Solar photocatalysis: A green technology for *E. coli* contaminated water disinfection. Effect of concentration and different types of suspended catalyst

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**A B S T R A C T**

Photocatalytic and photolytic disinfection of *Escherichia coli* in water was studied under natural sunlight using different types of photocatalyst (TiO₂, P-25, PCS00, Ruana and Bi₂WO₆) at different concentrations. The solar photo-inactivation yielded complete inactivation results, which varied with the solar light intensity. Meanwhile, dark control samples in the lab (temperature constant at 25 °C) remained at constant concentration and dark samples outside laboratory showed a decrease due to the mild solar heating occurred during the experiments. The adding of any kind of photo-catalyst to the water accelerated the bactericidal action of solar irradiation and led to complete disinfection (until detection limit). The photocatalytic disinfection efficiency was not enhanced by the increase of catalyst concentration above 0.5 g/L for P-25, PCS00 and Bi₂WO₆, where about 10⁶ CFU/mL were completely inactivated within 5 min, 30 min and more than 150 min of solar exposure under clear sky, respectively. An increase of the concentration to 1 g/L slightly decreased the total inactivation time. Rutile (Ruana) catalyst behaves differently, optimal concentration was lower than for the other titania materials; agglomeration of particles occurred as the concentration of catalyst increases. Durability of photocatalytic treatment and chemical analyses of inorganic anions and cations have also been investigated.

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1. Introduction

In recent years, there is an urgent need for an economical, practical, non-toxic and safe method for drinking and waste water purification. Natural sunlight is one of the promising means for water disinfection mainly in countries with a high degree of solar radiation.

Solar water disinfection (known as SODIS) is an ancient technology that uses the effect of solar UV radiation for the inactivation and destruction of micro-organisms in drinking water. SODIS was first studied in 1980 by Acra et al. [1], who used polyethylene bags and variety of containers made of transparent glass or plastic with different colours and shapes filled with contaminated water and exposed to direct sunlight. The results confirm the effectiveness and feasibility of the solar decontamination of water in small quantities (≤3 L) where a zero coliform count/ml was attained in about one hour. Later in 1990 Acra et al. [2] demonstrated that water can be decontaminated by solar radiation on a large scale using continuous-flow systems.

Since then, water disinfection using natural and/or artificial sunlight is widely studied for the inactivation of a wide range of bacteria (*E. coli*, *Streptococcus faecalis*, *Shigella dysenteriae*, *Cryptosporidium*, *Enterococcus faecalis*, *Salmonella*, *Pseudomonas* and *Coliform*) [3–8].

The principle of SODIS is to use a combination of irradiation by direct sunlight and solar heating to kill the pathogens in contaminated drinking water. This simple procedure to disinfect drinking water using transparent PET–bottle (polyethylene terephthalate) and/or glass containers has been tested in different sunny regions with high solar irradiance. Many families from different regions use the SODIS method to treat their drinking water as in South Africa, Cameroon, Senegal and India [9].

In 1996 Joyce et al. [3] showed that, in highly turbid water (200 NTU (nephelometry turbidity units)) the heating effect of sunlight could produce complete inactivation of *E. coli* within 7 h only if the water temperature reached at least 55 °C. Turbidity above 200 NTU
absorbs approximately 99% of the incident radiation within the first centimetre of optical path.

Although, SODIS is an effective, low cost method that would be more appropriate for application in the third world countries for improving their water quality, many factors can affect its efficiency as:

- The daily and seasonal variation of sun intensity which increases time exposure of treated water [7].
- Risk of bacteria regrowth during the post-treatment water storage [10,11].
- Presence of waterborne pathogens resistant to sunlight.

Water turbidity will reduce the penetration of light through highly turbid samples. The presence of suspended solids will also prevent light penetration and may facilitate bacterial regrowth, especially in the presence of sufficient amount or dissolved organic matter in the water under moderate temperatures if minimal solar power densities are not reached [12]. Some of the main disadvantages of SODIS include the lack of quality assurance for the end user and high levels of non-compliance by the users. To increase the efficiency of this method (SODIS), some additives are added as the oxidant H2O2 and/or O3, as well as the addition of a catalyst (TiO2, Fe2O3, ZnO, etc.).

Photocatalytic inactivation of bacteria was firstly investigated by Matsunaga et al. in 1985 [13]; where TiO2–Pt photocatalyst illuminated by near-UV radiation was used to inactivate microbial cells in water. Since then, there has been a great deal of interest in the disinfection applications of this process, many including disinfection of drinking water in industrial and healthcare environments. Several contributions show the inactivation of different microorganisms as (viruses, fungi and bacteria) with titania, where E. coli and TiO2 P25 are the most studied microorganism and catalyst [14–29]. Recent reviews present the capabilities and mechanisms of TiO2 for water disinfection [30–32], also using solar energy as source of UVA photons [33]. In fact, the photocatalytic process is effective via solar irradiation even if TiO2 anatase (which is the most efficient photocatalyst) absorbs only 4% of solar spectrum.

