

## Inactivation of *Escherichia coli* in Water by TiO<sub>2</sub>-assisted Disinfection using Solar Light

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Estudou-se a desinfecção de águas de abastecimento por fotocatalise heterogênea usando um reator de fluxo em um sistema composto por uma placa de vidro com TiO<sub>2</sub>P 25 (Degussa) imobilizado e luz solar como fonte de radiação. Foram utilizados dois modos de operação do reator: passagem única e recirculação. Os experimentos foram conduzidos utilizando inicialmente uma água preparada em laboratório e posteriormente água coletada em poços e lagos de uma região próxima à Campinas, SP. Estudou-se a influência de fatores, tais como, o modo de operação do reator, a cor e turbidez da água, os quais influenciam significativamente na eficiência fotocatalítica de descontaminação e, portanto, na viabilidade da aplicação do processo. Em dias ensolarados, alcançou-se uma redução na carga bacteriológica de cerca de 100% do valor inicial de *Escherichia coli* ( $2 \times 10^3$  NMP per 100 mL) para soluções de água sintética, e, 80% do valor inicial de *Escherichia coli* ( $16.6$  to  $22.2 \times 10^3$  MPN per 100 mL) para água *in natura*, através da fotocatalise heterogênea usando TiO<sub>2</sub>.

TiO<sub>2</sub>-assisted heterogeneous photocatalysis and photolysis were evaluated for the disinfection of water samples using a glass reactor with immobilized TiO<sub>2</sub> (catalyst), solar light and *E. coli* as an indicator microorganism of the efficiency of disinfection. Parameters such as color and turbidity of the water, level of coliform bacteria (by the Colilert® method), inclination angle of the solar reactor, solar light intensity, flow rate and retention time were controlled during the experiments. Two different operational modes were used for the solar reactor: single pass mode and recirculation mode. First, synthetic water was used in the disinfection tests as a model system; second, tests were conducted using natural samples specifically groundwater collected from a lake and a well. In bacterial suspensions in synthetic water in the absence of color and turbidity, heterogeneous photocatalysis was responsible for the reduction of approximately 100% of the initial concentration of *E. coli*. Only a 56.5% reduction was obtained by photolysis during the same solution recirculation time, which indicated a better efficiency using the catalyst. From the natural samples, total inactivation was not achieved in the studied cases. However, photocatalysis using TiO<sub>2</sub>/solar light was shown to be quantitatively efficient in the destruction of the total coliforms in water, reaching values up to around 80% inactivation in natural waters with initial levels of total coliforms ranging from 16.6 to  $22.2 \times 10^3$  MPN per 100 mL.

**Keywords:** disinfection, *E. coli*, TiO<sub>2</sub>, heterogeneous photocatalysis

### Introduction

Water quality and human health are considered essential for preventing endemic diseases and improving the quality of life. Especially in subtropical areas of the world, pathogenic bacteria represent a severe danger to the human population: they should therefore not be underestimated.<sup>1</sup>

Deficiencies in water supply systems associated with illnesses acquired by ingestion of contaminated water are responsible for approximately 30% of all cases of death in Latin America, a level six times higher than those in developed nations. In Brazil, more than 60% of all hospital admissions are attributable to the lack of sanitation in communities.<sup>2</sup>

Conventional technologies, such as chlorination, are usually used to remove microorganisms from contaminated water. Although these control systems have

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important application, alternative disinfection methods have been developed due to growing concerns regarding the toxicity of residual chlorine. UV (ultraviolet) irradiation has become one of the most important alternatives to chlorination for disinfection throughout the world.<sup>3</sup>

The ability of UV radiation to disinfect water has been known since the beginning of the twentieth century however, due to problems related to the technology and unreliability of the equipment, this process was abandoned for some time. Once these problems were solved, the treatment of water with UV radiation gained popularity, mainly in European countries. It has been reported from previous research that solar energy can be used effectively in water disinfection because inactivation of microorganisms is accomplished either by heating water to a high temperature (usually over 70 °C) or by exposing it to ultraviolet solar radiation especially in the near UV range (300-400 nm).<sup>4</sup> However, once the sunlight consists of about 3% ultra-violet light, an alternative means to improve the efficiency of process is to use a photocatalyst that is excitable by the energy of photons provided by sunlight.

