Comparative study of Gram-negative bacteria response to solar photocatalytic inactivation

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Received: 22 February 2018 / Accepted: 28 May 2018 / Published online: 3 June 2018
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Abstract

Solar photocatalytic inactivation of Gram-negative bacteria with immobilized TiO₂-P25 in a fixed-bed reactor was modeled with simplified kinetic equations. The kinetic parameters are the following: the photocatalytic inactivation coefficient ($k_{d,QUV}$), the initial bacterial reduction rate (A) in the contact with the disinfecting agent, and the threshold level of damage (n) were determined to report the effect of Q_{UV} /TiO₂-P25 on bacterial cultivability and viability and to compare the response of bacterial strains to photocatalytic treatment. In addition, the integration of the reactivation coefficient (C_r) in the photocatalytic inactivation equation allowed evaluating the ability of bacterial reactivation after photocatalytic stress. Results showed different responses of the bacteria strains to photocatalytic stress and the ability of certain bacterial strains such as *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC4114 to resuscitate after photocatalytic treatment.

Keywords Photocatalysis · TiO₂-P25 · Solar irradiation · Inactivation kinetic · Reactivation

Introduction

In arid countries, re-using domestic wastewater for agricultural and industrial activities has been proposed as a strategy for suitable water use and resource conservation. However, the use of these effluents must be carefully considered, as they could contain high levels of pathogenic agents and harmful chemical compounds (Rengifo et al. 2010). Over the last 20 years, environmental remediation has emerged as a high national and international priority for the disinfection of contaminated water from hazardous pollutants.

Chlorine-based technologies have long been used as disinfection processes for drinking water supplies and also for the tertiary treatment of wastewater. However, these technologies are becoming of increasing concern due to the founding of recent studies indicating the formation of potentially harmful chloro-organic disinfection by-products (DBPs) during the treatment process (Nassar et al. 2017). For this reason, new disinfection technologies are currently under development.

Methods such as ozone and ultraviolet light are equally effective and less toxic. However, these techniques are expensive and often technically difficult to apply in less-favorable conditions. In this context, a semiconductor photocatalysis has emerged as a very attractive, environmentally friendly technology for water disinfection, especially considering the possibility of using solar light to drive the photocatalytic process.

Many authors consider TiO₂ as the most effective material for catalytic photodegradation due to its low cost, high efficiency, and long-term photostability (Mills and Lee, 2002).

A titanium dioxide (TiO₂) nanoparticle has attracted great interest in a wide range of applications such as photocatalysis, nanomedicine (Sapizah et al. 2012), sterilization (Mills et al. 2013), anti-fogging materials (Mills et al. 2013), lithography (MacFarlane et al. 2011), degradation of organic compounds (Hashimoto et al. 2005; Tatsuma et al. 2002), and prevention of metal corrosion (Zsilák et al. 2014; Vamathevan et al. 2002). In the last decade, TiO₂ has been widely used as a

Responsible editor: Suresh Pillai

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self-cleaning and self-sterilizing material for coating many clinical tools including sanitary ware, food table and cooking ware, and items for use in hospitals (Rtimi et al. 2015; Rtimi 2017).

Once the TiO_2 photocatalyst is activated by light with an energy equal or greater than the bandgap of the semiconductor, electron (e^-)/hole (h^+) pairs are generated in the conduction and in the valence bands, respectively. Then, some of these pairs migrate to the photocatalyst surface. In the primary stage, e^- reacts with O_2 adsorbed at the photocatalyst surface to generate superoxide $\text{O}_2^{\cdot-}$ radicals while h^+ react with water to produce hydroxyl $\cdot\text{OH}$ radicals. These hydroxyl radicals are able not only to mineralize organic pollutants but also to affect several microorganisms, including viruses, bacteria, spores, and protozoa (Wang et al. 2015).

The contact between bacteria and nanoparticles such as TiO_2 is considered according to many researches as the key point of the bacterial inactivation (Gogniat et al. 2006). Therefore, TiO_2 can induce a bactericidal effect in the dark when adsorbed on the surface of the bacterial cell (Huang et al. 2000). Other reactive oxygen species (ROS), such as superoxide ions ($\text{O}_2^{\cdot-}$ or $\text{HO}_2^{\cdot}$), are less effective against bacteria, due to the negative charge which prevents them from penetrating bacteria cell membranes (Rengifo et al. 2010; Caballero et al. 2009).

The first research on the bactericidal effect of TiO_2 photocatalytic reactions was conducted on *Escherichia coli* (Matsunaga et al. 1985) and subsequently has been intensively conducted on a wide spectrum of organisms including viruses, bacteria, and fungi (Silva et al. 2017).

Commercial TiO_2 Degussa P-25 has been used in most previous studies (Chong et al. 2009). Most of the reports about photocatalytic treatment of water sources have used TiO_2 slurries (Pablos et al. 2011). However, the difficulty of separation and reuse of nanostructures TiO_2 (10–30 nm) from treated water often limits its real application (Caballero et al. 2009). Numerous material engineering solutions have been investigated in order to resolve this problem. One of the more prominent solutions is the immobilization of the TiO_2 nanoparticles onto an inert carrier to retain its quantum effects in the form of a thin film, while allowing ease of separation from the treated water (Chonga et al. 2011). The thin-film fixed-bed reactor (TFFBR) is one of the most used solar photoreactor which has received an increasing interest as a suitable commercial application (Spasiano et al. 2015).

