

PHOTOCATALYTIC INACTIVATION OF *Clostridium perfringens* AND COLIPHAGES IN WATER

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Abstract - This study presents results of the photocatalytic inactivation of two groups of microorganisms: spores of the anaerobic bacterium *Clostridium perfringens* and coliphage. A cylindrical reactor impregnated with titanium dioxide and irradiated with ultraviolet light (15 W) was used. Parameters such as color, turbidity, hydraulic detention time (HDT) and initial concentration of microorganisms were evaluated in relation to the efficiency of the inactivation process. According to the experiments with the bacterium *C. perfringens*, the reduction in number of microorganisms was higher than 98% after an irradiation time of 152 seconds, independent of color and turbidity. For solutions with low turbidities efficiency of the coliphage inactivation reached approximately 100% between 89 and 104 HDT, while this value was 98% for solutions with higher turbidities.

Keywords: photocatalytic inactivation, photolytic inactivation, *Clostridium perfringens*, coliphage.

INTRODUCTION

Contaminated water and a lack of basic sanitation procedures 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 visits are blamed on the lack of sanitation in communities (Garrido, 2000).

The presence of microorganisms and the production of toxic products in the traditional process of chlorination for disinfection of potable water are considered serious problems. Alternatives to the chlorination method are being developed, mainly in an attempt to reduce the formation of

trihalomethanes (THM) and any other product that presents a threat to human health.

According to Borges et al. (2001), during of the process water disinfection with free chlorine, hypochlorous acid (HClO) is formed and in contact with the bromide anion is oxidized to hypobromous acid (HBrO). Both hypochlorous and hypobromous acids as well as the anions resulting from the dissociation (ClO⁻ and BrO⁻) can react with organic matter found in water to form THM.

The process of disinfection of water utilizing UV radiation has been known since the beginning of the twentieth century, but due to problems related to the technology and reliability of the equipment, the process was abandoned for some time. Once these

problems were solved, the treatment of water with UV (ultraviolet) radiation gained popularity, mainly in European countries. Unlike, disinfectants chemical UV radiation acts physically, mainly on the nucleic acids of microorganisms, producing photochemical reaction, and thereby inactivating viruses and bacteria (Daniel et al., 2001).

During the past years, the advanced oxidation process (AOP) has been studied to determine its a viability. It is considered an oxidation process in which mainly hydroxyl radicals (OH^\bullet) are generated, which have a greater oxidation potential and thus the ability to react with organic compounds and microorganisms (USEPA, 1998; Daniel, 2001).

Heterogeneous photocatalysis (HP), na AOP which utilizes a semiconductor under UV radiation, has a higher disinfection potential than a UV lamp alone (Sjorgren and Sierka, 1994). In heterogeneous photocatalysis, hydroxyl radicals are formed and can react with most biological molecules (Dorfman and Adams, 1973) in addition to a wide range of anthropogenic organic compounds (Al-Ekabi and Serpone, 1988; Buxton et al., 1988; Graze and Peyton, 1988; Nogueira and Jardim, 1998; Ziolli and Jardim, 1998).

When titanium dioxide (TiO_2) normally, in its allotropic form, anatase, absorbs light at a higher energy than its band gap, the electrons of the valence band are excited and transferred to the conduction band, creating an electron-hole pair. The photogenerated electrons react with the dissolved oxygen in the water, and the hole, which is also photogenerated, reacts with water molecules adsorbed on the TiO_2 particles, creating mainly the reactive species OH^\bullet , HO_2^\bullet and H_2O_2 . These photogenerated substances are strong oxidizing agents (Blesa, 2001).

In 1985, Matsunaga et al. proposed a "new concept of photochemical sterilization of water," in which microbial cells could be inactivated using heterogeneous photocatalysis. Suspensions of TiO_2 spiked with platinum (TiO_2/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*, *S. cerevisiae* and *E. coli* was achieved in 60, 60 and 120 minutes of irradiation, respectively.

It is believed that the inactivation of the microorganisms using TiO_2 , irradiated with ultraviolet light is attributed to the inhibition of the cellular respiratory process by the photoelectrochemical oxidation of coenzyme A (CoA). However, this mechanism is not yet totally understood. Besides the hydroxyl radicals (OH^\bullet), certainly other active species such

as O_2^\bullet , HO_2^\bullet and H_2O_2 also participate in the process (Matsunaga et al., 1985; Kikuchi et al., 1997; Blesa, 2001).