In recent years there has been remarkable progress in Advanced Oxidation Technologies (AOT) based on the generation (by whatever means) of highly reactive intermediates (e.g. hydroxyl radicals) that initiate a sequence of reactions resulting in the destruction of pollutants in water, air and soil. However, well-established advanced oxidation processes such as ozonation and photochemical oxidation by hydrogen peroxide, are hampered by high costs resulting from the high input of energy and chemicals. However, among the AOT, heterogeneous photocatalysis using semiconductors as catalysts (UV/TiO<sub>2</sub>) employs photons of the ultraviolet range of the solar spectrum as the source of energy for the oxidation of organic compounds and for the disinfection.<sup>1</sup>

It is interesting to note that many laboratory studies have demonstrated the feasibility of the photocatalytic detoxification for almost all classes of hazardous compounds.<sup>5-10</sup>

In 1985, Matsunaga *et al.*<sup>11</sup> proposed a “new concept of photochemical sterilization of water” in which microbial cells could be inactivated using heterogeneous photocatalysis. Suspensions of TiO<sub>2</sub> spiked with platinum (TiO<sub>2</sub>/Pt) and microorganisms such as *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and *Escherichia coli* were irradiated with a halogen lamp for a period of 60 to 120 minutes. The complete inactivation of *L. acidophilus* and *S. cerevisiae* was achieved in 60 minutes of irradiation. For *E. coli* the same degree of inactivation required 120 minutes of treatment.

Following that work, several studies were performed using TiO<sub>2</sub> irradiated with UV light for the inactivation of microorganisms, mainly viruses and bacteria, in both photocatalytic and solar reactors.<sup>11-21</sup>

Although the mechanism of heterogeneous photocatalysis is not yet fully understood, the following steps are expected to occur: (i) through a direct mechanism, where the oxidation of organic compounds occurs directly at the holes in the valence band of the semiconductor (oxidation site); or (ii) through an indirect mechanism, when water molecules adsorbed on valence band holes of the semiconductor oxide form hydroxyl radical, <sup>•</sup>OH, and subsequently attack the organic compounds adsorbed onto the catalyst or in the vicinity of the interface. In general, the slow kinetics of the intermediate products may inhibit a faster adsorption process on the catalyst sites, supporting the hypothesis that the limiting step is the formation of the active species (hydroxyl radical, <sup>•</sup>OH) through reactions on the surface of the TiO<sub>2</sub>.<sup>22</sup>

The photochemical formation of peroxides occurs in the presence of oxygen in an aqueous medium and it acts as a precursor of many reactive species in light-driven reactions. The possibility that H<sub>2</sub>O<sub>2</sub> assists in the destruction of the bacteria cell cannot not be ruled out, since this reagent is used as a coadjutant in many oxidative processes. On the other hand, one has to consider that this compound undergoes dismutation during heterogeneous photocatalysis generating the superoxide radical-ion (O<sub>2</sub><sup>•-</sup>). This species is an important source of hydroxyl radical and avoids the recombination of the electron/hole pair in the catalyst, thus enhancing the redox process. As a result, the decomposition of H<sub>2</sub>O<sub>2</sub>, observed in the presence of TiO<sub>2</sub>, might represent an additional source of OH radical species, thus increasing its efficiency in the destruction of microorganisms present in the water.

The inactivation of the microorganisms using TiO<sub>2</sub>, irradiated with UV light is attributed to the inhibition of the cellular respiratory process by the photoelectrochemical oxidation of coenzyme A (CoA). However, this mechanism has not been fully elucidated. Besides the hydroxyl radicals (OH<sup>•</sup>), certainly other active species such as O<sub>2</sub><sup>•-</sup>, HO<sub>2</sub><sup>•</sup> and H<sub>2</sub>O<sub>2</sub> also participate in the process.<sup>11-13</sup>

The main objective of the present study was to evaluate and propose heterogeneous photocatalysis using TiO<sub>2</sub> under solar light for the disinfection of small volumes of natural waters under environmental conditions. In order to provide photocatalysis using TiO<sub>2</sub> nanoparticles in the form of a technology appropriate for people living in poor socio-economics conditions, a further goal of this work

was to produce a solar reactor with supported TiO<sub>2</sub> supported using materials and equipment that would be available in developing countries. The following topics were addressed in this paper: (i) *E. coli* inactivation by sunlight in the presence and absence of TiO<sub>2</sub>; (ii) single pass mode and recirculation mode in the solar reactor with immobilized TiO<sub>2</sub>; (iii) the influence of color and turbidity from the natural waters on the photocatalytic process.

## Experimental

### Preparation of the synthetic water

Synthetic water was formulated as recommended in APHA.<sup>22</sup> It was used in all experiments in order to standardize the tests with microorganisms. Table 1 shows the concentrations of the reagents added to deionized water in order to prepare the synthetic water. Table 2 shows the recommended and obtained values of several parameters for the water used in the experiments. The deionized water used for the preparation of the synthetic water must have an electrical conductivity of 1 μS cm<sup>-1</sup> at 25 °C. Color values of the synthetic water were about 10 color units (cu), which was obtained using 0.1% solution humic substance. Turbidity values of the synthetic water were about 3.0 nephelometric turbidity units (NTU), which was obtained using a 1.0% clay solution (sodium montmorillonite clay). These parameters were monitored with a spectrophotometer (DR4000, HACH) as recommended in APHA.<sup>23</sup> Samples were prepared at the beginning of the experiments and the turbidity measurement was carried out immediately after sample preparation, without altering the original sample