Therefore, these reactors can employ both direct and diffuse portions of solar radiation as a light source without the need to separate the photocatalysts from the purified water (Caballero et al. 2009). Indeed, the real application of this technology should avoid the separation step to allow a continuous water treatment. Indeed, employing the photocatalyst as a suspension or slurry makes the scaling up of the process difficult, as the TiO_2 has to be removed from the decontaminated water to be reused several

times. This problem presented a significant barrier to commercial application. The objective of this research was to develop a modeling approach to describe the kinetics of photocatalytic inactivation for solar photocatalytic reactor utilizing immobilized TiO_2 -P25. The overall goal was to build simple equations which could report the influence of the synergic effects of solar light radiation and TiO_2 -P25 on bacterial inactivation. The model will serve as a predictive tool to design disinfection systems, so that water can be disinfected quickly, efficiently, inexpensively, and without risk of recontamination with pathogenic bacteria.

Materials and methods

Photocatalyst

Degussa P-25 TiO_2 commercial powder (Degussa, Germany) was used as a photocatalyst for all experiments. Its crystalline structure consists of anatase (80%) and rutile (20%) and has a specific surface area of $50 \pm 15 \text{ m}^2/\text{g}$ and an average crystallite size of 30 nm.

Preparation of bacterial strains

Each bacterial strain (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 4114, and *Salmonella Typhi* ATCC 560) was grown overnight in nutrient broth NB medium under aerobic conditions at 37°C with constant shaking at 160 rpm. The bacterial growth was monitored by measurement of optical density at 600 nm. Then, for photocatalytic experiments, bacteria were cultured overnight ($\approx 18 \text{ h}$), collected by centrifugation at 4000 rpm for 4 min and washed twice with sterile demineralized water. Cell pellets were finally re-suspended in sterile saline buffer (NaCl 0.85%) and the cell density was adjusted spectrometrically (OD_{600}) to a final cell concentration of about 10^6 colony-forming units per milliliter (cfu/mL).

Deposition of TiO_2 -P25 on the glass plate

Commercial TiO_2 (Degussa P25) powder was suspended in deionized water with a concentration of 10 g/L; a few drops of nitric acid (HNO_3) are added up to pH 3 to positively charge the TiO_2 nanoparticles and better disperse the suspension. Then the ordinary glass plate (surface area of 0.8 m^2) was treated with a nitric acid bath (1 M) with a holding time of about half an hour and then rinsed with 20% solution of sodium hydroxide (NaOH). Alkaline treatment increases by one hand the density of surface OH groups, which react with the hydroxyls on the TiO_2 P25 surface, and secondly the roughness of the support to facilitate the attachment of the photocatalyst particles (Ismail 2011). The suspension was

sonicated for 10 min to avoid agglomerations. The mixture thus prepared is deposited on the glass plate by “spray” and heated in an oven at 100 °C. The process was repeated 10 times during a day until a homogeneous layer was obtained. Then the immobilized TiO₂ layer was calcined at 475 °C for 7 h (Ismail 2011); subsequently, it was rinsed three times with deionized water to remove unfixed particles before the photocatalytic experiment.

Photocatalytic inactivation of bacterial strains

After immobilization of TiO₂-P25 on the glass plate, the photocatalytic reactor was inclined at 30 °C (Fig. 1) (Bousselmi et al. 2002). Then 1 L of bacterial suspension (10⁶ cfu/mL) was recycled continuously from the tank by a pump (flow rate 12 L/h) to the surface of the glass plate where it is irradiated by solar light and returned to the tank and recycled again (Fig. 1). During experiments, the average of global accumulated incident UV radiation energy Q_{UV} was equal to 134 kJ/L for 60 min of solar UV irradiation. A control test was conducted with TiO₂-P25 in dark condition. The photocatalytic experiments with or without UV light were carried out under natural conditions with pH = 7 and a temperature of the day around 25 °C.

Radiation evaluation

The incident solar irradiance in the photoreactor was measured with a pyranometer (Dr. Hönle UV-Meter Hand-held Technology (UV-A (330–400 nm))). Since those experiments were done in different days where solar intensity change from day to other, bacterial inactivation was represented as a function of the accumulated UV (Q_{UV}) in the photoreactor per unit of treated water volume for given periods of time during the experiment: Q_{UV} (kJ/L).

Incident UV energy (Q_{UV}) was determined by the following expression (Malato et al. 2000).

$$Q_{UV,n} = Q_{UV,n-1} + \Delta t_n UV_n S_r / V_t; \Delta t_n = t_n - t_{n-1}$$

where Q_{UV} is the accumulated energy received by the reactor per liter of solution for each sample taken during the experiment, t_n is the experimental time for each sample, UV_n is the average solar ultraviolet radiation measured during Δt_n , S_r is the surface area (0.8 m²), and V_t is the total volume of the reactor (1 L). During experiments, the average of global accumulated incident UV radiation energy Q_{UV} was equal to 134 kJ/L for 60 min of solar irradiation.

Determination of viable and cultivable bacterial cells

Samples of bacterial suspension (1 mL) were taken at regular times of the photocatalytic experiments. Serial dilutions were

Fig. 1 Solar photocatalytic reactor with immobilized TiO₂

performed in sterile saline buffer (NaCl 0.85%) and then spread on Petri dishes containing Luria-Bertani medium (LB agar Sigma-Aldrich) to count the viable cultivable bacteria. After incubation for 24 h at 37 °C, the bacterial cells were numbered. Three Petri dishes were used at each sampling time.

Bacterial reactivation in dark conditions

To investigate the ability of bacteria to resuscitate or reactivate after rest time in darkness, photo-irradiated samples were transferred into separate sterile Petri dishes covered with foil to examine the bacterial potential to dark repair at room temperature. This part of experiment was carried out to verify the ability of post-photo-irradiated bacteria in presence of fixed TiO₂ to restore their solar light/TiO₂-P25-induced lesions by molecular mechanisms and especially with dark repair systems such as excision-repair mechanisms and SOS system.