It is important to conduct inactivation tests with *C. perfringens* because this microorganism produces endospores, which form a thick wall that functions as a resistant barrier to aggressive agents. It can survive in this way for a very long period in aquatic environments. It has low sensitivity to traditional disinfectants such as chlorine and chlorine dioxide. *C. perfringens* is considered an indicator of remote pollution mainly associated with human feces. Its presence is detected in feces and residual and polluted waters (CETESB, 1993; USEPA, 1999).

Several studies were performed using TiO_2 irradiated with UV light for the inactivation of microorganisms, mainly viruses and bacteria, in both photocatalytic and solar reactors (Matsunaga et al., 1985; Morioka et al., 1988; Ireland et al., 1993; Watts et al., 1995; Wei et al., 1994; Matsunaga and Okochi, 1995; Kikuchi et al., 1997; Dillert 1998; Maness et al., 1999; Melián et al., 2000; Senogles et al., 2001; Alam et al., 2001).

The objective of the present study was the use of heterogeneous photocatalysis as an alternative method to disinfect water spiked with spores of the anaerobic bacterium *Clostridium perfringens* and the coliphage bacteriophage.

MATERIALS AND METHODS

Assembly of the Photochemical Reactors

Each reactor was constructed with a borosilicate glass cylinder with an internal diameter of 3.8 cm and a length of 42.5 cm. A germicide lamp (15 W, $\lambda_{\text{max}} = 254$ nm and 2.5 cm e.d.) was fixed at the center of the reactor. The inside of the reactor had a working volume of 273 mL. The photoreactors were operated in a one-way ascending passage mode. The photocatalytic reactor was internally coated with a fixed bed of TiO_2 (Degussa P-25, $30 \text{ m}^2\text{g}^{-1}$, mainly anatase). The TiO_2 catalyst in its immobilized form was supported on the inner surface of the glass cylinder, as described by Takiyama (1995) (Figure 1a and 1b).

The complete system for disinfection was composed of a reservoir a capacity of 20 L of contaminated water, which was fed into the reactor by a peristaltic pump (Ismatec IPS-12). Hydraulic detention time (HDT) varied from 0 to 152 seconds. The system was also equipped with a mixing table to homogenize the water with suspended microorganisms before passage through the pump

(Figure 2). Monitoring of the microorganism concentration occurred at the reactor entrance (N_0) and exit (N).

The Effluent

In order to standardize the microorganism tests, a

synthetic formulated water was used (APHA, 1992) (Table 1).

It was necessary for the deionized water used for preparation of the solutions to have a theoretical value of $1 \mu\text{S}/\text{cm}$ and the final solution to have a hardness of $10 - 13 \text{ mg L}^{-1} \text{ CaCO}_3$, a pH of $6.4 - 6.8$, and total alkalinity of $10 - 14 \text{ mg L}^{-1} \text{ CaCO}_3$.