**Table 1.** Composition of the synthetic water used in the tests

| Reagent                         | Concentration/(mg L <sup>-1</sup> ) |
|---------------------------------|-------------------------------------|
| NaHCO <sub>3</sub>              | 12.0                                |
| CaSO <sub>4</sub>               | 6.0                                 |
| MgSO <sub>4</sub>               | 7.5                                 |
| KCl                             | 0.5                                 |
| NaOH                            | 200.0                               |
| KH <sub>2</sub> PO <sub>4</sub> | 4,000.0                             |

**Table 2.** Synthetic water specifications used in the experiments

| Parameter                                                | Recommended range, at 25 °C | Experimental values or ranges |
|----------------------------------------------------------|-----------------------------|-------------------------------|
| Hardness/mg equivalent CaCO <sub>3</sub> L <sup>-1</sup> | 10.0 - 13.0                 | 12.0                          |
| Total alkalinity/mg CaCO <sub>3</sub> L <sup>-1</sup>    | 10.0 - 14.0                 | 8.6                           |
| pH/pH units                                              | 6.4 - 6.8                   | 6.4                           |
| Color/ color units                                       | 10                          | 7 - 10                        |
| Turbidity/NTU                                            | 3.0                         | 2.2 - 3.5                     |

qualities, such as temperature or pH. Water pH was measured using an Orion pHmeter (model 370).

### Organisms and growth conditions

*Escherichia coli* strain ATCC13706 was obtained from the Institute of Biological Sciences at the University of São Paulo, Brazil (ICB/USP). Cultures were grown in Tryptonated Soy Broth (TSB) medium. The number of viable cells was determined by the COLILERT<sup>®</sup> method after appropriate dilutions onto buffered sterilized water after 12 h incubation at 37 °C ± 1. The sterility of the materials was ensured by autoclaving at 121 °C for 20 min.

An initial magnitude level of 10<sup>3</sup> cells *per* 100 mL was used at all experiments. It is important to mention that the experiments were followed by different initial concentrations of microorganisms due to the inherent difficulty of preparing the bacterial suspension.

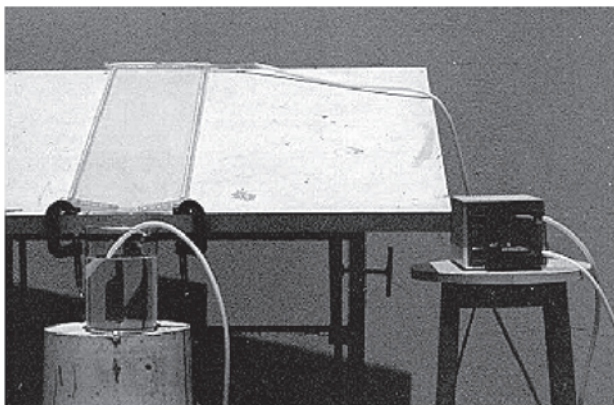
Microorganisms were monitored and quantified using two replicates during experiments in the solar reactor. The results were calculated and expressed in MPN *per* 100 mL (most probable number).<sup>23</sup>

### TiO<sub>2</sub> immobilization

All the experiments were carried out in an immobilized TiO<sub>2</sub> reactor, which was the same as that described by Nogueira and Jardim.<sup>24</sup> Titanium dioxide (Degussa P-25, predominantly anatase, specific area of 50 m<sup>2</sup> g<sup>-1</sup>, non-porous, 70:30 anatase form, BET surface area 50 m<sup>2</sup> g<sup>-1</sup>, 30 nm average particle size) was used as a photocatalyst. Immobilization of the catalyst was performed by applying a 10% (m/v) TiO<sub>2</sub> aqueous suspension of TiO<sub>2</sub> to flat borosilicate glass plates. The plate dimensions were 20 × 84 cm, resulting in a thin film of supported TiO<sub>2</sub> and an exposed area of 0.16 m<sup>2</sup>. The TiO<sub>2</sub> suspension was passed by gravity flow over the plate and dried with hot air for the immobilization of TiO<sub>2</sub>. This procedure was repeated several times until a homogeneous TiO<sub>2</sub> film, with about 10 g TiO<sub>2</sub> *per* m<sup>2</sup> was formed onto the glass.<sup>24</sup> Nogueira and Jardim observed that the titanium dioxide film remained stable during the flow of aqueous solutions and that no loss of photocatalytic activity occurred during 40 h of use.