The main aim of the study was to investigate the photocatalytic disinfecting activity of three samples of TiO2 containing mainly anatase phase (P25 and PC500), or containing mainly rutile phase (Ruana), and one sample of Bi2WO6: these two last materials having the advantage of absorbing more solar photons. The photocatalyst concentration was varied and results compared with solar disinfection (without catalyst) under natural sunlight. E. coli was used as model of microorganism. Bacterial re-growth following 72 h treatment, and the formation of anions and cations during the photocatalytic processes was also investigated. The role of solar mild heating due to the IR component of the solar spectrum will be discussed.

2. Experimental methods

2.1. Bacterial strain, cultivation and quantification

E. coli K-12 was used as the test organism in these experiments due to its wide spread use as a faecal indicator and its resistance to the bactericidal effects of solar irradiation relative to other bacteria (Pseudomonas, Shigella, and Salmonella) [34].

E. coli was generated from frozen stocks by streaking on to Luria Bertani (LB) (Sigma–Aldrich, USA) agar and incubated at 37 °C for 15–18 h. Single colonies were then inoculated in to two aliquots of 14 mL sterile LB broth (Miller’s LB Broth, Sigma–Aldrich, USA) and were incubated at 37 °C with constant agitation under aerobic conditions on a rotary shaker at 100 rpm for 18 h. E. coli suspensions were centrifuged at 3000 rpm for 10 min. The bacterial pellets were re-suspended in a same volume (14 mL) of phosphate buffered saline (PBS), solution yielding to a final concentration of 10^9 CFU/mL (Colony Forming Units per millilitre).

2.2. Solar stirred tank reactor

All experiments were carried out under natural solar irradiation at the Plataforma Solar de Almeria, Spain, located at 37° 84′ N and 2° 34′ W, in clear sunny days. Solar stirred tank reactors exposed to sunlight were borosilicate glass (DURAN, Schott, Germany) bottles magnetically stirred during all experiment with a total volume of 250 mL (Fig. 1). Glass covers (Schott) were used instead of plastic lids permitting the entrance of the solar radiation from all directions. Prior to solar exposure, bottles with 200 mL of NaCl (0.9 wt%) sterile solution were spiked with 200 μL of E. coli (10^9 CFU/mL) solution to obtain an initial bacterial concentration of 10^6 CFU/mL. Saline solution (NaCl, 0.9%) was used to avoid the bacterial osmotic stress induced by distilled water [22]. The photocatalyst was added as received from the manufacturer to the bacterial suspension, after few minutes of stirring in the dark for homogenisation the reactors were exposed to natural solar irradiation.

2.3. Photocatalyst

The experiments were performed with four different catalysts, TiO2 Evonik P-25, TiO2 PC500, TiO2 Ruana and Bi2WO6. Bismuth tungstate (Bi2WO6) is composed of micrometre-sized spherical polycrystalline particles of bismuth (65%) and tungstate (35%). Bi2WO6 has a hierarchical “flake-ball” shape and was prepared by a facile hydrothermal reaction without using any surfactants and polymers as structure-directing agents [35,36]. All catalysts were used in suspension under different concentrations of 0.05, 0.1, 0.5 and 1 g/L. Their characteristics are given in Table 1.

<table>
<thead>
<tr>
<th>Photocatalyst</th>
<th>Provider</th>
<th>Crystalline form</th>
<th>Crystallite size (nm)</th>
<th>Surface area (m^2/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-25</td>
<td>Degussa</td>
<td>Anatase (80%)</td>
<td>24 ± 2</td>
<td>50</td>
</tr>
<tr>
<td>PC 500</td>
<td>Millennium</td>
<td>Rutile (20%)</td>
<td>37 ± 3</td>
<td>326</td>
</tr>
<tr>
<td>Ruana</td>
<td>IRCELyon</td>
<td>Anatase (25%)</td>
<td>8 ± 3</td>
<td>157</td>
</tr>
<tr>
<td>Bi2WO6</td>
<td>Japan Laboratory</td>
<td>Bismuth (65%)</td>
<td>3 × 10^3</td>
<td>20</td>
</tr>
</tbody>
</table>
2.4. Photocatalytic experiments

The first sample (t = 0 min) was taken in the dark at room temperature and under agitation and then kept in the dark as a "control sample" to be analysed again at the end of the experiment to determine any decrease of E. coli concentration in the dark. Once the first sample was taken, the reactors were exposed to natural solar light under magnetical stirring at 100 rpm. All experiments were performed in triplicate (3 reactors for each condition). Solar photocatalytic and solar disinfection tests (without photocatalyst) were carried out simultaneously. Two additional experiments were also done in the dark in the absence of catalyst, one was placed outside with an opaque cover to permit the samples to heat due to environmental temperature and the other was kept in the lab in the absence of light at constant temperature. All experiments started between 10:00 and 11:00 a.m. local time, for 5 h exposure to sunlight.

