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Short communication

Evaluation of photocatalytic disinfection of crude water for drinking-water production

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Abstract

TiO_2-photocatalysis disinfection experiments were carried out with both *E. coli* in distilled water and natural water samples from the Cauca River (Cali, Colombia). The experiments performed with *E. coli* in distilled water showed no increase in cell concentration after the treated solution was left in the dark for 24 h. However, the experiments carried out with natural water samples showed drastic culturable cell concentration increase 24 h after stopping the irradiation. This shows the lack of residual effect of TiO_2-photocatalysis. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Solar; Disinfection; Photocatalysis; *Escherichia coli*; Crude water; Oxidative stress

1. Introduction

Chlorination is a widely used technique for the production of drinking-water. Although its bacterial inactivation effect has been proven, a great concern is that chemical risks could be enhanced due to by-products formed during the chlorination process [1]. Therefore, the necessity to find low cost, environmental friendly and sustainable alternatives to chlorination is of great interest.

Photocatalysis is a promising technology based on the interaction of light with solid semiconductor particles, and is able to produce highly oxidative species that not only destroy bacteria, but also destroys a large variety of chemical contaminants in water [2,3].

In the last decade, important works report strong and fast photocatalytic cell inactivation of *E. coli* [4–6] and different bacteriophages [7] in aqueous suspensions employing distilled water.

The disinfection potential of photocatalytic treatments was also reported for real waste water samples [8]. Photocatalytic inactivation rates of waste water were found to be slower in the presence of dissolved species [9] or suspended solids. In the latter case, disinfection is slightly improved with filtering but still remains less efficient than for distilled water samples [10]. Chlorination disinfection experiments carried out with waste water also demonstrate an interaction of suspended solids during the disinfection process [11].

These waste water disinfection experiments point out the important role played in the disinfection process by dissolved species and suspended solids. For example, embedded cells in suspended solid particles can lead to a decrease in chlorination efficiency. The same phenomena can reasonably occur with other kinds of disinfection treatment like photocatalysis. In photocatalytic treatments, suspended solids can lead to strong radiation absorption that decrease the photocatalytic quantum yield with a concomitant decrease in photocatalytic cell inactivation yields.

Even if numerous precedent works have demonstrated the bactericide effect of irradiated titanium dioxide, the bactericide activity after stopping the illumination remains undemonstrated. Indeed, some recent experiments conducted with urban waste waters show an increase in culturable cell concentration after photocatalytic treatment [12]. This issue is key to the viability of a disinfection process based on

Abbreviations: CW, crude water; CFU, colony forming unit; PCR, polymerase chain reaction; DO, Dissolved oxygen; SPC, standard plate count

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photocatalysis because a reliable water supply system requires that pathogenic agents are effectively destroyed.

The objective of this work is to investigate photocatalysis applied to crude water (CW) samples compared with *E. coli* in distilled water samples, both during and after the irradiation. This is in order to evaluate the feasibility of using titanium dioxide photocatalysis to produce clean drinking-water from crude river water and pretreated CW.

2. Materials and methods

Crude water. CW from the Cauca River (Cali, Colombia) was collected from an experimental pilot drinking-water plant [13]. The Cauca River has high levels of both microbiological and organic matter contamination coming from industrial waste water and agro-industrial runoffs. This experimental plant is operated by The Centro Inter-Regional de Abastecimiento y Remoción de Agua (CINARA), a research institute of the Universidad del Valle. Samples were collected at three different points of the process as indicated in Fig. 1: at the influent (A), after a first dynamic rough filter (B), and after a following ascendant flow rough filter. For each sample, the following parameters were determined directly at the treatment installations: turbidity, temperature, pH, suspended solids, real color, fecal coliforms (Fig. 2). The samples were processed at the Universidad del Valle,

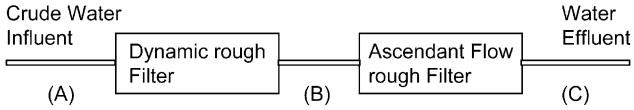

Fig. 1. Scheme of the drinking-water treatment plant. Samples collected at points A, B, C correspond to CW, primary effluent and secondary effluent, respectively.

Date	Experiment	turbidity [NTU]	temp. [C]	pH	susp. solids [mg/L]	real color [UPC]
25.10.00	Sample A	77	22	6.8	100	91
30.10.00	Control, sample A	120	22	6.8	242	128
14.11.00	Sample B	47	22	7.2	29	82
22.11.00	Sample C	32	22	6.7	7.4	45

Fig. 2. Table of sample properties.