### Irradiation experiments

The TiO<sub>2</sub> immobilized glass plate (Figure 1) was placed on a support that faced towards the equator. The solutions containing the microorganisms were pumped by a peristaltic pump (Ismatec IPS-12), to the top of the plate and flowed by gravity to the bottom while illuminated by solar light.<sup>24</sup>



**Figure 1.** Photocatalytic solar reactor and peristaltic pump.

Two experiments were performed: (i) single pass mode, in which 1 L solutions were passed over the plate and collected at the bottom. The retention time was changed with the inclination angle of the plate, geometric dimensions of the plate and the thickness of the fluid film. Flow rates were varied from 2, 4, 8, 16, 32 and 64 L h<sup>-1</sup> to evaluate the influence of flow rate and retention time in 1.8° inclination angle of plate (Table 3); and, (ii) recirculation mode, where 1 or 2 L of solutions were re-

circulated. The exposed irradiation time of bacteria to solar light was different from the total time of experiment in the recirculation experiments, since the reaction takes place that supports the catalyst. Irradiation time is given by the following equation:  $t_{\text{irradiation}} = t_{\text{total}} \times V_{\text{reactor}} / V_{\text{total}}$ .

In the single pass experiments, water samples were withdrawn before and after irradiation and at about 15 min intervals in the recirculation experiments, in order to quantify the microorganism population. Photolysis experiments were also carried out in the absence of the photocatalyst in the same kind of reactor, and control experiments were carried out in the absence of the photocatalyst and the light.

### Solar light intensity measurements

Solar light intensity was measured using a Cole Parmer Radiometer (model VLX 3W) at 365 nm at the same inclination angle as the plate. This wavelength was chosen due to absorption of TiO<sub>2</sub> in anatase form. The average light intensity was calculated for each experiment. All the experiments were performed under clear sky conditions at the University campus located in Campinas, Brazil (23° south latitude).

### Freshwater samples

Freshwater samples were collected from the Chico Mendes lake and from a well that supplies water to a small district near the University both locations are situated in Campinas, Sao Paulo. Table 4 shows the specifications of these samples.

## Results and Discussion

For all experiments, the efficiency of disinfection is given by  $-\log(N/N_0)$ , which refers to the concentration ratio of the *E. coli* level in the water measured at any time, N, compared to the initial concentration, N<sub>0</sub>. The higher the calculated value the more pronounced reduction in the *E. coli* level, and, therefore, the better the inactivation efficiency.

**Table 3.** Retention time variation with the flow rate for solar reactor

| Flow rate / (L h <sup>-1</sup> ) | Retention time / s |
|----------------------------------|--------------------|
| 2                                | 104 ± 23           |
| 4                                | 65 ± 06            |
| 8                                | 49 ± 07            |
| 16                               | 30 ± 05            |
| 32                               | 17 ± 02            |
| 64                               | 10 ± 01            |

**Table 4.** Characteristics of the natural water used in experiments in solar reactor

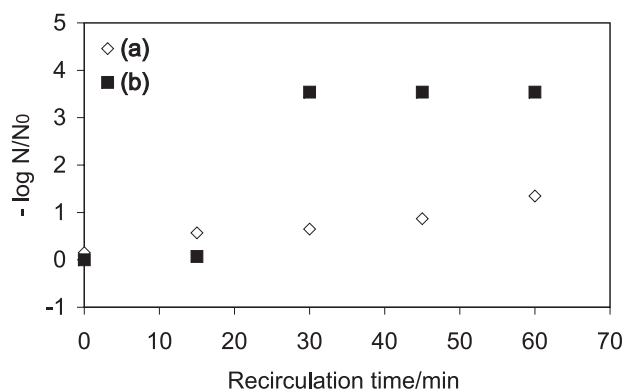
| Parameter, at 25 °C                                     | Natural waters from |                        |
|---------------------------------------------------------|---------------------|------------------------|
|                                                         | Well                | Lake                   |
| Total alkalinity / mg CaCO <sub>3</sub> L <sup>-1</sup> | 44.23               | —                      |
| pH / pH units                                           | 7.2                 | 7.0                    |
| Color / color units                                     | 1                   | 10                     |
| Turbidity / NTU                                         | 1.2                 | 15.2                   |
| Total coliforms, MPN per 100 mL                         | 16.6                | 5.04 × 10 <sup>5</sup> |

### *E. coli* inactivation: photolysis versus photocatalysis experiments

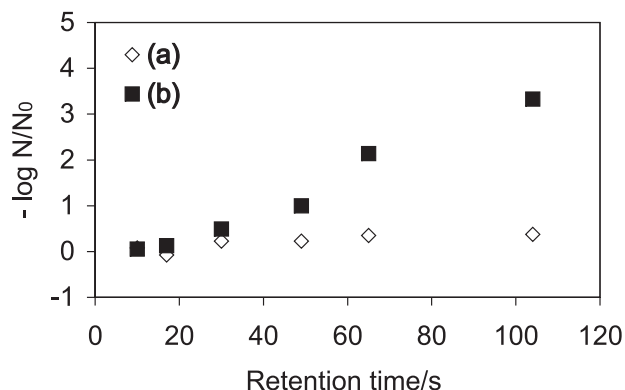
The results obtained from photolysis and heterogeneous photocatalysis experiments of *E. coli* in synthetic water using the solar reactor are presented in Figure 2 (for the solution recirculated by 1 h at a flow rate of 4.9 mL s<sup>-1</sup>) and in Figure 3 (for the single pass mode). During the experiments, the average solar intensity was 1.4 mW cm<sup>-2</sup>.