Results and discussion

Kinetics of photo-inactivation reactions

A series of experiments were carried out to highlight the effects of solar photocatalytic treatment on bacterial cultivability and viability. The number of surviving bacteria was expressed as the ratio (survival ratio) of the number of viable and

cultivable bacteria remaining after exposure to experimental conditions (N) to the number of the initial viable bacteria (N_0) ($\log N/N_0$). Figure 2 shows the photocatalytic Gram-negative bacilli: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 4114, and *S. typhi* ATCC 560 inactivation with immobilized TiO₂-P25.

This figure shows that the bactericidal rates were enhanced when we have combined the effect of TiO₂-P25 with solar UV and were increased by increasing irradiation time. The synergic effect of TiO₂ and solar UV irradiation had a remarkable effect on bacterial cultivability. However, we cannot neglect the bactericidal effect of TiO₂ without solar UV irradiation. In addition, the result also showed that fixed TiO₂-P25 induces low bacterial inactivation in the dark (0.7 to 1 log unit) (Lifen et al. 2009) were reported that pure TiO₂ had a high germicidal activity. Indeed, from microscopy observations, it was found that yeast cells on fresh TiO₂ were deformed under specific experimental conditions of pH, TiO₂ concentration, and suspended TiO₂.

The bactericidal effect of this semiconductor was highlighted only in the presence of solar UV irradiation after of a contact time of 60 min ($Q_{UV} = 134$ kJ/L) with solar UV/ TiO₂-P25. The bacterial strains *E. coli*, *P. aeruginosa*, and *S. typhi* showed a cultivability inactivation superior to 5 log10.

The antimicrobial photo-biological activity of TiO₂ on bacterial cells using solar UV irradiation has been attributed to the generation of very active free radical species called ROS (reactive oxygen species) (Acevedo et al. 2011). In fact, hydroxyl radicals (HO·) and superoxide anions (O₂^{•−}) are considered as the main generated species in the anodic and cathodic pathways, respectively, of photocatalytic processes in the presence of oxygen (Fujishima et al. 2000). Both species are known to be highly reactive with biological samples. Other oxygen reactive species have been also proposed, including hydrogen peroxide (H₂O₂), hydroperoxyl radical (HO₂·), and singlet oxygen (¹O₂) (Fujishima et al. 2000). Chromosomal aberration by DNA lesion caused by photoexcited TiO₂ was also reported.

Moreover, during solar UV radiation, the combination of indirect effect of UV_A (320–450 nm) radiation with direct germicidal action of UV_B (290–320 nm) can be lethal to cells respectively, through the photosensitization of endogenous chromophores such as co-enzymes or cytochromes, which could damage essential enzymes to bacteria growth and the blockage of DNA replication and RNA transcription (Pigeot et al. 2012). However, low solar irradiation can easily affect solar disinfection causing bacterial regrowth by photorepair mechanisms (Schuch et al. 2009).

In particular, some studies have demonstrated that the decrease of cultivability results from alteration in the bacterial metabolism due to the deleterious action of solar UV light in combination with TiO₂. Indeed, the cell membrane is the primary site of reactive photogenerated oxygen species attack, leading to lipid peroxidation (Kiwi and Nadochenko, 2005). The combination of cell membrane damage and further

oxidative attack of intracellular components ultimately lead to cell death (Rincon and Pulgarin, 2004). Other studies have suggested that the mode of action is the photooxidation of coenzyme A, leading to the inhibition of cell respiration and thus to cell death (Bonetta et al. 2013).

Knowing that the surface of TiO₂ is amphoteric and consequently, the charge of surface is pH-dependent (Piscopo et al., 2001). For the TiO₂-P25, the pH zpc is close to 6.3. Degussa P-25 has a negative charge at pH = 7. Thus, bacterial inactivation by the photocatalytic process may be due to free radicals in solution, not to radicals on the surface of the photocatalyst. This can be rationalized based on the electrostatic interaction between the TiO₂ surface and the cell surface. In fact, at pH 7, the surface of TiO₂ (isoelectric point, 6.3) and the outer membrane of bacteria are negatively charged (Cho et al. 2005) which lead to an electrostatic repulsion between the TiO₂ surface and bacteria. Consequently, adsorption of bacteria onto the surfaces of the TiO₂ particles is not favored at this pH, and the direct contact between the cells and the illuminated TiO₂ surface should be minimal. Cho has reported that the photochemical removal of coliform bacteria was unaffected by the pH of the solution in the range of 6–8 pH units (Cho et al. 2005). Rahmani et al. have reported that the electrostatic interaction at the TiO₂/water interface could be important in many cases of photocatalytic degradation of charged substrates (Rahmani et al. 2009); it is a relatively weak force, which can be dominated by other factors (Robertson et al. 2005). Thus, the initial pH of the water did not play an important role within a range of 5.5–8.5 pH units (Dheaya et al. 2009).

The performance of a disinfection system is directly relied on the knowledge of the inactivation rate of a target or indicator organism by the disinfectant. For photocatalysis, the synergistic effect of catalyst concentration and solar light intensity on the rate of the process determines the most efficient combination of contact time and the dose to be employed. Currently, most of these information were obtained from bench-scale studies and extrapolated with a series of empirical models which do not adequately describe photocatalytic disinfection.

The most common application is the Chick-Watson (C-W) model used primarily to fit inactivation data with first-order decay or modified for data with an initial lag, and whose general expression is given by this equation (Watson 1908):

$$N/N_0 = \text{Exp} (-k C^n t) \quad (1)$$

where N/N_0 is the reduction in the bacterial concentration, k is the disinfection kinetic constant, C is the disinfectant concentration with $C = d \times Q_{UV}$, d is the catalyst concentration (g/L) (for our experimental condition (fixed-bed reactor), the disinfectant concentration (TiO₂-P25) during photocatalysis was supposed to be constant), and Q_{UV} is the accumulated UV

Fig. 2 Monitoring of bacterial cultivability in contact with immobilized TiO₂-P25 in the dark and with solar UV irradiation/TiO₂-P25 conditions. **a** *E. coli* ATCC25922, **b** *P. aeruginosa* ATCC4114, **c** *S. typhi* ATCC560, and **d** comparison of the photocatalytic effect on the three strains

energy (kJ/L), t is the time (min), and n is the threshold level of series-event model; n is equal to 1 for the first-order C-W model.