The temperature of samples inside the reactors was measured during all experiments with a platinum resistance temperature sensor.

2.5. UVA solar radiation measurement

Solar UVA radiation was measured by a global UV radiometer (Model CUV4, Kipp & Zonnen, the Netherlands) with a typical sensitivity of 264 μV/W/m², and a central wavelength of 300–400 nm which provides data in terms of incident WUV/m². QUV was calculated [17] for comparison of solar test results. It estimates accumulated UV energy in the solar bottles reactor per unit of treated water volume for given periods of time (Eq. (1)).

\[
Q_{UV} = \sum_{n} \bar{U}V_{n-1} A_t (t_n - t_{n-1})
\]  

where \( t_n \) is the experimental time for n-sample, \( \bar{U}V_{n-1} \) is the average solar ultraviolet radiation measured during the period \( (t_n - t_{n-1}) \), \( A_t \) is the illuminated reactor surface, and \( V_t \) is the total water volume.

2.6. Chemicals analysis

At different times of photocatalytic disinfection, water samples were taken and filtered using a 0.45 μm Millipore filters to be analysed. The formation of anions and cations during the process was determined using a Dionex DX-120 ion exchange chromatograph. For cation analysis, a Dionex Ion Pac CS16 (5 mm x 250 mm) column was used with H₂SO₄ (11 mmol/L) as eluent at a flow rate of 1 mL/min. The analysis of anions was performed by using a Dionex Ion Pac AS14A (4 mm x 250 mm) column with Na₂CO₃ (8 mmol/L) and NaHCO₃ (1 mmol/L) as eluent (1 mL/min).

3. Results

3.1. Solar mild heating effect on E. coli inactivation in the absence of photocatalyst

Sunlight provides UVA-Vis photons and also heating due to the IR spectrum, which are the main microbial decontaminating factors. In order to separate the effects of these two factors, and study only the thermal effect of sunlight, reactors were covered by aluminium paper and exposed outside to the solar irradiation. Before solar exposition, a control sample was taken and kept at room temperature (24–26 °C) in the dark. Another bottle reactor was maintained inside in lab-temperature for monitoring the E. coli cultivability in the dark.

The temperature profiles of the water in bottles reactors and the variation of the E. coli concentration during dark experiments inside and outside the laboratory were represented as a function of the sunlight exposure time and shown in Fig. 2.

Fig. 2 shows that E. coli concentration remained almost stable in dark condition inside the laboratory where the temperature was maintained constant during the experiment (around 25 °C). Outside laboratory, the water temperature increases due to IR and a positive effect of the solar mild heating on the inactivation of bacteria was observed. A thermal increase from 25 to 42 °C improved the E. coli inactivation, which reveals their sensitivity to mild temperatures. During the first two hours of heating where the water temperature was below 40 °C, no bacterial inactivation was detected; whereas above 42 °C bacterial inactivation rate gradually increased until a total 2.5-log decrease at the end of the experiment (5 h in the dark).

We note also, that during the last two hours of the experiment, the temperature of the solution increased very slowly, but the E. coli concentration continued to decrease indicating that the temperature reached (42 °C) was high enough to lead at bacteria inactivation.

McGuan et al. [4] have studied the roles of optical and thermal inactivation mechanisms by simulating conditions of optical irradiance and temperature. They found that the thermal inactivation of E. coli was negligible at temperatures below 40–45 °C, being sufficiently important only at water temperatures higher than 45 °C.

Fujikawa et al. [37] found that the constants rates k of E. coli destruction increase with temperatures, with a value of 6.55 × 10⁻³ L/s at a temperature of 56 °C which increases to 4.7 × 10⁻² and 2.69 × 10⁻¹ at respectively 60 °C and 64 °C.