*E. coli* inactivation by solar light occurs in photolysis such as in photocatalysis experiments as shown in Figure 2 and Figure 3. However, the inactivation by solar light is strongly enhanced by presence of TiO<sub>2</sub> in both operation modes of the solar reactor: recirculation mode (Figure 2) and single pass mode (Figure 3).

Figure 2 shows that after just 30 min heterogeneous photocatalysis was responsible for the reduction of approximately 99.97% (-log N/N<sub>0</sub>=3.54, below the limit of detection) of the initial concentration of *E. coli* whereas



**Figure 2.** *E. coli* inactivation in synthetic water using solar reactor in recirculation mode. (a) Photolysis (without TiO<sub>2</sub>), synthetic water with *E. coli* (N<sub>0</sub>= 12.70×10<sup>3</sup> MPN per 100 mL); (b) Heterogeneous photocatalysis using TiO<sub>2</sub>, synthetic water with *E. coli* (N<sub>0</sub>= 3.50×10<sup>3</sup> MPN per 100 mL).



**Figure 3.** *E. coli* inactivation in synthetic water using solar reactor in single pass mode. (a) Photolysis (without TiO<sub>2</sub>), synthetic water with *E. coli* (N<sub>0</sub>= 11.87×10<sup>3</sup> MPN per 100 mL); (b) Heterogeneous photocatalysis using TiO<sub>2</sub>, synthetic water with *E. coli* (N<sub>0</sub>= 2.13×10<sup>3</sup> MPN per 100 mL).

only 77.61% (-log N/N<sub>0</sub>=0.65) inactivation was achieved in the photolysis process. The plot indicates that in the first 20 min of solution recirculation on the surface of the solar reactor, there was a sharp increase in the inactivation of the bacteria; after this time stabilization of the remaining bacteria was observed. With the reactor working in single pass mode (Figure 3), a similar result in the maximum ratio -log (N/N<sub>0</sub>) was observed with a retention time of 104 s. The heterogeneous photocatalysis process reached up to 99.95% (-log N/N<sub>0</sub>=3.33) of *E. coli* inactivation in a short solar irradiation time whereas photolysis reached only 58.31% (-log N/N<sub>0</sub>=0.38), showing again a higher rate of inactivation of the microorganisms in TiO<sub>2</sub>-assisted disinfection.

Comparing the results obtained in the experiments, it can be concluded that the disinfection is a consequence of both the direct action of the sunlight on the microorganisms as well as the photocatalytic action of the excited photocatalyst particles. It is well known that sunlight is able to inactivate microorganisms due to the synergistic effect of the UV and heating of water by infrared radiation.<sup>25</sup> In agreement with literature, the action of the photocatalytic process was more effective than the action of solar light alone.<sup>25,26</sup> There is a synergistic effect of sunlight and supplementary oxidative species generated by the photoactivation of TiO<sub>2</sub>. The action of the radicals and other oxidative species on the bacterial cell membrane leads to the perturbation of different cellular processes and ultimately to bacterial inactivation and death.<sup>27</sup>

The results are in agreement with the ones reported by Bekbölet,<sup>28</sup> who observed that TiO<sub>2</sub> suspension under black light illumination resulted in 100% of inactivation of *E. coli* in 60 min.

The residence time of water in a solar reactor is of primary importance to any water treatment process. The residence time decreases as the flow rate is increased and consequently the total volume of water. Six different flow rates, 2, 4, 8, 16, 32 and 64 L h<sup>-1</sup> were evaluated (Table 3). Figure 3 shows that the lowest flow rate produced the highest retention time of the bacteria under solar radiation as well as the highest efficiency of *E. coli* reduction.

The flow rate selected to carry out the experiments could be an important factor because stress from movement of the fluid in which bacteria are dispersed could cause their inactivation.<sup>26</sup> Experiments were also carried out in the dark in order to evaluate the effect of the stress due to the flow rate only compared to the result obtained by the combination of the flow rate and solar light. The results of control experiments showed no significant bactericidal activity for either operating mode of the solar reactor.