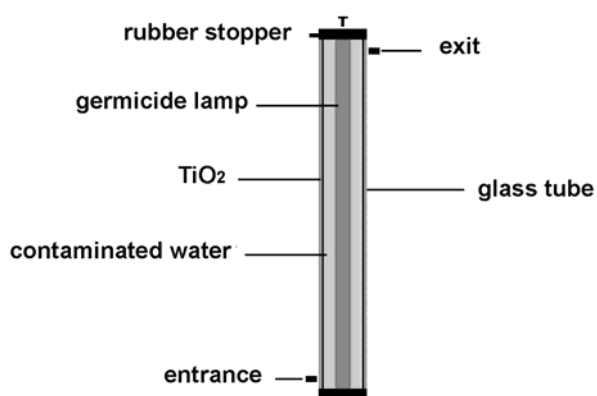


Figure 1a: Photocatalytic Reactor Design

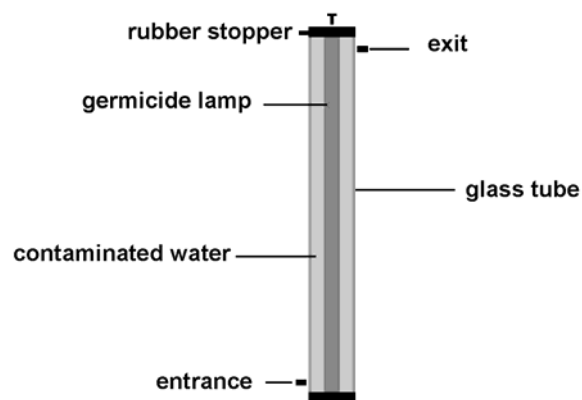


Figure 1b: UV Reactor Design

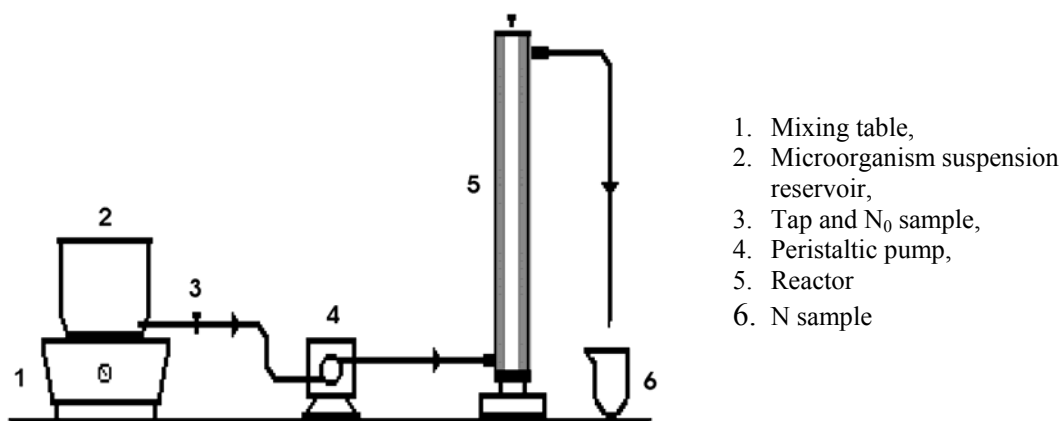


Figure 2: Experimental System Design

Table 1: Composition of the aqueous solution used in the tests: reagents and concentrations.

Reagent	Concentration (mg L^{-1})
NaHCO_3	12
CaSO_4	6.0
MgSO_4	7.5
KCl	0.5
NaOH	200
KH_2PO_4	4000

C. perfringens

C. perfringens is usually found in the human intestine and in other animals as a simple symbiote. Its presence in water is a sign of fecal contamination. It can cause gastrointestinal distress, food poisoning and gaseous gangrene among other effects. Morphologically it is a short stick rod, from 1.0 to 1.5 μm wide by 4 to 8 μm long, sometimes filamentous, with slightly rounded, gram-positive and motionless extremities (Bier, 1982). The strain of *C. perfringens* spores was obtained from the Institute of Biological Sciences at the University of São Paulo, Brazil (ICB/USP).

Microorganisms were monitored and quantified by the multiple tubes technique. The culture medium DRCM (differential medium enriched with *Clostridium*) was used for the presumptive test, anaerobically incubated for 48 h \pm 3 h at 35 $^{\circ}\text{C} \pm 1$ $^{\circ}\text{C}$. The samples showing turbidity or darkening due to reduction in the sulfite in the medium, forming sulfide, were considered positive (CETESB, 1993).

In the confirmation test, Litmus milk was used as the culture medium and the samples were also incubated under the conditions mentioned above. The samples showing pink coloration due to the coagulation of the milk casein and the production of bubbles due to fermentation were considered positive (CETESB, 1993).

The results were calculated and expressed in MPN/100 mL (most probable number), (APHA, 1992).

Coliphage

Coliphage is a bacteriophage that infects bacteria of the coliform group and replicates through them and is always present in study samples in which *E. coli* is isolated (Daniel et al., 2001). According to Pelczar et al. (1996), it is necessary to provide live host cells to isolate and grow viruses in the laboratory. The bacteria viruses are easily isolated and grown in young bacterial cultures in active growth. A modified TSA culture medium (tryptic soy agar) solidified on petri dishes was utilized. As the infected coliform bacteria were lysed, visibly clear areas known as plaques developed in the lawn of confluent bacterial growth. The coliphages were grown in a strain of the bacteria *E. coli* (ATCC 13706). Strains of both coliphages and *E. coli* were obtained from ICB/USP.

The determined number of microorganisms was according to standard methods: by counting the plaques on each petri dishes and recording them. The number of plaques was obtained for 100 mL of sample. The sum of the plaques on four dishes was then multiplied by 5. The final result was expressed

as CFU/100 mL (colony-forming units), (APHA, 1992).

