PREs in each cell.

Most studies on photoreactivation involve the use of visible light from artificial sources such as fluorescent lamps that emit light at 360 nm [15,24,38,18,25,28,4] and halogen lamps emitting between 400 nm and 800 nm [10]. However, very few studies of this type concern the use of natural sunlight. UV solar radiation is able to inactivate microorganisms due to the synergistic effect of the UV light and heating of water by infrared radiation. The UV wavelengths that reach the earth's surface are classified as UV-A (320–400 nm) and UV-B (290–320 nm). UV-B radiation can cause direct DNA damage by inducing the formation of DNA photoproducts [26] and UV-A radiation has a bacteriostatic effect and, in some water matrixes, sunlight could produce dissolved oxygen, leading to highly reactive oxygen species in the presence of dissolved oxygen [7,29]. UV-A also has a repair effect on DNA and RNA damaged by the action of UV-B radiation on exposure to natural sunlight and the effect of UV-C upon artificial exposure.

Likewise, the majority of photoreactivation studies involve the use of collimated beam tests with low fluences and optimal conditions for light exposure for repair, such as a thin layer of fluid [14].

Very few studies have focused on modelling the reactivation processes and in most cases the process is simply described [12,17,13,39,23,25,10]. Other authors have tried to model jointly the inactivation and reactivation phases [15,31,2,20]. In 1996 Kashimada et al. developed a photoreactivation model to predict the reactivation phase for *Escherichia coli* with a saturation-type first order reaction. In 1999 Tosa and Hirata, used the Kashimada model to study the photoreactivation of *E. coli* exposed at fluorescent lamps by comparing experiments with and without reactivation and established a relation between microorganism concentration and UV dose. In 2002, Beggs used the Tosa and Hirata experimental data to describe a theoretical model for quantifying the photoreactivation process and the photolysis rates for *E. coli* strains. In 2007, Nebot et al. studied the photoreactivation and dark repair of bacteria – total coliforms, faecal coliforms and *faecal streptococci* – in order to develop a kinetic model that would allow the prediction of the reactivation after UV disinfection depending on the UV-C dose applied.

In the majority cases, the photoreactivation had been studied in controlled conditions using only UV-A, but the photoreactivation with real conditions of sunlight (including UV-A and UV-B radiation) had not been studied and the decay phase after reactivation phase had not been observed. Therefore, it is necessary to investigate and to apply new kinetic models that can predict reactivation processes and provide a better understanding of the factors that affect this interesting phenomenon. For this reason, the principal objective of the work described here was to propose a new kinetic model to explain the photoreactivation of microorganisms after UV disinfection in real conditions of sunlight exposition.

2. Materials and methods

2.1. UV irradiation

2.1.1. Laboratory plant

UV disinfection experiments were carried out at laboratory scale in the system described by Vélez-Colmenares et al. [35]. This system has a UV germicidal emission from a low pressure lamp of 2.6 W and the time of exposure to radiation can be assumed to be the hydraulic residence time because the dispersion coefficient obtained is 0.05 and this is considered the optimal design for an ultraviolet disinfection reactor [32,33,34]. Disinfection experiments were performed with pure cultures of two strains of *E. coli* in synthetic wastewater.

2.1.2. Pilot plant

The disinfection experiments with real wastewater were performed in a Pilot Plant made by Trojan Technologies S.L., Canada. The UV channel received the water from the unfiltered secondary effluent of the Municipal Wastewater Treatment Plant of Jerez de la Frontera (Spain).

The UV Pilot Plant treatment was performed with two horizontal lamps directed into an open-channel with a flow rate in the range 4–15 m^3/h. The unit consisted of a single stainless steel UV lamp bank (22 cm × 7.5 cm × 122 cm) and a double transition box (49 cm × 23 cm × 47 cm). The bank contained two low pressure lamps, each with a length of 914 mm and a production of 78 W UV-C at 254 nm. Lamps were positioned parallel to the fluid flow direction and encased in a 60 mm diameter quartz sleeve.

2.2. Microorganisms, culture media and culture conditions

E. coli was selected due to its strictly fecal character and as it is one of the most common biological indicators of efficient water disinfection [39] and is governed by all water quality regulations. The following commercial strains of *E. coli* were employed in a laboratory plant: ATCC 15597, derived from *E. coli* K-12, and ATCC 11229, a host bacteriophage, both of which are commonly used as indicators in disinfection studies [25]. These strains were obtained from the American Collection of Microorganisms and Cell Cultures provided by LGC Standards. An initial volume of 1 mL of bacteria from frozen stock was shaken for 10 s, resuspended in 30 mL of Tryptic Soy Broth (TSB) and incubated for a period of 24 h at 37 °C. The cells were centrifuged at 3000 rpm for 10 min [25] and the resulting pellet was washed in a 10% peptone solution to ensure complete removal of the culture medium. Finally, each pellet was resuspended in 50 mL of sterile milli-Q water and, depending on the requirements of the study, the bacteria solution was diluted or concentrated. This process guaranteed an initial bacterial concentration between 10^5 and 10^{11} colony forming units per 100 mL (CFU/100 mL).

The initial concentration of commercial strains of *E. coli* before ultraviolet radiation exposure was estimated by the construction of an experimental relationship between turbidity and microbiological concentration. This estimation subsequently was verified by membrane filtration. Turbidity measurements (nephelometric units, NTU) were carried out with a Eutech TN100 portable turbidimeter (range 0.1–1000 NTU).

Each test started with 10 L of buffered and sterilized milli-Q water, to which was added a determined volume of a concentrated nutrient medium to achieve a synthetic wastewater of 35 mg/L O_2 of COD (Table 1) and a specific concentration of enriched microbiological culture.

The membrane filtration method specified by Standard Methods for the Examination of Water and Wastewater [1] was always