3.2. Effect of the natural solar UV irradiance on E. coli inactivation in the absence of photocatalyst

Experiments using natural solar UV irradiation at different intensities were performed at different days in summer (July 2010). These experiments were run from 10:45 a.m. to 15:45 p.m., when the solar irradiance reaches the highest levels. The results of the effect of sunlight on E. coli inactivation were represented as a function of the local time (Fig. 3a) and the accumulated UV energy QUV (Fig. 3b). Temperature (°C) was also monitored during experiments (Fig. 3c).

Fig. 3a shows that E. coli inactivation rates were enhanced in the presence of solar radiation and that the inactivation increases with
Fig. 3. Effect of the natural solar UV intensity on E. coli inactivation as a function of (a) Local time and (b) UV-dose ($Q_{UV}$), $C_0 = 10^7$ CFU/mL. Open symbols are dark controls. DL is the detection limit for the experiments; 4 CFU/mL. Insert Fig. 3b (c) TiO$_2$ measurement during E. coli inactivation.

the UV-dose. The inactivation rates observed varied as a function of the UV irradiance of each day, leading to a complete inactivation (from $10^6$ CFU/mL to DL (Limit of detection) in all cases. The higher the solar irradiance the shorter treatment time was required (SODIS 3) for a total inactivation, and vice versa. About 3 h are needed in the case of SODIS 3, while the experiment performed under the lowest irradiance (SODIS 1) requires at least 5 h achieving same result (Fig. 3a).

The three inactivation curves present a shoulder, the E. coli inactivation is very slow during the first hours of irradiation, followed by a faster linear-phase of the inactivation rate. This kinetic profile has been reported before for solar water disinfection studies [38]. The shoulder was attributed to first resistance phenomenon due to the self-defence and auto-repair mechanisms of E. coli.

Since those experiments were done in different days where solar intensity change from day to other, E. coli inactivation was represented as a function of the accumulated UV energy $Q_{UV}$ (Fig. 3b) for better comparison. Fig. 3b shows that the three curves are not totally superposed suggesting that solar disinfection can be also influenced by thermal processes. However, the differences in the temperature of the three experiments are negligible (Fig. 3c), the germicidal action is mainly due to solar radiation in the near UV range (300–400 nm) [1], which depends on the solar irradiance intensity. However, a synergistic effect can be established when the optical and thermal inactivation processes are combined during solar disinfection leading to a total destruction of E. coli.

The bacterial inactivation rate depends of the intensity of sunlight, seasonal variations and cloud cover, the effective range of wavelengths of light, and the time of day [2], and of water temperature and its modification through the solar exposure.

Aca et al. [2] have reported that UV-A and early visible wavelength (320–450 nm) of the sunlight spectrum were able to generate reactive oxygen species (ROS) which cause strand breakage and base changes in DNA. These reactive oxygen species can also disrupt protein synthesis; and attack bacterial cells contributing to the destruction of microorganisms [10].

Moreover, UV-B (290–320 nm) is known to induce direct damage to DNA via formation of the cyclobutane pyrimidine dimer (CPD) photoproduct [39]. The formation of these dimers alters gene expression and inhibit DNA replication, and causes genetic mutation [40].

Different authors [3,4] have reported that solar disinfection is based on the pasteurizing effect of solar irradiation at mild high temperatures (40–55 °C), and the synergy effect caused by both factors at same time causing complete and irreversible inactivation of most bacterial pathogens. However, low solar irradiation and water turbidity can negatively affect the solar disinfection efficiency, causing only partial bacterial inactivation, favouring bacterial regrowth by photorepair mechanisms [5,6,8].

3.3. Influence of the load of photocatalysts (TiO$_2$ Evonik P25, PC 500, Ruana and Bi$_2$WO$_6$) on E. coli inactivation

As previously observed, the use of only solar radiation for water disinfection was able to completely inactivate E. coli but it needed a long exposure time. To reduce this time, and for a practical application of this technique, different types of photocatalysts (TiO$_2$ Evonik P25, PC 500, Ruana and Bismuth tungstate (Bi$_2$WO$_6$) were added to the solution in order to study their efficacy on E. coli inactivation under natural solar irradiation.

For all catalysts, the optimal concentration was experimentally evaluated testing the photocatalytic disinfecting activity against E. coli for different concentrations ranged from 0.05 to 1 g/L under natural sunlight (Figs. 4–7). Before sunlight exposure, control samples were taken from reactors for initial analysis of viable bacteria concentration, then kept at room temperature (24–26 °C) in the dark to be analysed again at the end of the experiment. Results were represented in the degradation curves (Figs. 4–7) and named as ‘dark controls’. During solar photocatalytic treatment water samples were analysed at different exposure times varying from 5 to 30 min. For each catalyst, simultaneous solar disinfection experiments were also done and represented on each graph (‘SODIS’) for comparing with photocatalytic results.