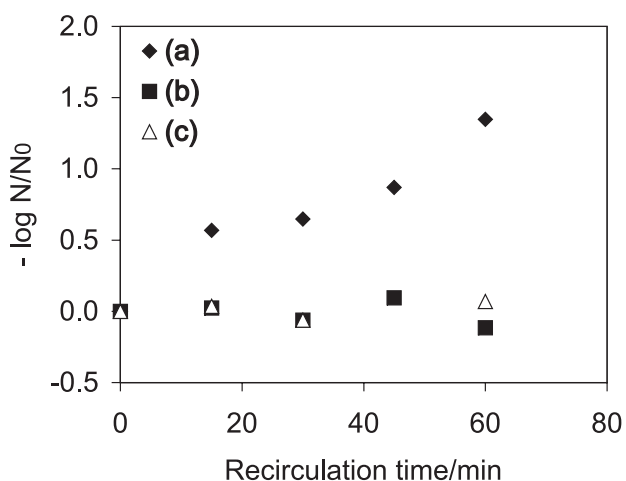


Because of the lower disinfection rates observed, the photolysis experiments were not further pursued. Efforts were instead concentrated on the heterogeneous photocatalysis experiments, which yielded better results due to the accelerating the action of light. In addition, Fernández *et al.*<sup>26</sup> verified that photolysis does not completely deactivate *E. coli* since bacteria re-growth was detected. They also indicated that solar light disinfection was improved by the photocatalytic action of TiO<sub>2</sub> under solar radiation, enabling complete bacterial death<sup>26</sup> this approach was successful and efficient enough to stimulate us to pursue its the application in photocatalysis for drinking water disinfection.

#### *Influence of humic substance, clay, and adsorption process on TiO<sub>2</sub> surface in E. coli level*

In order to determine if adsorption occurred on the TiO<sub>2</sub> surface or if any decrease in *E. coli* level was caused by processes other than photo-inactivation, *E. coli* solutions (10<sup>3</sup> cells *per* 100 mL) in synthetic water with no humic substance and clay were recirculated for 1 h at flow rate of 5.2 mL s<sup>-1</sup>, both in the presence and absence of solar irradiation (Figure 4, plot a and plot b respectively). When solar light was used, its intensity was over the course of the entire experiment, and its average value was 2.3 mW cm<sup>-2</sup>.

When the *E. coli*-contaminated water was not exposed to irradiation (no photo-degradation took place), no change in the *E. coli* level was observed. This behavior indicated that there was no loss or adsorption of microorganisms onto the TiO<sub>2</sub> surface, clearly indicating that TiO<sub>2</sub> assisted



**Figure 4.** (a) *E. coli* in synthetic water ( $N_0=8.90\times 10^3$  MPN/100 mL) during TiO<sub>2</sub>/solar irradiation in recirculation mode; (b) *E. coli* in synthetic water ( $N_0=3.50\times 10^3$  MPN/100 mL) passing reactor with TiO<sub>2</sub> immobilized in dark; (c) *E. coli* in synthetic water ( $N_0=1.71\times 10^3$  MPN/100 mL) added humic acid (color 9 uC) and clay (turbidity 2.9 NTU) passing reactor without TiO<sub>2</sub> immobilized in solar irradiation.

photo-oxidation is the major process involved in the removal of *E. coli* in water.

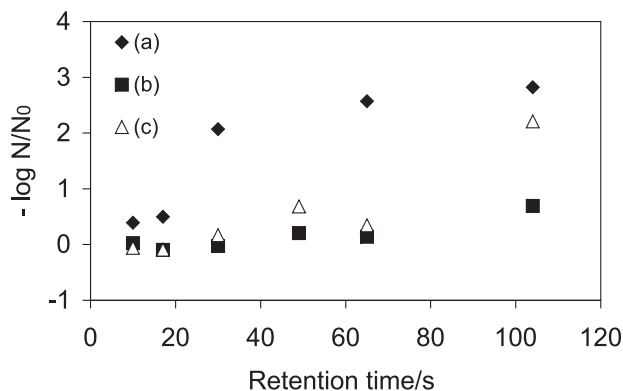
Since it was possible that the presence of humic substance and clay might inhibit or compete with the heterogeneous photocatalysis process by a faster adsorption process on the catalyst sites, an additional experiment was also carried out in the absence of irradiation and in which humic substance and clay were added to the *E. coli* solution (Figure 4, plot c). Solutions with color 9 uC and turbidity 2.9 NTU were used in such experiments, and the results show that neither humic acid nor clay are not toxic to microorganisms supporting the hypothesis that the disinfection efficiency results only from the heterogeneous photocatalysis.

The lower disinfection rates observed in the humic acid and clay-containing solutions can be explained by the competition between the microorganisms and the organic and inorganic compounds for the holes or surface-bound hydroxyl radicals generated at the photocatalyst surface upon irradiation.

#### *Photo-inactivation experiments using synthetic water in single pass mode*

Figure 5 shows the results for inactivation of *E. coli* in synthetic water using the solar reactor in single pass mode for two different color and turbidity values of the microbial suspension: color 10 uC and turbidity 2.6 NTU (plot b) and color 7 uC and turbidity 2.2 NTU (plot c), obtained by adding humic substance and clay. The average solar intensity was 1.8 mW cm<sup>-2</sup> during the experiment.