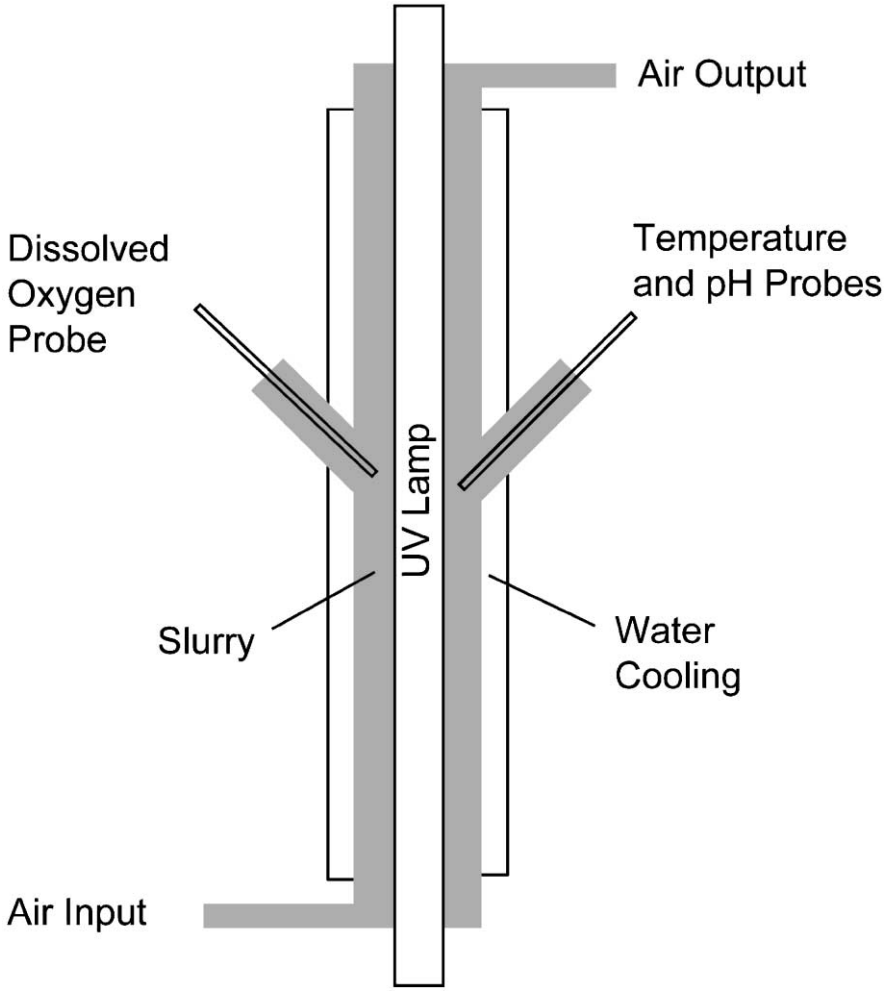

Fig. 3. Scheme of the reactor.

Laboratory of Microbiology within maximum 2 h after collection.

E. coli strain. For experiments carried out with synthetic water, an *E. coli* strain was isolated and purified from the Cauca River. High cell concentrations, 10^9 cell/ml, were reached by inoculating 10 ml of triptic soy broth (TSB, Mikrobiologie, 1.05459, Merck) and incubating for 24 h with stirring at 35 °C.

Reagents. For the experiments carried out with *E. coli*, the TiO_2 slurry consisted of 0.5 g of TiO_2 (P-25, Degussa) in 1-l of distilled water solution. This solution was then inoculated with 10 ml of the above cell preparation. Thus, initial cell concentrations were in the range 10^7 cell/ml. For the experiments carried out with natural water, the TiO_2 slurry consisted of 0.5 g of TiO_2 in 1-l of sampled water solution.

The reactor. The reactor was kept in the vertical position throughout the experiments and consists of a black lamp in a double-glass jacket that defines two compartments as shown in Fig. 3. The inner compartment, where the photocatalytic treatment occurred, allows the CW containing TiO_2 to be in direct contact with the lamp surface. The external compartment was for water-cooling. The reactor contains a gas input at the bottom and output at the top with other inlets for sampling and pH, dissolved oxygen (DO), and temperature sensors. The reactor was wrapped in aluminum foil to isolate it from ambient light. An air stream was pumped into the reactor and was kept constant at 2 l/min. This gas flow was high enough to provide good agitation inside the reactor.