For every photocatalyst four concentrations were tested in two different days: 0.05 and 0.1 g/L were tested one day and 0.5 and 1 g/L the following day. For each catalyst concentration, three replicates experiments and a solar disinfection test were done simultaneously. Each point in the curves represents an average of triplicates with the standard deviation as error bar. Solar UVA irradiance, which varied constantly and from one day to another, was measured during all experiments. Water temperature was also monitored during all experiments, and represented in Figs. 4–7.

To compare the influence of catalyst concentrations on E. coli inactivation, all results were represented as a function of the accumulated UV energy in the photoreactor per unit of treated water volume for given periods of time during the experiment: $Q_{UV}$ (KJ/L).

For all photocatalysts tested, the solar inactivation of E. coli was strongly enhanced by the presence of the different types of photocatalysts. The treatment times required to completely inactivate
E. coli were dramatically reduced compared to solar disinfection. The enhancement observed depend on the type and concentration of catalyst, in agreement with the finding of Gumy et al. [41], who have reported that the inactivation of E. coli is enhanced by the presence of different types of suspended TiO₂ (Evonik P-25, Millennium PC-100 and PC-500, Tayca AMT-100 and AMT-600). Dark control samples showed that the inactivation was due to the photocatalytic process and not to other stress factors like the osmotic stress during the experiment. As mentioned before, in the absence of catalyst the bacterial inactivation was very slow (‘shoulder effect’) during the first hour of illumination, followed by a faster bacterial decrease afterwards. This ‘shoulder’ shape of the inactivation curves disappears or becomes less important in the presence of photocatalysts, indicating that the production of OH⁻ radicals during the process rapidly overcomes the self-defence mechanisms of the bacteria causing rapidly its destruction.

Fig. 4a and b shows that for Evonik P25, the total E. coli inactivation was reached using the four different catalysts concentrations under different times. The use of 0.5 and 1 g/L of Evonik P25 has given almost the same result, where about 10⁶ CFU/mL were completely inactivated within 5 min. At lower concentrations of TiO₂ 0.05 and 0.1 g/L a supplementary time of respectively 15 and 30 min was necessary to reach a 6-log bacterial kill off. During the first min of irradiation, the active species generated begin an external to the cells membrane causing serious damage to the bacterial outer membrane leading to the loss of its permeability [16,21].

Although the increase of catalyst concentration from 0.05 to 1 g/L increases the suspension opacity, the disinfection capacity was not affected and a total E. coli inactivation was also reached at 1 g/L. Accordingly, 0.5 g/L was considered as the optimum concentration of TiO₂ Evonik P25. In this optimum condition (0.5 g/L), 0.7 kJ/L of the accumulated UVA energy Qₜᵥ was needed for a complete inactivation of the bacteria (Fig. 4b).

Fig. 5a shows that PC500-based disinfection was successfully achieved for all catalyst concentrations (0.05, 0.1, 0.5, and 1 g/L), where about 10⁶ CFU/mL were decreased till DL within different exposure times. The total inactivation time decreased as catalyst concentration increased, from 50 min at 0.05 g/L to 30 min at 0.5 g/L. However, further increase of the concentration than 1 g/L had a slight negative effect on the inactivation time due to a light screen effect of the catalyst, indicating that 0.5 g/L was the most effective concentration.

For all PC 500 concentrations tested, the photocatalytic inactivation rate was observed to be decreased in the last stage of the disinfection process. For example, with 0.5 g/L, almost 75% of the survival E. coli (4.5-log) was inactivated in the first 15 min, and 25% (1.5-log) in the following 15 min, suggesting the competition between the viable and non viable cells and by-products formed during the photocatalytic process [29]. The same behaviour was also observed with the photocatalytic disinfection of the fungal F. solani with TiO₂ Evonik P25 under natural solar irradiation in a small volume of distilled water (200 mL) [42]. Fig. 5b shows that the amount of UVA accumulated energy required for complete E. coli
inactivation in the optimum conditions (0.5 g/L of PC 500) was equal to 1.6 kJ/L.

In presence of Bi₂WO₆ (Fig. 6a and b) a complete inactivation of bacteria was also achieved. However, the time necessary to observe a complete inactivation was more important than this one obtained in presence of anatase structures. Moreover, the presence of this catalyst at low concentration (0.05 g/L) did not significantly accelerate the solar inactivation results.

As found with TiO₂ Evonik P25 and PC 500, a similar behaviour was also observed with Bi₂WO₆, where the photocatalytic disinfection efficiency was enhanced by the increase of photocatalyst concentration until 0.5 g/L, where about 7 kJ/L was needed for a total inactivation of bacteria against 12 kJ/L in presence of 0.05 g/L. However, the increase of catalyst concentration to 1 g/L needed more energy for total bacterial inactivation.