Reduction of the *E. coli* inactivation efficiency was observed (Figure 5) with increasing color and turbidity values of the solution. Lower efficiency in *E. coli*



**Figure 5.** *E. coli* inactivation in synthetic water varying color and turbidity values using solar reactor in single pass mode. (a) Synthetic water with *E. coli* ( $N_0=2.72\times 10^3$  MPN per 100 mL); (b) Synthetic water with *E. coli* ( $N_0=2.96\times 10^3$  MPN/100 mL), color 10 uC and turbidity 2.6 NTU; (c) Synthetic water with *E. coli* ( $N_0=5.38\times 10^3$  MPN per 100 mL), color 7 uC and turbidity 2.2 NTU.

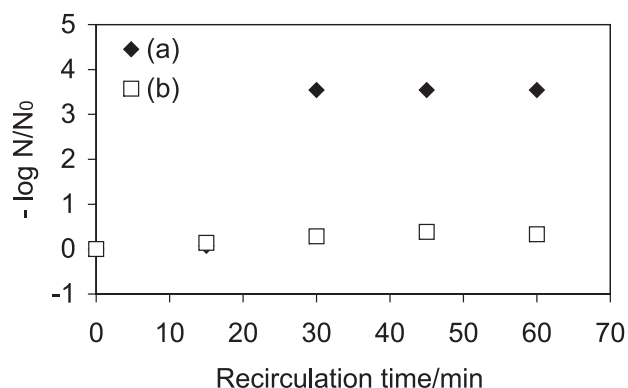
inactivation was observed in suspensions with the highest color and turbidity values. This was probably a result of a decrease in light passing through the solution, and the consequently lesser activation of the catalyst. It is relevant to point out that humic substances can be initially degraded to a complex mixture that appears to be extremely recalcitrant to further degradation. In addition, saturation of the catalyst surface results in a decrease in of active sites available for adsorption and reaction with the microorganisms as this mechanism is assumed to be the major one responsible for the kinetics of degradation.

Although the pronounced reduction of transparency of the water this effect has shown to be not very significant if evaluated in terms of percentage when the inactivation reached up to 99.85% ( $-\log N/N_0=2.82$ ) of the initial concentration of *E. coli*, without addition of humic substance and clay. Values up to 99.38% ( $-\log N/N_0=2.21$ ) and 79.58% ( $-\log N/N_0=0.69$ ) in the presence of humic substance and clay, respectively, shows that the reduction in the initial number of microorganisms (inactivation) was always significant.

#### *Photo-inactivation experiments using synthetic water and natural water in recirculation mode*

Figure 6 shows the results obtained for inactivation of *E. coli* in synthetic and natural water, plot (a) and (b) respectively, using the solar reactor in recirculation mode at a flow rate of 4.9 mL s<sup>-1</sup>. The synthetic water, used as a reference solution, did not contain humic substance or clay. The values of color (15 uC), turbidity (15 NTU), and total coliforms ( $N_0=22.2\times 10^3$  MPN per 100 mL) of the lake water were relatively high.

As previously mentioned color and turbidity significantly affect the efficiency of inactivation.



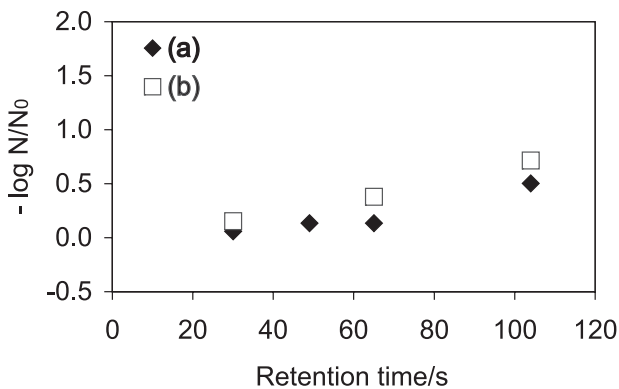
**Figure 6.** *E. coli* inactivation in synthetic and natural water using TiO<sub>2</sub>/solar reactor in recirculation mode. (a) Synthetic water with *E. coli* ( $N_0=3.50\times 10^3$  MPN per 100 mL); (b) Natural water (from lake) with total coliforms ( $N_0=22.2\times 10^3$  MPN per 100 mL), color 15 uC and turbidity 15.0 NTU.

Particulate matter in suspension can affect the process of water disinfection because light scattering occurs, thereby serving as an ultraviolet blocking filter for microorganisms. Similar to the particles that cause turbidity, the microbial aggregates can affect the efficiency of disinfection because they serve as a support for pathogenic organisms, protecting against the dispersing radiation. Normally, disinfection using UV light is efficient in waters with low turbidity. In surface waters, however, the turbidity effect is more significant. In the case of heterogeneous photocatalysis, the active surface of the TiO<sub>2</sub> reactor can be reduced by the adsorption of particulate material, including aggregates of microorganisms. In fact, the *E. coli* inactivation rate could be dependent on the physical-chemical parameters of the water, as well as on the presence of biogenic and anthropogenic compounds and adverse environmental conditions. Previous studies on heterogeneous photocatalysis using simple model samples have focused on the degradation of individual pure compounds, with few references to complex mixtures or to real samples. For real samples, total inactivation could require a much longer time. The results indicate that the efficiency of the photocatalytic process is drastically affected as the complexity of the sample is increased. In spite of this, it is interesting to note that even working with a real sample (natural water), there was a significant decrease in total coliforms from the initial level, approximately 53.23% ( $-\log N/N_0=0.33$ ) inactivation.