Monitored Variables

In order to evaluate reactor efficiency in the disinfection process for both microorganisms, tests varying the color and turbidity of the microbial suspension were carried out. Humic acid was added (0.1% solution) in order to vary the color of the solution, and a 1.0 % clay solution was used to modify the turbidity (sodium montmorillonite clay). These variables were monitored with a spectrophotometer (DR4000, HACH). Three tests of inactivation with the species *C. perfringens* and three tests of inactivation with coliphages were carried out. The pH of the bacterial solution was measured before and after passing it through the heterogeneous photocatalytic reactor.

RESULTS AND DISCUSSION

Figures 3 to 5 show the results obtained in the present work for inactivation of the anaerobic bacterium *C. Perfringens* for different colors and turbidities values of the microbial suspension.

It is important to mention that in the tests conducted with *C. perfringens*, the pH value did not change after the bacterial suspension through the passed heterogeneous photocatalytic reactor, showing an average value of 6.9 ± 0.1 . Watts et al. (1995) used heterogeneous photocatalysis with natural and artificial light for the inactivation of bacteria of the coliform group and poliovirus I. No differences in photocatalytic disinfection rates were found when the assays were conducted in the pH range of 5 to 8.

Melián et al. (2000) described that at pH 5 and TiO_2 and UV light, an increase in the rate of inactivation of total coliform compared to that in the bacterial suspension without the photocatalyst was observed; however at pH 7.76, there is no significant difference between the two processes. In a study of inactivation of *E. coli*, Kikuchi et al. (1997) showed that at pH 4, there is a higher efficiency than at pH 7.4, at least for one hour of UV irradiation with TiO_2 . This effect is caused by the formation of oxidant species such as HO_2^{\bullet} and H_2O_2 .

The efficiency of disinfection is shown as $-\text{Log } N/N_0$ versus HDT, where N_0 is the initial concentration of the microorganism and N is the concentration at each HDT.

As shown in the figures, efficiency values for the experiments were similar, showing a reduction in the initial number of microorganisms of over 98%. It is important to mention that this was accomplished in spite of the different initial concentrations of

microorganisms, due to the inherent difficulty of preparing the bacterial suspension.

At an HDT of 17 seconds a pronounced increase in inactivation of the bacteria occurred; after this time there was a trend towards stabilization for the remaining number of bacteria or a decrease in the *C. perfringens* inhibition rate, as demonstrated in Figures 3 to 5.

None of the tests performed achieved complete inactivation of the microorganisms, probably because a longer period of exposure is necessary. However, the reduction in the initial number of microorganisms was always significant, justifying experiments with longer exposure times due to the resistance mechanism of this species.

In parallel tests performed with ultraviolet radiation but without TiO_2 , a lower efficiency in inactivation of *C. perfringens* was observed in suspensions with differing amounts of color and turbidity than that obtained by the heterogeneous

photocatalytic process. This effect was larger for shorter HDTs.

Due to their resistance to the chlorination process and to adverse environmental conditions, *C. perfringens* spores have been suggested as a safe surrogate indicator of disinfectant activity for *Cryptosporidium parvum* and other hardy pathogens in water (Venczel et al., 1997).

Due to the correlations between coliphage and coliform bacteria found in water and the higher resistance of coliphages to chlorine, they can be used as indicators of the sanitary quality of water, thus indicating the presence of both pathogenic viruses and coliform bacteria in water with fecal contamination. Another important aspect to be considered with respect to coliphages is necessary to the short time test to provide which results, ranges from 4 to 6 hours (Daniel et al., 2001). Other common microorganisms, such as bacteria of the coliform group, show a response period of 24 hours.

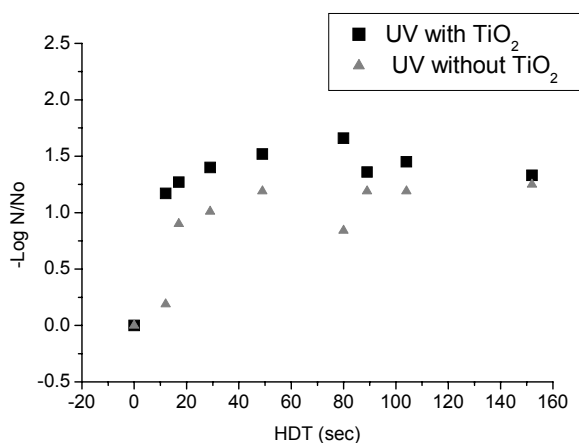


Figure 3: UV radiation and UV-TiO₂ in inactivation of *C. perfringens* ($N_0 = 430$ NMP/100 mL, without the addition of color and turbidity to the water, dose ranges from 137.88 mW s cm^{-2} to 1746.48 mW s cm^{-2} and from 131.88 mW s cm^{-2} to 1670.48 mW s cm^{-2} for UV and UV-TiO₂, respectively).