These results are in agreement with the findings of Saito et al. [14] suggesting that the increase of catalyst concentration increased the turbidity and then reduced the efficiency of cell inactivation. In the case of Bi₂WO₆, the particles sizes of 3 μm had more important effect on the turbidity than the TiO₂ particles, which had sizes of the order of 8 ± 3 nm for PC500 and ~30 nm for Evonik P25.

In the presence of Bi₂WO₆, and during the first 20–30 min (depending of the concentration) the number of bacterial cells remained almost constant, and then decreased significantly towards the end. After this induction period, E. coli cells started to decrease significantly indicating that bacteria cannot overcome oxidant stress as the anti-stress enzymes were no longer able to protect their membrane against oxidation leading to their subsequent death. The relatively large size of the powder (around 3 μm) may protect bacteria cells from light during the initial exposure time. Moreover, for the photocatalytic inactivation of bacteria, a physical contact between particles of catalyst and bacteria cells may be necessary. In such case, a relatively large size of particles is disadvantageous, especially for Bi₂WO₆ which has a hierarchical “flakeball” shape. This flake-ball structure was kept in the initial stage, but collapsed during the reaction to give relatively high photocatalytic activity afterward [43].

In presence of Ru ana catalyst, which contained mainly rutile structure, a different behaviour was observed (Fig. 7a). With 0.5 and 1 g/L, E. coli inactivation rates were very fast at the beginning and then decreased; almost 3-log reduction of E. coli was reached during the first 30 min where the accumulated UV-energy was about 2.5 kJ/L. Following this QUV value, the inactivation of bacteria becomes slow; the photocatalytic disinfection efficiency decreased and the process became less effective than with the small concentrations of Ru ana (0.05 and 0.1 g/L), suggesting that the few active bacteria remaining in the irradiated water were in competition for OH* with both the inactivated bacteria and the metabolites released during the photo-process [18]. Nevertheless, the best inactivation was shown with a concentration of 0.05 g/L where a 6-log kill (from ~10⁶ CFU/mL to the detection limit) was achieved within 90 min, corresponding to an accumulated UV-energy of about 10 kJ/L (Fig. 7b).

The greater amounts of photocatalyst present can limit light incoming into the reactor. A screen effect of photocatalyst may occur. In the case of this catalyst, we observed that it was not well dispersed in solution and seemed to form aggregates, which
may explain the decrease of its activity beyond 0.05 g/L. To better understand this phenomenon DLS measurements (Dynamic Light Scattering) were done (Table 2). We observed that as the concentration of catalyst increased, the size of the agglomerate rose too, leading to a decrease of the photocatalytic efficiency due to a decrease on the light inside the reactor at the interaction with the micro-organism.

As a summary, for the three photocatalysts Evonik P25, PC500 and Bi2WO6, the optimal concentration found was 0.5 g/L, while the smallest concentration used, 0.05 g/L, was less effective because it was not sufficient to absorb all photons, and less radicals OH• responsible of cell inactivation were generated.

The action of the radicals on the bacterial cell membrane induced a perturbation of different cellular processes before their death [44]. Nevertheless, this small concentration of photocatalyst (0.05 g/L) was experimentally found as the optimum for Ruana photocatalyst, as this depends strongly on the optical properties of the particles used in suspension.

Wei et al. [15] have investigated the influence of TiO2 concentration on the rate of E. coli inactivation (∼10⁶ cells/mL), in the range from 0.1 to 1 g/L under constant illumination. They have found that cell cultivability decreased gradually with the increase of TiO2 concentration, where a dose of 1 g/L TiO2 caused complete inactivation of the bacteria in less than 30 min. According to several contributions, the optimal TiO2 concentration reported in the literature for E. coli inactivation studies was ranged from 0.25 to 1 g/L [15,16,18].

### Table 2

<table>
<thead>
<tr>
<th>Ruana concentration (g/L)</th>
<th>Agglomerate size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>550</td>
</tr>
<tr>
<td>0.1</td>
<td>646</td>
</tr>
<tr>
<td>0.5</td>
<td>653</td>
</tr>
<tr>
<td>1</td>
<td>780</td>
</tr>
</tbody>
</table>

Fig. 8. Absorption spectrum of TiO2 Evonik P25, PC500, Ruana and Bi2WO6.
However, in photocatalytic degradation of organic compounds, the optimal TiO$_2$ concentration was found, using the same experimental conditions, to be higher. In the literature, the initial rate ($r_0$) for bacterial inactivation as well as for organic compound degradation increased with the amount of catalyst until reaching a plateau [16,45].