The C-W model was modified to consider the initial reduction (A) in the contact with the disinfecting agent:

$$N/N_0 = A \exp(-k C^n t) \quad (2)$$

where A is the initial bacterial reduction rate in the contact with the synergic action between cumulate solar UV radiation (Q_{UV}) and the catalyst TiO₂-P25.

$$A = (N_C + N_d)/\mu \quad (3)$$

where N_C is the number of CFUs after the first contact with solar UV radiation and catalyst TiO₂-P25, N_d is the initial number of CFUs in the dark condition in the presence of semiconductor, and μ_t is the adsorption coefficient in the fixed bed of TiO₂ that is assumed to be negligible due to the negative charge of TiO₂-P25 at pH = 7.

In this C-W model, the reduction in enumerated bacteria was proportional to the contact time. This correlation, however, cannot be applied for every instance, due to various factors such as the reactor configuration, solar intensity change from day to other, bacterial response (dose/response), inactivation

mode, and the bacterial resistance to disinfectant used which might cause severe non-linearity characteristics. Thereby, the incorporation of an additional empirical parameter was made to accurately account the photocatalytic inactivation mechanism.

$$N/N_0 = A \exp(-k C^n t^m) \quad (4)$$

m is the empirical parameter that controls the deflection of the inactivation rate. If m is greater than 1, there is an increase in the inactivation rate and vice versa. This variation depends essentially of water quality and the photoreactor configuration.

$$k = k_d \left(\frac{d}{Q_{UV}} \right) (k_d + k_{QUV}) \quad (5)$$

where k is the disinfection kinetic constant, $k_{(d,QUV)}$ is the global photocatalytic inactivation coefficient, k_d and k_{QUV} are the disinfection kinetic constant related separately to the catalytic action and solar UV effects, d is the catalytic concentration (g/L), and Q_{UV} is the accumulated UV energy (kJ/L).

In the studied strains, the time required for bacterial inactivation by photocatalysis depends of the type of microorganism. Indeed, the time required for the inactivation of nearly

99.99% of cultivable bacteria bacterial was estimated at 30 min of irradiation for the indicator of biological contamination bacteria, *E. coli*, and about 40 min for pathogenic bacteria, *S. typhi*, against 45 min of exposure time for nosocomial bacteria, *P. aeruginosa*.

Based on the kinetics parameters determined according to the C-W model (Fig. 1; Table 1), the photocatalytic inactivation coefficient (k) that represents the slope of inactivation curve was more important for *E. coli* ATCC 25922 ($k = 0.19$) strain than for *S. typhi* ATCC 560 ($k = 0.17$) and *P. aeruginosa* ATCC 4114 ($k = 0.15$). According to Pagan et al. (1999), the greater the coefficient k , the lower the bacterial resistance to the applied disinfectant. Thus, we can deduce the photocatalytic sensibility of *E. coli* followed by *S. typhi* and we can estimate from Fig. 2d that *P. aeruginosa* is the most tolerant to a solar UV with fixed TiO₂-P25.

The results represented in Table 1 show that the reduction of bacterial cultivability rate (A) in the initial contact with the disinfecting agent was more significant for *P. aeruginosa*. Thus, this strain was estimated to be the most sensitive bacteria in the retention of cultivability in the initial contact with the disinfectant. However, *E. coli* was qualified to be more resistant in the retention of cultivability, followed by *S. typhi*.

Thus, based on photocatalytic inactivation, *P. aeruginosa* was the most resistant or tolerant strain to photocatalytic activities. This difference in bacterial behavior or response was probably due to an inter-specific difference concerning the cell growth strategy, the response to the environmental stress, the metabolism activity, and the genetic regulation and flexibility.

To describe bacterial inactivation behavior, series-event (SE) kinetic model with modification was used in addition to C-W model with modification. The inactivation process was modeled as a progression of discrete damage levels. The microorganism was assumed to be inactivated at a threshold level of damage (Fig. 3, Table 2).

Each step is characterized by first-order kinetics. Each damage level D_i has a kinetic constant k_i and n is the threshold level of damage.

By assuming that the kinetic constant is the same at each level, the following generalized expression can be derived for the series-event model (Severin et al. 1984).

$$\frac{N}{N_0} = A \exp(-K \times C^n t^m) + \ln \frac{(1 + \sum_{i=0}^{n-1} \times C^n t^m) i}{i!} \quad (6)$$

Table 2 illustrates the ability of the series-event model to report bacterial response behavior. The results indicated the absence of change in the kinetic constants (k and A) but the use of this model can report the series-events undergone by tested bacteria.

Table 1 Kinetic parameter for tested bacterial strains according to the first-order Chick-Watson model (C-W) with modification

Bacterial strain	A	k
<i>E. coli</i> ATCC 25922	0.03	0.19
<i>P. aeruginosa</i> ATCC 4114	0.94	0.15
<i>S. enterica typhi</i> ATCC 560	0.07	0.17

For example, to respond to solar UV with fixed TiO₂-P25, *E. coli* was chosen to pass through different damage levels (D_1 to D_4). The accumulation of damages has been realized by keeping the cultivability in initial contact with disinfectant (solar UV/TiO₂-P25). However, *P. aeruginosa* was adapted to another strategy. Indeed, this bacteria strain was decreased in the bacterial cultivability at the initial contact with the disinfectant (solar UV/TiO₂-P25) as a response to stress. Thereby, the damage level determined for this bacteria was $n = 1$ (D_1).