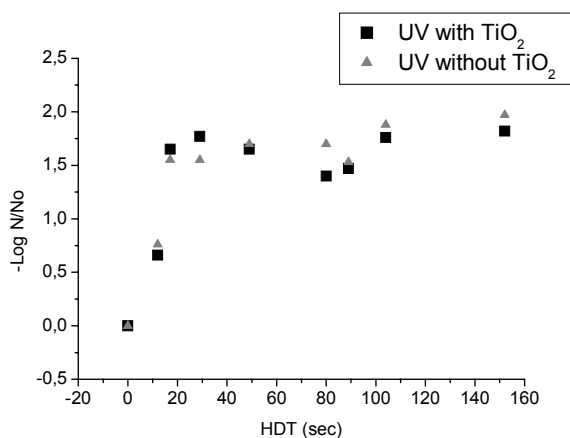


Figure 4: UV radiation and UV-TiO₂ in inactivation of *C. perfringens* ($N_0 = 1600$ NMP/100 mL, color 10 uC and turbidity 2.8 NTU, dose ranges from 137.64 mW s cm^{-2} to 1743.44 mW s cm^{-2} and from 131.64 mW s cm^{-2} to 1667.44 mW s cm^{-2} for UV and UV-TiO₂, respectively).

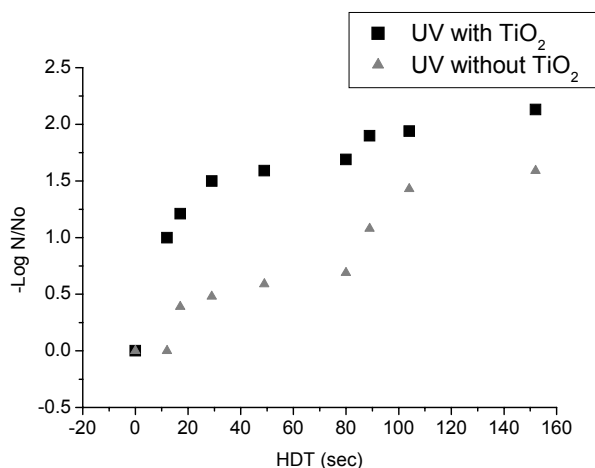


Figure 5: UV radiation and UV-TiO₂ in inactivation of *C. perfringens* ($N_0 = 540$ NMP/100 mL, color 29 uC and turbidity 29.0 NTU, dose ranges from 136.80 mW s cm⁻² to 1732.80 mW s cm⁻² and from 130.80 mW s cm⁻² to 1656.80 mW s cm⁻² for UV and UV-TiO₂, respectively).

Figures 6 to 8 show the test results obtained for inactivation of the bacteriophage in the microbial suspension using different color and turbidity values.

As previously stated, in the experiments with *C. perfringens*, the pH values did not change after the bacterial suspension passed through the heterogeneous photocatalytic reactor. In the experiments performed with coliphage, the average pH value was 6.6 ± 0.1 .

The efficiency values for all the cases studied were similar. There was a reduction in microorganism concentration of 98% for the first HDT, even though this comparison was made with different initial concentrations of microorganisms. It's worth mentioning that with no color or turbidity, with 7 uC and 2.1 NTU, respectively, in the bacterial suspension, efficiency reached approximately 100% (with HDTs of 89 to 104 seconds).

There was a pronounced increase in the inactivation of bacteriophage up to an HDT of 12 s. After this time, the remaining coliphage concentration tended to stabilize, or the rate decreased, as shown in Figures 6 to 8. This phenomenon was also observed in experiments with *C. Perfringens* as the test organism.