The pH probe (N 6480, Schott, Germany) was connected to a pH-meter (8417 N, Hanna instruments). The DO probe (CellOx 325, WTW, Germany) was connected to an oxymeter (Oxi 330, WTW, Germany). The temperature probe was a standard thermocouple. The irradiation source was a 40 W black light lamp (F40BLB, General Electrics, USA).

Method. The reactor tube was filled with 1-l of TiO_2 slurry in the dark, and the agitation setup turned on. The solution was then irradiated continuously for 1.5 h. After complete irradiation, the UV-source was switched off and the solution was kept in the same experimental conditions for 48 h. The solution was kept between 25 and 27 °C, and saturated in oxygen during the whole experiment. The control experiment was carried out in the same experimental conditions but without any irradiation.

Cell inactivation was monitored with standard plate count (SPC) methods during irradiation, after 24 and 48 h. TSB-agar and eosin methyl blue agar (EMB-agar, Mikrobiologie, 1347, Merck) were used as universal and selective media, respectively.

Serial dilutions were carried out in 9 ml tubes containing 0.1% peptone solution at pH = 7. Petri dishes were inoculated with 0.1 ml samples at the surface and further incubated for 24 h at 35 °C. A verification count was carried out after 48 h of incubation.

3. Results and discussion

3.1. E. coli in distilled water assays

The experiment consisted of two parts: the first being continuous irradiation of the sample for 1.5 h and a second period, where the reactor was left in the dark. Air stream agitation was kept constant during the whole experiment. Cell concentrations were monitored during both parts with SPC method.

The slurry consisted of a 10^6 cell/ml suspension of *E. coli* in TiO_2 in distilled water solution. The solution was saturated with oxygen and irradiated for 1.5 h. The control experiment was carried out in the same experimental conditions, but without irradiation.

Fig. 4 shows the variation of the log of *E. coli* concentration as a function of time. Initially, a fourfold order of magnitude inactivation was achieved during the irradiation period. After switching the light off, a slow decrease was observed in the next 24 h. The culturable cell concentration remained unchanged with this simple model as shown in Fig. 4. Results carried out under similar experimental conditions (not shown), displayed no observable bacterial concentration change even after 48 h. Inactivation yields immediately after irradiation were of 99.98 and 99.995 in TSB and EMB, respectively.

3.2. Crude water assays

Experiments were performed with samples collected from three different points of a pilot-scale drinking-water installation (Fig. 1). These filters are used as pretreatment in the drinking-water production chain. Their function is to reduce the concentration of suspended solids, turbidity, real color and fecal coliforms of the influent before passing into other slow-rate filters. In the first dynamic rough filter, the CW

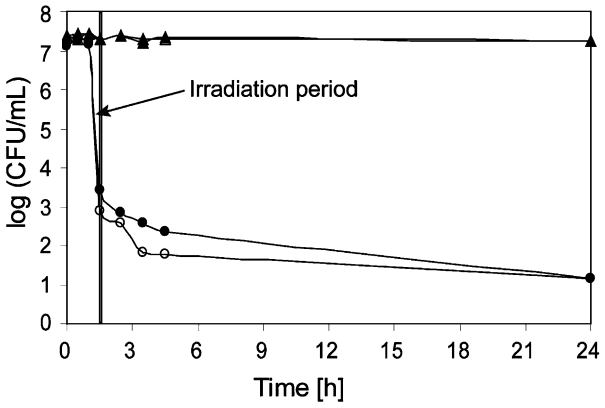

Fig. 4. Inactivation of *E. coli* in distilled water suspension as a function of time with 1.5 h of irradiation in: (●) TSB, and (○) EMB. The broken vertical line indicates the end of the illumination and the beginning of the dark period. Control experiment was carried out without illumination in: (▲) TSB, and (△) EMB.