We can conclude that keeping or losing bacterial cultivability can be related to a bacterial adaptation to overcome the stress effects. Thus, the loss of cultivability is not constantly the synonym of sensibility and vulnerability and, in the same means, the retention of cultivability cannot reveal the resistance or tolerance of bacteria. The bacterial growth modality is directly related to the response of each species to the stress.

Post-irradiation events after the photocatalytic treatment

To study the efficiency of the photocatalytic process UV/TiO₂ (Degussa P-25) fixed on a glass plate, we studied the post-irradiation events after the photocatalytic treatment.

To semi-quantify the reactivation level of post-irradiated bacteria in dark condition, a log ratio was determined according to a modified version of the Lindauer and Darby (1994) equation with modification:

$$C_r = \log N_r / N_{Q_{UV/TiO_2}} \quad (7)$$

where C_r is the coefficient of reactivation, N_r = number of viable and cultivable bacteria after a rest time in the darkness, UV irradiation and N_{UV/TiO_2} is the number of viable and cultivable bacteria after photo-disinfection process with immobilized TiO₂.

According to Lindauer and Darby (1994), the reported log values have ranged from 1 to 3.4. When the C_r was inferior to 1, there was no reactivation; if C_r ranged between 1 and 3.4, we can conclude that the reactivation occurred in the darkness or/and in visible light; when C_r was superior to 3.4, there were no disinfectant effects and the cells grew naturally without any environmental stress. In effluents from the studied photoreactor and according to the result of *E. coli* and *P. aeruginosa*, a slight recovery was observed after

Fig. 3 Response of tested bacteria to solar photocatalytic treatment with immobilized $\text{TiO}_2\text{-P25}$, and monitoring of bacterial cultivability according to Chick-Watson (C-W) and series-event (SE) models. **a** *E. coli* ATCC 25922. **b** *P. aeruginosa* ATCC 4114. **c** *S. typhi* ATCC 560

photocatalytic treatment during the subsequent 24 h in dark conditions. Indeed, C_r was equal to 1.07 for *E. coli* and 1.22 for *P. aeruginosa* (Fig. 4), where C_r is the coefficient of reactivation, $C_r = \log N_r/N_{\text{UV/TiO}_2}$; N_r = number of viable and cultivable bacteria after a rest time in the darkness; UV irradiation and $N_{\text{UV/TiO}_2}$ are the number of viable and cultivable bacteria after photo-disinfection process with immobilized TiO_2 .

After solar UV/ TiO_2 treatment, the density of cultivable bacteria (*E. coli* and *P. aeruginosa*) was increased. This enhancement can be explained by the fact that biological systems have evolved some molecular mechanisms to

appropriately respond to the environmental stresses which have damaged proteins and DNA (Marugán et al. 2008). In this case, to respond to UV damage, bacteria generally possess molecular mechanisms to restore DNA lesions. Indeed, bacteria can evolve four main mechanisms in the repair of UV radiation-damaged DNA, including photoreactivation,

Fig. 4 Coefficient of bacterial reactivation (C_r) determined after a rest time in dark condition

Table 2 Kinetic parameter for tested bacterial strain according to series-events model (SE) with modification

Bacterial strain	<i>n</i>	<i>A</i>	<i>k</i>
<i>E. coli</i> ATCC 25922	4	0.14	0.14
<i>P. aeruginosa</i> ATCC 4114	1	0.94	0.15
<i>S. typhi</i> ATCC 560	2	0.138	0.13

Fig. 5 Bacterial kinetic of **a** *P. aeruginosa* ATCC4114 and **b** *E. coli* ATCC25922 after integration of coefficient of reactivation (C_r) in the kinetic models: Chick-Watson model (C-W) and series-events model (SE)

nucleotide excision repair (NER), mutagenic DNA repair (MDR), and recombinational DNA repair (Errol et al. 2006). These results can affect the effectiveness and the durability of solar photocatalytic treatment with fixed titanium dioxide device.

Bacterial resuscitation after the photocatalytic treatment could be explained by the fact that, under oxidative stress, their cells can enter into a viable but non-cultivable (VBNC) state.

The result of no cultivability after TiO_2 photocatalytic treatment under solar light does not always represent a total bacterial death. In fact, the ROS produced during the photocatalytic process can induce oxidative stress on the microorganisms, causing the cells to enter a VBNC state.

Solar UV irradiation and attacks by oxidative species generate changes in the permeability of the lipid membrane and/or modify the bacterial DNA, leading to the loss of cultivability while viability remains unaltered (Ben Said et al. 2010). When the oxidative stress ends (dark conditions), the microorganisms recover their cultivability. In addition, the growth state of bacteria (exponential or stationary) varies in time. Depending on this parameter, some bacteria could persist under photocatalytic conditions and consequently, their recovery rate in the dark could also be influenced. This hypothesis can explain the recovery of *E. coli* and *P. aeruginosa* after a rest time in the darkness.

In addition, the decrease in the accumulated UV energy (Q_{UV}) and the fluctuation of the visible spectral composition of sunlight during exposed day can affect directly the solar photo-inactivation, and photoreactivation as well as the bacterial behavior in the subsequent dark period.