Watts et al. (1995) conducted water disinfection experiments in water containing coliform bacteria and viruses using heterogeneous photocatalysis. For those tests, TiO₂ (250 mg L⁻¹) was used as the photocatalyst and was irradiated with solar or black light. Coliform bacteria were more resistant than poliovirus I to both solar and black light. In the case of the bacteria, 150 minutes of exposure time to

black light were necessary to reduce activity one hundred times, while the same reduction for the poliovirus I was reached in 30 min of exposure to solar or black light.

Sjogren and Sierka (1994) studied the photocatalytic inactivation of a bacteriophage (MS2) that had *E. coli* as its host. The initial MS2 concentration approximately was 10⁴ CFU/mL. A 15 W UV lamp with a λ of 365 nm and an intensity of 2 mW cm⁻² was used. With TiO₂ efficiency was 95%, while after the addition of 2 μ mol L⁻¹ of ferrous sulfate this value reached 99%. This increase was attributed to the Photo-Fenton reaction.

In the present study, using UV radiation and TiO₂, 89 seconds of radiation of the coliphage suspension with no color or turbidity were enough for total inactivation. (Figure 6). At low color and turbidity values, this time increased to approximately 104 s (Figure 7). The bacteriophage test suspension wasn't completely disinfected at the higher color and turbidity values, even at the maximum exposure time of 152 s (Figure 8). This result suggests that a longer period of exposure to UV radiation is required.

Particulate matter in suspension can affect the process of water disinfection because light scattering occurs, serving as an ultraviolet ray protection shield to 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 subterranean waters of low turbidity. In superficial waters, however, the

turbidity effect is more significant (USEPA, 1999). In the case of heterogeneous photocatalysis, the active surface of the TiO_2 reactor can be reduced by the adsorption of particulate material, including aggregates of microorganisms.

It is important to mention that all experiments were carried out with a very short exposure time, in the range of seconds, thereby differing from several studies that were carried out over times of several minutes to hours for both the coliphage and *C. perfringens* (Watts et al., 1995; Morioka, et al., 1988; Kikuchi, et al., 1997; Melián et al., 2000).

In the experiments where samples with no color or turbidity were employed (Figure 6), apparently efficiency was similar in both processes; however, the initial concentration of microorganisms used in the experiment with UV- TiO_2 was larger than that used with the UV method alone. The efficiency of the heterogeneous photocatalytic reactor in inactivation of the coliphage was higher than the efficiency obtained when only ultraviolet radiation was employed, mainly with low turbidity values (Figure 7). Efficiency was similar in both processes when microbial suspensions had higher turbidity values (Figure 8).

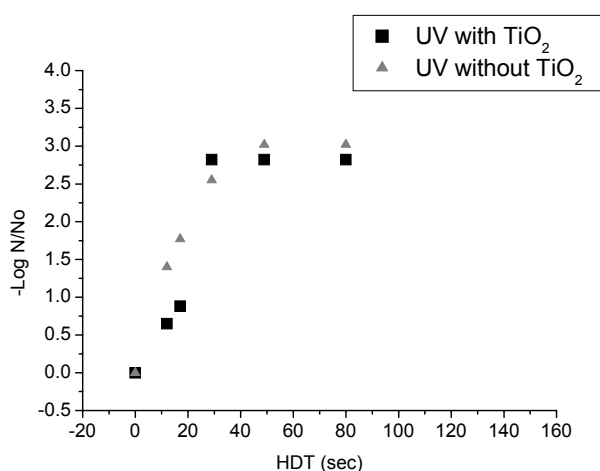


Figure 6: UV radiation and UV- TiO_2 in inactivation of coliphage ($N_0 = 13180$ CFU/100 mL, dose range from $131.88 \text{ mW s cm}^{-2}$ to $879.20 \text{ mW s cm}^{-2}$ for UV- TiO_2 and $N_0 = 10580$ CFU/100 mL, dose range from $137.88 \text{ mW s cm}^{-2}$ to $919.20 \text{ mW s cm}^{-2}$ for UV radiation, without addition of color and turbidity)