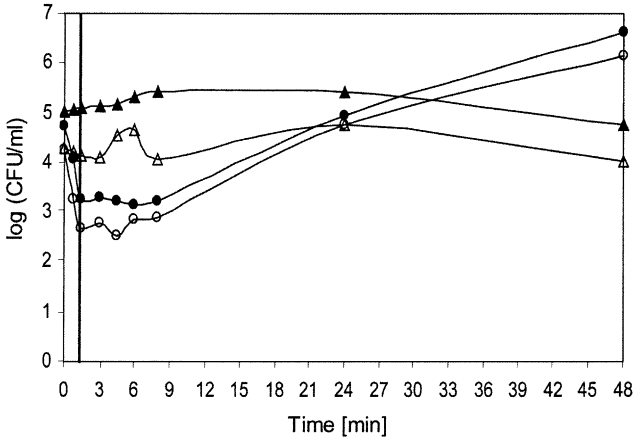

Fig. 5. Inactivation of a CW, sample A, as a function of time, with 1.5 h of irradiation in: (●) TSB, and (○) EMB. The broken vertical line indicates the end of the illumination and the beginning of the dark period. Control experiment was carried out without illumination in: (▲) TSB, and (△) EMB.

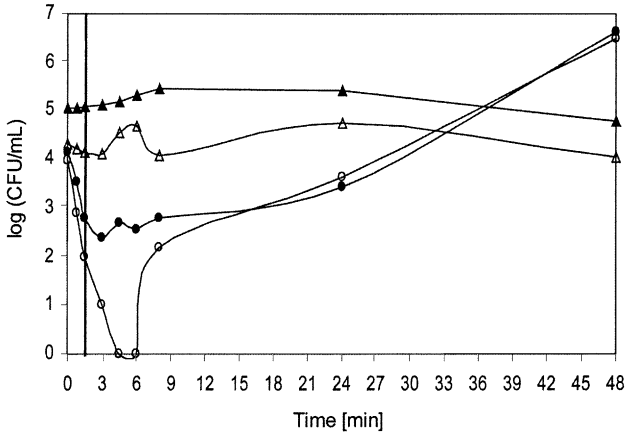

Fig. 6. Inactivation of primary effluent, sample B, as a function of time with 1.5 h of irradiation in: (●) TSB, and (○) EMB. The broken vertical line indicates the end of the illumination and the beginning of the dark period. Control experiment was carried out without illumination in: (▲) TSB, and (△) EMB.

passes slowly over a sand bed with a fraction of it being filtered through the sand, while the excess was returned to the input. The second filter consists of a horizontal three-layer sand bed in which the primary effluent is filtered vertically.

Sample A. With CW collected at point A in Fig. 1, a strong bacterial inactivation from 5×10^4 to 2×10^3 CFU/ml was observed after 1.5 h of irradiation. The culturable cell concentration remained unchanged for a latent period after irradiation was stopped. Fig. 5 shows that after 1 day in the dark, the culturable concentration in the reactor returns to initial value. After 48 h in the dark, cells reached 4×10^6 CFU/ml, thus final concentration was found to be higher than the initial one. Very similar results were obtained with both selective and non-selective media, indicating the lack of residual effect of TiO_2-photocatalysis in CW.

The control experiment, shown in Figs. 5–7 for comparison, was carried out in the same experimental conditions, but omitting irradiation. It showed slight culturable cell concentration increase within the first 24 h and a slight decrease within the second 24 h as shown in Figs. 5–7. This slight increase can be explained through the higher nutrients availability for the bacteria in the reactor as in natural conditions due to agitation. However, the culturable cell concentration after 48 h was always lower than that initially due to the reduced concentration of natural nutrients in a batch reactor.

Sample B. With sample collected at point B in Fig. 1, the culturable bacterial concentration decreased from 1.4×10^3 to 500 CFU/ml (less than one order of magnitude) in the non-selective medium, after 1.5 h irradiation. After a latent period of several hours, the population increased to reach 20% of the initial culturable cell concentration after 24 h and reached 4×10^6 CFU/ml after 48 h. In the selective medium, the culturable cell concentration showed a decrease from 10^4 to 10^2 CFU/ml during the irradiation and from 10^2 to less than 10 CFU/ml (detection limit). However, a strong increase in culturable cell concentration was observed during the next 20 h to reach half of the initial culturable cell concentration. After 48 h, the same culturable cell concentration was reached as that found for the non-selective medium. Thus, final cell concentrations was actually higher as shown in Fig. 6.