#### *Photo-inactivation experiments using natural water in single pass mode*

Due to the low practicality of recirculating small volumes of water in a reactor, other tests were performed using natural water in single pass mode. Figure 7 shows the results for inactivation of total coliform in natural water using solar reactor in single pass mode for two natural samples (from a lake and from a well). The characteristics of these samples are described in Table 4. Well water had low values of total coliforms ( $N_0=16.60$  MPN per 100 mL), color (1 uC) and turbidity (1.2 NTU), as compared to lake water, which contained higher values of total coliforms ( $504\times 10^3$  MPN per 100 mL), color (5 uC) and turbidity (10.6 NTU).

Although the samples were taken from natural sources, there was a significant decrease in the initial total coliform level: approximately 80.95% ( $-\log N/N_0=0.72$ ) and 68.38% ( $-\log N/N_0=0.50$ ) inactivation, were obtained for



**Figure 7.** *E. coli* inactivation in natural waters using solar reactor in single pass mode. (a) Well water with *E. coli* ( $N_0 = 16.60$  MPN per 100 mL), color 1 uC and turbidity 1.2 NTU; (b) Lake water with *E. coli* ( $N_0 = 504 \times 10^3$  MPN per 100 mL), color 5 uC and turbidity 10.6 NTU.

the well and lake water samples, respectively. These results are especially relevant because they indicate that the low efficiency of  $\text{TiO}_2$ -assisted disinfection achieved in natural waters is not only related to values of total coliforms, color and turbidity, but also to other environmental conditions that may not provide the necessary conditions for a complete inactivation or to yield an adequate reduction of the initial number of microorganisms.

## Conclusions

Different suggestive points can be presented for the application of the proposed process in developing countries. Exposing a water sample to solar radiation can disinfect the contaminated water by ultraviolet radiation alone. However, heterogeneous photocatalysis with supported- $\text{TiO}_2$  has been demonstrated to be more efficient for *E. coli* disinfection than photolysis. Results show that in pure bacteria suspension (no color and turbidity), heterogeneous photocatalysis was responsible for the almost complete inactivation of *E. coli* in a relative short time (around 30 min in recirculation mode and a retention time of 104 s operating in single pass mode). The almost complete inactivation of *E. coli* in synthetic water indicates that photocatalysis represent an attractive means to decontaminate water. However, color and turbidity are parameters that impede the performance of solar disinfection. For solutions presenting some degree of color and turbidity, pronounced reductions in inactivation rates were observed. A lower efficiency of inactivation of *E. coli* was observed in suspensions that had the highest color and turbidity values, probably the result of the decrease in light passing through the solution, thereby reducing the activation of the catalyst.

In the case of natural water samples, total inactivation was not achieved in the studied cases. The maximum efficiency obtained varied from 53.23 to 80.95%, for initial microorganisms concentration range of 17 to 504,000 MPN per 100 mL. However, this inactivation range should be significant depending on the initial level of the microorganisms and also depending on its use.

Considering all the aspects discussed in this paper and the continuous demand for sanitation technology with concomitant production of wastewater, heterogeneous photocatalysis using  $\text{TiO}_2$  must be considered as an attractive technology; however; the effects of organic and inorganic compounds in natural waters on photocatalytic disinfection should be carefully examined in order to assess the applicability of the process to drinking water purification in remote localizations of developing countries. Thus, at the present state of development, photocatalysis will be (on an economical basis) restricted to small batches (e.g., households or small communities) and to water with low initial color and turbidity. In addition, the total volume of a natural water that can be purified within one solar day depends of the irradiated area (solar reactor area).

The inactivation of *Escherichia coli* in water by  $\text{TiO}_2$ -assisted disinfection using solar light may be used to improve water quality in small communities far from cities, communities that have deficiencies in water supply or that consume untreated ground water of inadequate quality. In tropical countries such as Brazil, this alternative might be extremely useful because of the poor sanitation system in many regions, mainly in the North of the country where less than 10% of sewage and less than 80% of water are treated.

It is important to emphasize that  $\text{TiO}_2$  films can be produced without access to specialized laboratory equipment and that the solar reactor can be used on the roofs of houses.

However, Lonnen *et al.*<sup>29</sup> demonstrated that photolysis and photocatalysis are not effective for the inactivation of cysts of the waterborne protozoan pathogen *A. polyphaga* or for the spores *B. subtilis*.

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