We can also observe a reactivation of bacteria even during photocatalytic treatment. Indeed, between $t = 20$ min and $t = 30$ min, *P. aeruginosa* presented a reactivation (Fig. 3). This bacteria resilience during this period can be explained by the cells' ability to restore their UV-induced lesions by molecular photo-dependent mechanism called photoreactivation. This

mechanism repair was thought to be an important component of the bacterial arsenal in the repair or reversal of UV-mediated DNA damage (Ben Said and Otaki, 2012). Photoreactivation in bacteria involves a single enzyme called photolyase which binds CPDs and, in a light-dependent step, monomerizes the CPD and dissociates from the repaired lesion (Ben Said and Masahiro, 2013). Indeed, UV_A is essential for photoreactivation, although it also has lethal and sublethal effects on microorganisms (Oguma et al., 2001). Thus, when the value of log ratio (C_r) was inferior to 1, we can deduce that the lethal effects of Q_{UV} radiation take over its beneficial effects but when C_r was superior to 1, the inactivation effect of photocatalytic can be slowed down, and thereby, we can integrate this log ratio in the inactivation equation as well:

$$N/N_0 = A (1 - 1/C_r) \left[\text{Exp} \left(-k (C^n t^m) \right) \right] \quad (8)$$

Figure 5 shows the bacterial kinetic of *P. aeruginosa* and *E. coli* after integration of C_r in the kinetic model. We can note a modification of the initial bacterial reduction rate in the contact with solar UV radiation and TiO_2 -P25 for both tested bacteria that showed a reactivation after photocatalytic treatment where, for *E. coli*, A' was equal to 0.082 instead of 0.14 determined with SE model and for *P. aeruginosa*, A' was equal to 0.38 instead of 0.94 determined with C-W model. The difference between the two kinetic values ($A-A'$) represents the potential bacteria rate able to resuscitate after photocatalytic treatment.

The purpose of C_r rate integration in the photocatalytic inactivation equation was to consider an eventual reactivation after photocatalytic treatment in real scale and to find a solution to overcome the inconvenient related to this interesting technology.

According to Fig. 4, no recovery was observed for the tested *S. typhi* after 24 h in the darkness (C_r equal to 0.52). In this case, only *S. typhi* completed loss of viability and cultivability; other tested bacteria were not affected. Thus,

the behavior in the dark of *S. typhi* suggests that during photocatalytic disinfection, radicals and other oxidative species produced by illuminated TiO₂-P25 induced damage that can in certain cases get worse in the dark, generating a “residual effect” of the photocatalytic treatment. For this reason in post-treated *S. typhi* suspension, bacterial cells continued to decrease in the dark. In this case, the DNA repair mechanism became less active rendering the irreversible *S. typhi* inactivation. In other words, the bacterial injury generated by photocatalytic treatment continued to enhance in the dark.

For this delayed process, we have applied the term of “residual effect” but it is not necessarily induced by the residual presence of any active oxidative compound. In a previous article, we have reported that this “residual effect” in the dark was dependent on the light intensity previously applied (Marugán et al. 2008).

Conclusion

The photocatalytic solar UV/TiO₂ system was tested for its bactericide action against three bacterial strains:

E. coli ATCC 25922, *P. aeruginosa* ATCC 4114, and *S. typhi* ATCC 560. The comparison of different kinetic parameters determined according to the C-W model and series-events model (k , A) for the studied bacteria showed a difference in each tested bacterial response to a disinfectant agent. This difference in bacterial behavior or response was probably due to an inter-specific difference concerning the cell growth strategy, the response to the environmental stress, the metabolism activity, the genetic regulation and flexibility, etc.

After solar UV/TiO₂ treatment, bacterial strains (*E. coli* and *P. aeruginosa*) showed an ability to overcome photocatalytic stress, determined by the reactivation coefficient (C_r). This reactivation can be related to the fact that the biological systems have evolved mechanisms to appropriately respond to environmental stresses. These results can affect the effectiveness and the durability of solar photocatalytic treatment with fixed titanium dioxide device. However, no recovery was observed for tested *S. typhi* after 24 h in the darkness. In this case, only *S. typhi* complete loss of viability and cultivability. Thus, the behavior in the dark of *S. typhi* suggest that during photocatalytic disinfection, radicals and other oxidative species produced by illuminated TiO₂ induce damage that can in certain cases get worse in the dark, generating a “residual effect” of the photocatalytic treatment.

Enhancing the photocatalytic process efficiency remains a challenge and a subject of extensive research. In the end of this paper, we can propose many solutions such as (i) the increase of retention time, (ii) the change of photoreactor conception, and (iii) the addition of supplement step applied before or after solar UV-TiO₂.

Funding information This work is partially supported by the Tunisian-French project PHC Utique CMCU 14G0821.