Rincón et al. [16] have reported that at high TiO$_2$ concentrations (>1 g/L) the low penetration of the light in the major part of the solution led to a lower photoactivity of the catalyst and the direct action of light on bacteria was also reduced. They have also reported that at high TiO$_2$ concentrations, terminal reactions (Eqs. 2 and 3) could also contribute to the decrease of the efficiency of the process. According to Eq. (1), OH$^*$ will readily dimerised to form H$_2$O$_2$ producing afterwards HO$_2^*$ which was less reactive

\[ \text{OH}^* + \text{OH}^* \rightarrow \text{H}_2\text{O}_2 \]  \hspace{1cm} (2)

\[ \text{H}_2\text{O}_2 + \text{OH}^* \rightarrow \text{H}_2\text{O} + \text{HO}_2^* \]  \hspace{1cm} (3)

3.4. Comparison of the solar photocatalytic activity of different types of suspended catalyst on E. coli inactivation

For all the catalysts and the concentrations used (Fig. 8), TiO$_2$ Evonik P25 was found to be the most efficient photocatalyst, despite of its lower absorption of visible light compared to Ruana and Bi$_2$WO$_6$ (Fig. 9) and its smaller specific surface area (50 m$^2$/g) compared to Ruana (157 m$^2$/g) and PC500 (326 m$^2$/g). Even though used fixed, TiO$_2$ Evonik P25 has shown the best photocatalytic inactivation rate compared to rutile and anatase used alone [16]. However, in the case of hyper-adherent E. coli, PC500 was found better than TiO$_2$ P-25 [19].

Regardless the concentration used, the highest photocatalytic efficiency was obtained with Evonik P-25: while if we consider the time required for a complete E. coli inactivation the classification of the others photocatalysts varied. At low concentration 0.05 g/L of photocatalyst, Ruana was more effective than PC500 and Bi$_2$WO$_6$. This can be explained considering the agglomeration of this catalyst in our experimental condition, which might also explain that a lower Ruana concentration was necessary to absorb all photons. For higher concentrations (0.1, 0.5, and 1 g/L), PC500 (100% anatase) was shown to be the second most efficient catalyst after Evonik P25. The highest activity of anatase compared with rutile could be explained by a higher aptitude of anatase (i) to photoadsorb oxygen, in $\text{O}_2^-$ and $\text{O}^-$ forms; (ii) to photodesorb it; and (iii) to have a low relative electron–hole recombination rate [46]. In addition, Ruana was not very well dispersed during the experiments, which may increase the loss of light into the photo-reactor due to light by scattering at high concentrations of catalyst.

The photocatalytic disinfection may be influenced by different parameters, the absorption of photons is an important phenomenon but it is not the only key parameter. In fact, the contact between microorganisms and the catalyst is necessary in the photocatalytic process [19,47] to have a good photocatalytic result; these contacts depend not only on the surface electrical charge and the point of zero charge (PZC) of the catalyst but also on the microorganism surface properties. Gumy et al. [47] have shown a correlation between the E. coli inactivation efficiency and the PZC of the TiO$_2$, where a better inactivation was shown with TiO$_2$ Evonik P25 (PZC = 7) compared to PC500 (PZC = 6.2). These results indicate also that the specific surface area of the catalyst had not a strong influence on the bacterial inactivation rate: the surface of TiO$_2$ Evonik P25 (50 m$^2$/g)$<PC500$ (326 m$^2$/g), although TiO$_2$ Evonik P25 showed the best disinfection efficiency. Moreover, the surface of Bi$_2$WO$_6$ (20 m$^2$/g)$<Ruana$ (157 m$^2$/g), but Bi$_2$WO$_6$ was more efficient than Ruana (except at 0.05 g/L).

Other parameters may also play an important role in the photocatalytic process, as the dispersion of catalyst particles in the solution which was difficult for Ruana and Bi$_2$WO$_6$ which collapsed during the reaction; this fact induced important changes on the surface area of catalyst when was dispersed in water reducing the active sites of the catalyst available for the photocatalytic disinfection process and therefore reducing the photocatalytic inactivation rate. Moreover, the penetration of very small TiO$_2$ particles into bacterial cells could be considered improving direct attack on the intracellular components and cell death [43]. Also, the density of superficial defects favouring the recombination of electrons with positive holes might be another factor influencing this activity.

Rincon et al. [16] investigated the influence of the crystalline form of immobilized TiO$_2$ on E. coli inactivation and they have found that fixed rutile was more efficient than anatase. They explained this by several facts, i.e. the stronger absorption of rutile in the near ultraviolet (360–400 nm) than anatase ($<380$ nm); and its higher refractive index allowing more light penetration into the bacterial suspension.