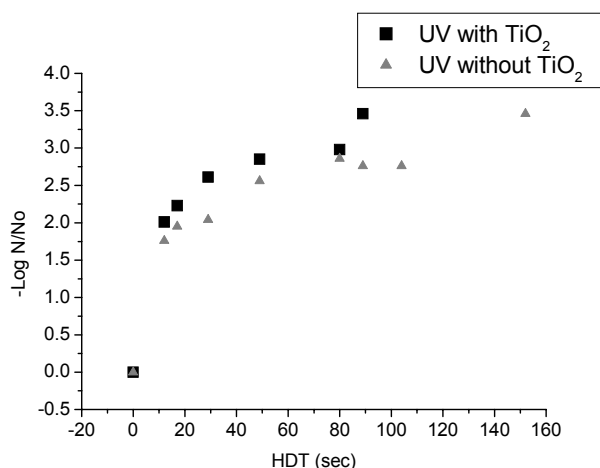


Figure 7: UV radiation and UV- TiO_2 in inactivation of coliphage ($N_0 = 28920$ CFU/100 mL, color 7 uC and turbidity 2.1 NTU , dose ranges from $137.76 \text{ mW s cm}^{-2}$ to $1744.96 \text{ mW s cm}^{-2}$ and from $131.76 \text{ mW s cm}^{-2}$ to $1141.92 \text{ mW s cm}^{-2}$ for UV and UV- TiO_2 , respectively).

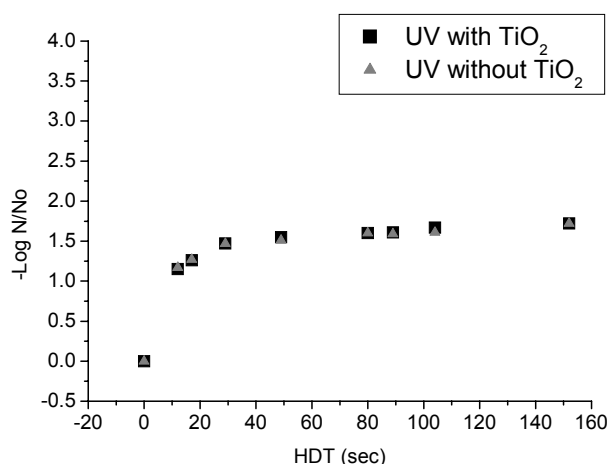


Figure 8: UV radiation and UV-TiO₂ in inactivation of coliphage ($N_0 = 148100$ CFU/100 mL, color 23 uC and turbidity 26.3 NTU, dose ranges from 136.80 mW s cm⁻² to 1732.80 mW s cm⁻² and from 130.80 mW s cm⁻² to 1656.80 mW cm⁻² for UV and UV-TiO₂, respectively).

CONCLUSION

The results demonstrate the real potential of microorganism inactivation in aqueous solutions using UV irradiation and TiO₂ as the photocatalyst. It is important to mention that compared to the processes using either irradiated TiO₂ or UV irradiation alone, the combined process (UV-TiO₂) increased the efficiency of inactivation for the microorganisms studied.

In the case of the anaerobic bacterium *C. perfringens* spores, total inactivation was not achieved in any of the three cases studied. The maximum efficiency obtained varied between 98 and 99.9%, depending on the color and turbidity of the bacterial solution. However, the inactivation rate was high up to 17 s HDT, achieving more than 90% inactivation; beyond this exposure time there was a tendency to stabilize (Figures 3, 4 and 5).

In bacteriophage suspension with no color and turbidity, total inactivation occurred at an HDT of 89 s (Figure 6). At intermediate values, this time was 104 s (Figure 7). Inactivation was not total for the highest color and turbidity values used at the maximum HDT value of 152 s (Figure 8). However, it did reach an efficiency higher than 98%.

Even though inactivation of approximately 100% (below the limits of detection) was not achieved for the microorganisms in some cases, it is important to mention that the irradiation times for the suspensions were very short, within a range of seconds, differing from other studies where this time varied from several minutes to several hours.

The reactor used in this study is cheap and easy to operate. It could be redesigned for use on a practical scale with higher flow rates, hydraulic detention times, and UV intensity. However, the redesign could create some potential problems for further operations. For example, when using higher flow rates there is a potentially rate-limiting step resulting from microorganism mass transfer to the photoactivated TiO₂ surface.

The photocatalytic process can be used for the disinfection of potable and residual water, inactivating even more pathogenic organisms than the ones studied, e.g., *Cryptosporidium* sp. and *Giardia* sp. cysts, as described in other studies.

NOMENCLATURE

λ_{\max}	maximum wavelength
e.d.	external diameter
m ² g ⁻¹	surface area
μS/cm	electrical conductivity
mW cm ⁻²	radiation intensity
μmol L ⁻¹	concentration

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