References

- Acevedo A, Carpio EA, Rodríguez J, Manzano MA (2011) Disinfection of natural water by solar photocatalysis using immobilized TiO₂ devices: efficiency in eliminating indicator bacteria and operating life of the system. *J Sol Energy Eng* 134:011008. <https://doi.org/10.1115/1.4005338>
- Ben Said M, Masahiro O (2013) Enhancement of ultraviolet water disinfection process. *Afr J Biotechnol* 20:2932–2938. <https://doi.org/10.5897/AJB12.1479>
- Ben Said M, Otaki M (2012) Development of a DNA-dosimeter system for monitoring the effects of pulsed ultraviolet radiation. *Ann Microbiol* 62:1339–1344. <https://doi.org/10.1007/s13213-012-0562-0>
- Ben Said M, Masahiro O, Hassen A (2010) Detection of viable but non cultivable *Escherichia coli* after UV irradiation using a lytic Q β phage. *Ann Microbiol* 60:121–127. <https://doi.org/10.1007/s13213-010-0017-4>
- Bonetta S, Bonetta S, Motta F, Strini A, Carraro E (2013) Photocatalytic bacterial inactivation by TiO₂-coated surfaces. *AMB Express* 3(1): 59. <https://doi.org/10.1186/2191-0855-3-59>
- Bousselmi L, Ghrabi A, Ghazzi K, Zayani G, Ennabli M (2002) Solar photocatalytic treatment of textile wastewater: possibilities and limitations in the Tunisian context. *Proceedings of International Symposium on Environmental Pollution Control and Waste Management (EPCOWM'2002)* 804–812
- Caballero L, Whitehead KA, Allen NS, Verran J (2009) Inactivation of *Escherichia coli* on immobilized TiO₂ using fluorescent light. *J Photochem Photobiol A-Chem* 202:92–98. <https://doi.org/10.1016/j.jphotochem.2008.11.005>
- Cho M, Chung H, Choi W, Yoon J (2005) Different inactivation behaviors of MS-2 phage and *Escherichia coli* in TiO₂ photocatalytic disinfection. *Appl Environ Microbiol* 71(1):270–275. <https://doi.org/10.1128/AEM.71.1.270-275.2005>
- Chong MN, Lei S, Jin B, Saint C, Chow CWK (2009) Optimisation of an annular photoreactor process for degradation of Congo Red using a newly synthesized titania impregnated kaolinite nano-photocatalyst. *Sep Purif Technol* 67:355–363. <https://doi.org/10.1016/j.seppur.2009.04.001>
- Chong MN, Jin B, Saint CP (2011) Bacterial inactivation kinetics of a photo-disinfection system using novel titania-impregnated kaolinite photocatalyst. *Chem Eng J* 171:16–23. <https://doi.org/10.1016/j.cej.2011.03.024>
- Dheaya MAA, Patrick SMD, McMurray TA, Byrne JA (2009) Photocatalytic inactivation of *E. coli* in surface water using immobilised nanoparticle TiO₂ films. *Water Res* 43:47–54. <https://doi.org/10.1016/j.watres.2008.10.015>
- Errol CF, Graham CW, Wolfram S, Richard DW, Roger AS, Tom E (2006) DNA repair and mutagenesis. *ASM Pres* 687–698. <https://doi.org/10.1128/9781555816704>
- Fujishima A, Rao TN, Tryk DA (2000) Titanium dioxide photocatalysis. *J Photochem Photobiol. C: Photochem Rev* 1:1–21. [https://doi.org/10.1016/S1389-5567\(00\)00002-2](https://doi.org/10.1016/S1389-5567(00)00002-2)
- Gogniat G, Thyssen M, Denis M, Pulgarin C, Dukan S (2006) The bactericidal effect of TiO₂ photocatalysis involves adsorption onto catalyst and the loss of membrane integrity. *FEMS Microbiol Lett* 258: 18–24. <https://doi.org/10.1111/j.1574-6968.2006.00190.x>
- Hashimoto K, Irie H, Fujishima A (2005) TiO₂ photocatalysis: a historical overview and future prospects. *J Appl Phys* 44:8269–8285. <https://doi.org/10.1143/JJAP.44.8269>