3.5. E. coli regrowth after stopping sunlight exposure

For a practical application of the photocatalytic disinfection process, it was important to verify if there was any bacterial regrowth during the subsequent dark period after the solar treatment by monitoring the viable bacteria concentration during water storage. Actually, two mechanisms of DNA reparation can be established after cells damaging: the first photo–repair mechanism occurs after exposition of damaged (but not killed) cells to light wavelengths between 300 and 500 nm [48]. The second reactivation mechanism takes place in the dark and is based in the excision–resynthesis and post-replication repair processes [16].

In this study, different samples were kept in the dark after complete inactivation (when DL was reached), to be re-plated and analysed later after 24, 48 and 72 h.

Contrary to the SODIS treatment, where a regrowth phenomenon may be observed [16,17], no bacterial regrowth was observed during the subsequent dark period (24, 48 and 72 h) using the different types of photocatalyst (TiO$_2$ P25, PC500 and Ruana) which is agreement with others contributions [16,44,49]. The absence of bacterial regrowth after photocatalytic treatment indicates that hydroxyl radicals, other reactive oxygen species or quite simply the presence of nanoparticles caused lethal, and sub-lethal damages that cannot be repaired during the subsequent dark period inducing cells death.

Huang et al. [44] have demonstrated that TiO$_2$–treated cells continued to lose their cultivability even at dark after UV light exposure.
suggesting that TiO₂ particles remaining in the slurry may still retain their bactericidal activity.

3.6. Formation of anions and cations during bacterial destruction

Anions and cations released during *E. coli* inactivation using different types of suspended catalyst were monitored; sodium, ammonium, potassium, magnesium, calcium, chloride, sulphate and nitrate as indicators of damage to bacterial cells. No formation of sodium, magnesium, calcium, chloride, sulphate and nitrate was observed during the first hours of photocatalytic inactivation of bacteria with different types of suspended catalysts. Only ammonium (Fig. 10a) and potassium (Fig. 10b) were formed with varied quantities depending on catalyst. Ammonium was already detected during *E. coli* inactivation using suspended TiO₂ Evonik P-25 [20,21]. This cations can be formed by the photocatalytic oxidation of amino acids [50] which composed the protein present in cells membranes.

The observation of the evolution of K⁺ during bacterial inactivation is in agreement with many previous studies [14,51,52]. Some authors have demonstrated that the loss of K⁺ was followed by the loss of cell viability [51,52], whereas others authors [14] have suggested that other disorders in cell membrane occurring after or with the K⁺ loss can also cause cell death.

Cell membrane damage may open a way to the leakage of inner bacterial components, as an increase in intracellular calcium ions due to the greater permeability of cell membrane to calcium ions was observed [53]. However, in our study cases, no calcium was detected among products formed during photocatalytic inactivation of *E. coli*.

No correlation was observed between inactivation and release of K⁺ or ammonium. Actually, in the case of TiO₂ Evonik P25 their detection were less important than this one detected with the others photocatalysts, suggesting that the oxidation of proteins of the membrane and the perforation of membrane (leakage of K⁺) did not the principal cause of bacterial inactivation.

4. Conclusion

The main microbial decontaminating factors using solar energy are UVA photons and heating due to the IR spectrum. However, we noticed that UV-solar bactericidal effect (without photocatalyst) was much more important than the solar mild heating. Of course, dose of UV plays an important role as observed during all our experiment. The addition of any kind of photocatalyst like TiO₂ P-25, PC500, Ruana, Bi₂WO₆ to the water accelerated the bactericidal action of solar irradiation. The irradiation time required to completely disinfect water depend on the type and concentration of catalyst. In presence of TiO₂ P-25, PC500 and Bi₂WO₆ the optimal catalyst concentration was 0.5 g/L, while for rutile 0.05 g/L was the optimal concentration. This behaviour can be explained considering the particle size of rutile and its agglomeration which increase the turbidity of the water suspension. For all concentrations used, Evonik P25 was found to be the most efficient photocatalyst. However, it is difficult to explain its best efficiency and why rutile and bismuth oxide absorbing more visible light is found less active. Compared to SODIS process, the presence of catalyst avoids any kind of bacterial regrowth at 24 h, 48 h and 72 following the solar treatment.

During the photocatalytic solar disinfection processes studied in this work, a release of potassium and formation of ammonium was observed in different concentrations depending on the catalyst used, but not correlated with their efficiency for water disinfection. The detection of these two cations in presence of photocatalysts indicates that damage of membrane occurs.

References