Sample C. The quality of the water improves as it passes trough the filters, and with sample collected at point C in Fig. 1. The culturable bacterial concentration decreased from 740 to 240 CFU/ml in the non-selective medium and from 160 to 20 CFU/ml in the selective medium, after 1.5 h irradiation. After a latent period of 24 h, a strong increase of the culturable cell concentration was observed within the next 24 and at 48 h the culturable cell concentration reached approximately 5×10^5 and 2.5×10^4 CFU/ml in

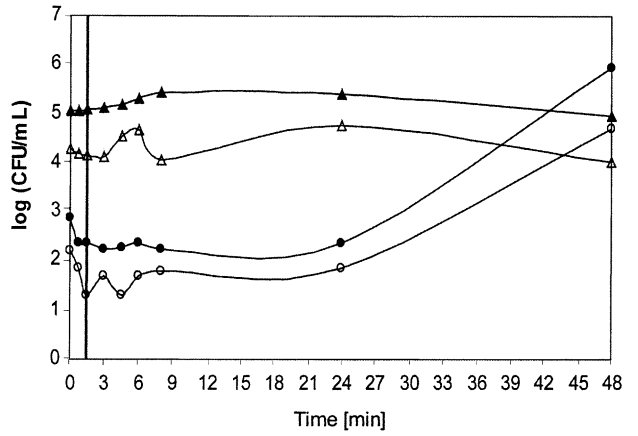

Fig. 7. Inactivation of secondary effluent, sample C, as a function of time with 1.5 h of irradiation in: (●) TSB, and (○) EMB. The broken vertical line indicates the end of the illumination and the beginning of the dark period. Control experiment was carried out without illumination in: (▲) TSB, and (△) EMB.

non-selective and selective media, respectively, as shown in Fig. 7.

For sample C, inactivation yields immediately after irradiation were 70 and 85% in TSA and EMB, respectively, which is very low in relation with the 96% (TSA), 96% (EMB) and 95% (TSA), 99% (EMB) found for samples A and B, respectively.

A detailed analysis of the mechanisms responsible for these phenomena is beyond the scope of this work. However, several mechanisms can be postulated to occur:

- Regrowth: some bacteria that were injured by the treatment are then able to regrow at the detriment of dead cells. The bacteria that grow fastest will then recolonize the treated solution.
- Reactivation: some bacteria that lost their culturability during the illumination can activate other bacterial resistances [14,15] to stressful conditions and recover their culturability after being in more favorable conditions.
- Activation: microorganisms that were present in the sample in inactivated forms before the irradiation activate under the stress condition and, therefore, contribute to culturable cell concentration after the treatment.

Photocatalytic treatment does not kill all the microorganisms and lacks residual toxicity. Thus providing that the changes introduced in the CW still allow this medium to sustain at least the same bacterial population as that before the treatment, then an opportunity exists for the more resistant microorganisms to grow and recolonize the freshly treated solution.

Three hypothesis can be made in order to explain why the culturable cell population was higher than the initial one:

- If the real cell population present in the non-treated solution was higher than the observed culturable cell population, the solution could then be able to sustain a higher culturable cell concentration. So, if after the treatment, the microorganisms that develop fastest are culturable, then the culturable cell population would be greater than the initial one.
- The stressful conditions could activate other bacterial metabolic mechanisms.
- The photocatalytic treatment might provide new sources of nutrients by action on the present biomass and organic compounds. Nutrients could be released from dead cells and/or could be produced by degradation of organic compounds.

It is probable that a real treatment would be a mixture of these phenomena. Their respective contributions would be influenced by the experimental conditions. The influence of these phenomena in the culturable cell concentration increase is of great importance for further real applications of photocatalysis in CW.

A further avenue of study would be to monitor the total amount of DNA present both before and after treatment. This should permit the direct observation of regrowth phenomena, assuming that any increase of DNA is due to the presence of additional bacteria. This would demonstrate if a real increase in cell concentration occurs after treatment or if only an increase in culturable cell concentration takes place.

Multiplex polymerase chain reactions (PCRs) offer another possibility to follow simultaneously the quantity of microorganisms. However, the use of PCR for quantitative measurements still suffer sever problems [16].

4. Conclusion

Photocatalysis applied to CW and pretreated water has been shown to be effective during irradiation, which is consistent with the literature, since a drastic cell inactivation was observed during the irradiation source was on. However, the lack of residual bactericide effect allowed a drastic culturable cell concentration increase after the treated water is left in the dark for several hours.

On the other hand, experiments carried out with *E. coli* in distilled water showed no increase in culturable cell concentration after photocatalysis. As a result, caution should be taken when making predictions based on this simple model as they are not necessarily representative of natural CW samples.

These results show the need for more information about the post-treatment period. A further understanding of the different possible interactions between toxic oxidative species and bacteria at cellular level is of great importance. Identifying the physical conditions or biological factors responsible for this increase in culturable cell concentration would allow pretreatment installations to be improved.

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