- Huang Z, Maness PC, Blake DM, Wolfrum EJ, Smolinski SL, Jacoby WA (2000) Bactericidal mode of titanium dioxide photocatalysis. *J Photoch Photobiol A* 130:163–170. [https://doi.org/10.1016/S1010-6030\(99\)00205-1](https://doi.org/10.1016/S1010-6030(99)00205-1)
- Ismail M (2011) Preparation et caractérisation de nouveaux matériaux pour les réactions de dépollution photocatalytique de l'eau dans le visible. Thèse à l'Institut National Polytechnique de Lorraine, ENSIC Nancy France
- Kiwi J, Nadtochenko V (2005) Evidence for the mechanism of photocatalytic degradation of the bacterial wall membrane at the TiO₂ interface by ATR-FTIR and laser kinetic spectroscopy. *Langmuir* 21:4631–4641. <https://doi.org/10.1021/la046983l>
- Lifen L, Barford J, King Lun Y (2009) Non-UV germicidal activity of fresh TiO₂ and Ag/TiO₂. *J Environ Sci* 21:700–706. [https://doi.org/10.1016/S1001-0742\(08\)62327-X](https://doi.org/10.1016/S1001-0742(08)62327-X)
- Lindauer KG, Darby J (1994) Ultraviolet disinfection of waste water: effect of dose on subsequent photoreactivation. *Water Res* 28:805–817. [https://doi.org/10.1016/0043-1354\(94\)90087-6](https://doi.org/10.1016/0043-1354(94)90087-6)
- MacFarlane JW, Jenkinson HF, Scott TB (2011) Sterilization of microorganisms on jet spray formed titanium dioxide surfaces. *Appl Catal B Environ* 106:181–185. <https://doi.org/10.1016/j.apcatb.2011.05.023>
- Malato S, Blanco J, Richter C, Fernández P, Maldonado MI (2000) Solar photocatalytic mineralization of commercial pesticides: oxamyl. *Sol Energ Mat Sol C* 64:1–14. [https://doi.org/10.1016/S0927-0248\(00\)00037-4](https://doi.org/10.1016/S0927-0248(00)00037-4)
- Marugán JO, Grieken RV, Sordo C, Cruz C (2008) Kinetics of the photocatalytic disinfection of *Escherichia coli* suspensions. *Appl Catal B: Environ* 82:27–36. <https://doi.org/10.1016/j.apcatb.2008.01.002>
- Matsunaga R, Tomodam T, Wake NH (1985) Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol Lett* 29:211–214. <https://doi.org/10.1111/j.1574-6968.1985.tb00864.x>
- Mills A, Lee SK (2002) A web-based overview of semiconductor photochemistry-based current commercial applications. *J Photoch Photobiol A* 152:233–247. [https://doi.org/10.1016/S1010-6030\(02\)00243-5](https://doi.org/10.1016/S1010-6030(02)00243-5)
- Mills A, Hepburn J, Hazafy D, O'Rourke C, Krysa J, Baudys M, Zlamal M, Bartkova H, Hill CE, Winn KR, Simonsen ME, Søgaard EG, Pillai SC, Leyland NS, Fagan R, Neumann F, Lampe C, Graumann T (2013) A simple, inexpensive method for the rapid testing of the photocatalytic activity of self-cleaning surfaces. *J Photoch Photobiol A Chem* 272:18–20. <https://doi.org/10.1016/j.jphotochem.2013.08.004>
- Nassar R, Mokh S, Rifai A, Chamas F, Hoteit M, Allskandarani M (2017) Transformation of sulfaquinoxaline by chlorine and UV light in water: kinetics and by-product identification. *Environ Sci Pollut Res*:1–10. <https://doi.org/10.1007/s11356-017-0814-4>
- Oguma K, Katayama H, Ohgaki S (2001) Photoreactivation of *Escherichia coli* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Appl Environ Microbiol* 68:6029–6035. <https://doi.org/10.1128/AEM.68.12.6029-6035.2002>
- Pablos C, VanGrieken R, Marugán J, Moreno B (2011) Photocatalytic inactivation of bacteria in a fixed-bed reactor: mechanistic insights by epifluorescence microscopy. *Catal Today* 161:133–139
- Pagan R, Manas P, Raso J, Condon S (1999) Bacterial resistance to ultrasonic waves under pressure at nonlethal (manosonication) and lethal (manothermosonication) temperatures. *Appl Environ Microbiol* 65:297–300
- Pigeot-Rémy S, Simonet F, Atlan D, Lazzaroni JC, Guillard C (2012) Bactericidal efficiency and mode of action: a comparative study of photochemistry and photocatalysis. *Water Res* 46:3208–3218. <https://doi.org/10.1016/j.watres.2012.03.019>
- Piscopo A, Robert D, Weber JV (2001) Influence of pH and chloride anion on the photocatalytic degradation of organic compounds Part I. Effect on the benzamide and para-hydroxybenzoic acid in TiO₂ aqueous solution. *Appl Catal B: Environ* 35:117–124
- Rahmani AR, Samarghandi MR, Samadi MT, Nazemi F (2009) Photocatalytic disinfection of coliform bacteria using UV/TiO₂. *J Res Health Sci* 9:1–6
- Rengifo HJA, Pulgarin C, Machuca F, Sanabria J (2010) TiO₂ photocatalytic inactivation under simulated solar light of bacterial consortia in domestic wastewaters previously treated by uasb, duckweed and facultative ponds. *Quim Nova* 33:1636–1639. <https://doi.org/10.1590/S0100-40422010000800003>
- Rincon AG, Pulgarin C (2004) Field solar *E. coli* inactivation in the absence and presence of TiO₂: is UV solar dose an appropriate parameter for standardization of water solar disinfection. *Sol Energy* 77:635–648. <https://doi.org/10.1016/j.solener.2004.08.002>
- Robertson JMC, Robertson PKJ, Lawton LA (2005) A comparison of the effectiveness of TiO₂ photocatalysis and UV_A photolysis for the destruction of three pathogenic micro-organisms. *J Photoch Photobiol A* 175:51–56. <https://doi.org/10.1016/j.jphotochem.2005.04.033>
- Rtimi S (2017) Indoor light enhanced photocatalytic ultra-thin films on flexible non-heat resistant substrates reducing bacterial infection risks. *Catalysts* 7:57. <https://doi.org/10.3390/catal7020057>
- Rtimi S, Pulgarin C, Sanjines R, Kiwi J (2015) Kinetics and mechanism for transparent polyethylene-TiO₂ films mediated self-cleaning leading to MB dye discoloration under sunlight irradiation. *Appl Catal B Environ* 162:236–244. <https://doi.org/10.1016/j.apcatb.2014.05.039>
- Sapizah R, Shahidan R, Ainon H (2012) Inactivation of *Escherichia coli* under fluorescent lamp using TiO₂ nanoparticles synthesized via sol gel method. *Sains Malays* 41(2):219–224
- Schuch AP, Galhardo RS, Lima-Bessa KM, Schuch NJ, Menck CFM (2009) Development of a DNA-dosimeter system for monitoring the effects of solar-ultraviolet radiation. *Photochem Photobiol Sci* 8:111–120. <https://doi.org/10.1039/b810085c>
- Severin BF, Suidan MT, Engelbrecht RS (1984) Series-event kinetic model for chemical disinfection. *J Environ Eng* 110:430–439. [https://doi.org/10.1061/\(ASCE\)0732-9372\(1984\)110:2\(430\)](https://doi.org/10.1061/(ASCE)0732-9372(1984)110:2(430))
- Silva CR, Miranda SM, Lopes FVS, Silva M, Dezotti M, Silva AM, Faria JL, Boaventura RAR, Vilar VJP, Pinto E (2017) Bacteria and fungi inactivation by photocatalysis under UV_A irradiation: liquid and gas phase. *Environ Sci Pollut Res* 24:6372–6381. <https://doi.org/10.1007/s11356-016-7137-8>
- Spasiano D, Marotta R, Malato S, Fernandez-Ibanez P, Di Somma I (2015) Solar photocatalysis: materials, reactors, some commercial, and pre-industrialized applications. A comprehensive approach. *Appl Catal B: Environ* 170–171:90–123. <https://doi.org/10.1016/j.apcatb.2014.12.050>
- Tatsuma T, Kubo W, Fujishima A (2002) Patterning of solid surfaces by photocatalytic lithography based on the remote oxidation effect of TiO₂. *Langmuir* 18:9632–9634. <https://doi.org/10.1021/la026246u>
- Vamathevan V, Amal R, Beydoun D, Low G, McEvoy S (2002) Photocatalytic oxidation of organics in water using pure and silver-modified titanium dioxide particles. *J Photoch Photobiol A Chem* 148:227–233. <https://doi.org/10.4236/ojic.2013.31003>
- Wang W, Huang G, Yu JC, Wong PK (2015) Advances in photocatalytic disinfection of bacteria: development of photocatalysts and mechanisms. *34*:232–247. <https://doi.org/10.1016/j.jes.2015.05.003>
- Watson HE (1908) A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *J Hyg* 8:536–542
- Zsilák Z, Szabó-Bárdos E, Fónagy O, Horváth O, Horváth K, Hajós P (2014) Degradation of benzenesulfonate by heterogeneous photocatalysis combined with ozonation. *Catal Today* 230:55–60. <https://doi.org/10.1016/j.cattod.2010.10.051>