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# Inactivation and injury of total coliform bacteria after primary disinfection of drinking water by $TiO_2$ photocatalysis

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ABSTRACT

In this study the potential application of $TiO_2$ photocatalysis as primary disinfection system of drinking water was investigated in terms of coliform bacteria inactivation and injury. As model water the effluent of biological denitrification unit for nitrate removal from groundwater, which is characterized by high organic matter and bacteria release, was used. The injury of photocatalysis on coliform bacteria was characterized by means of selective (mEndo) and less selective (mT7) culture media. Different catalyst loadings as well as photolysis and adsorption effects were investigated. Photocatalysis was effective in coliform bacteria inactivation (91–99% after 60 min irradiation time, depending on both catalyst loading and initial density of coliform bacteria detected by mEndo), although no total removal was observed after 60 min irradiation time. The contribution of adsorption mechanism was significant (60–98% after 60 min, depending on catalyst loading) compared to previous investigations probably due to the nature of source water rich in particulate organic matter and biofilm. Photocatalysis process did not result in any irreversible injury (98.8% being the higher injury) under investigated conditions, thus a bacteria regrowth may take place under optimum environment conditions if any final disinfection process (e.g., chlorine or chlorine dioxide) is not used.



## 1. Introduction

Chlorine (as gas or hypochlorites), chlorine dioxide, ozone and UV radiation are commonly used for disinfection of drinking water. Chlorine and chlorine dioxide can be used as final disinfectants because they assure a detectable disinfectant residual inside the distribution network to make drinking water safe on microbiological point of view. On the opposite, UV radiation and ozone can only be used as primary disinfectant because they cannot ensure a detectable residual. Unfortunately, the addition of chemical disinfectants to water results in formation of toxic and/or potentially carcinogenic disinfection by-products (DBPs) [1–4]. A possible solution to solve the problem could be a primary disinfection system "by-products" free which may decrease both bacteria density and oxidant demand in order to control the formation of DBPs as final (chemical) disinfectant is added.

Photocatalysis has recently emerged as an alternative technology for bacteria inactivation [5–7] and organic compound oxidation [8–10]. When catalytic semiconductor particles are illuminated with near UV radiation ($\lambda < 400$ nm) electron–hole pairs ($e^-/h^+$) are generated. These pairs can migrate to the surface of semiconductor particle to form oxidizing species ($^\bullet$OH radicals). Due to its stability and low energy band-gap, $TiO_2$ is accepted to be one of the most suitable semiconductor for photocatalysis. Bacteria inactivation by $TiO_2$ photocatalysis was studied in aqueous *Escherichia coli* (*E. coli*) suspensions [5,7,11]; total inactivation was obtained within 60 min using 1 mg/mL of $TiO_2$, under black light illumination and initial concentration of $10^3$ cells/mL [5]. Although the most of studies available in the scientific literature deal with *E. coli* suspensions, recently naturally polluted waters [12] as well as on purposed bacteria-contaminated waters [13–15] were investigated too.

The disinfection process may be not so effective in killing bacteria, simply inactivating some of these (*breaktrough* phenomenon). Thus injured bacteria may restore their own vital functions as soon as environmental conditions in drinking water distribution system are suitable, increasing their number too [16]. Typically, the compliance control to check microbiological water quality in distribution system is carried out by detection of indicator mircoorganisms such as total coliforms, as well as a selective medium (typically mEndo) is used for their detection. However, injured bacteria are incapable of growth and colony formation under standard conditions because of structural and metabolic damage; as a result a significant portion of bacteria may not be detected leading to erroneous assessment of microbial water quality [17]. In order to detect injured bacteria too, less selective media were used [16–18]. Thus it is important to

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characterize the injury caused by disinfection system in order to verify the efficiency of the process and to avoid the bacteria regrowth inside the distribution network.

In this study the potential application of $TiO_2$ photocatalysis as primary disinfection system of drinking water was investigated in terms of coliform bacteria inactivation and injury. The water samples were taken from biological denitrification unit used for nitrate removal from groundwater. In this way experiments were performed on water samples rich in total coliform bacteria and organic matter. The repairing capacity of injured bacteria was determined using more selective (mEndo) and less selective (mT7) cultivation media.

## 2. Materials and methods

### 2.1. Water source

Water samples were taken from the effluent of biological denitrification (BD) pilot unit for nitrate removal from groundwater. The BD reactor was filled with cotton which acts as organic carbon source for denitrification process and provides the surface for biofilm growth [19]. However, the most important drawbacks of the reactor are related to bacteria and organic matter releases.

### 2.2. Photocatalytic reactor

Photocatalytic tests were performed filling in 100 mL cylindrical Pyrex reaction vessel (7.5 cm diameter, 3.5 cm height) with 50 mL water samples. The photoreactor was confined in a wooden box with internal walls covered with Al thin layer to assure reflected surface. A 125-W black light fluorescent lamp emitting radiation between 300 and 420 nm with a maximum at 350 nm was used as the light source. The reactor was illuminated from the top and mixing was assured by a magnetic stirrer. The light intensity was measured by spectrometer AvaSpec 2048 from Avantes as 1309 $\mu W\,cm^{-2}$. $TiO_2$ (P-25) from Degussa (Germany), was used as photocatalyst, upon 3 min sonification in a deionized water bath (Elma, Transsonic TS 540). In order to investigate the contribution of different mechanisms to bacteria inactivation a set of experiments including photocatalysis, adsorption, UV radiation as well as a control test were carried out in parallel using the same water sample (400 mL after shaking were shared in 100 mL samples): photocatalysis test was carried out according to the above mentioned procedure using 1.0 g $TiO_2$/L as catalyst loading; dark test was carried out under the same conditions of photocatalytic test but lamp was kept switched off; UV test was carried out under the same conditions of photocatalytic test but without any catalyst addition; control test (water sample was left without any treatment) was carried out in parallel.

### 2.3. Analytical measurements

The raw water samples taken from the effluent of BD pilot unit were analyzed for pH, organic matter (in terms of UV absorbance at 254 nm), nitrate and nitrite. A Hanna Instruments probe (model HI 8314) was used for pH measurements. Nitrate and nitrite were measured according to the respective procedures as given in the Standard Methods [20]. UV absorbance at 254 nm ($UV_{254}$), which is a surrogate parameter of TOC useful to characterize the aromatic fraction of natural organic matter [21–23], was measured using PerkinElmer Lambda 12 UV–vis spectrophotometer equipped with 1 cm quartz cell.

### 2.4. Bacterial count

Membrane filter method was used to count bacteria according to Standard Methods [20] using acetate cellulose type filter with 0.45 mm pore size. Total coliforms count was carried out using both mEndo and mT7 media from Oxoid; the plates were incubated at 37 °C for 24 h. For each one set of tests a fraction of water sample was held at room temperature and coliform bacteria were measured at the beginning and the end of the experiment as control. The results are expressed in colony forming units (CFU) per 100 mL. The following equation has been used to quantify the degree of injury in total coliform bacteria [24]:

$$\text{Injury}[\%] = \frac{[\text{mT7 (CFU/100 mL)} - \text{mEndo(CFU/100 mL)}] \times 100}{\text{mT7(CFU/100 mL)}}$$

## 3. Results and discussion

### 3.1. Characteristics of raw water samples

Water samples taken from the effluent of BD pilot unit were characterized by high variations in the selected parameters as showed in Table 1.

In a potential full scale application of the BD process, as nitrate concentration approaches to the limit set by the regulations, the reactor is expected to be put out of order to restore initial efficiency, but in this study, the system was also operated in the presence of high nitrate concentration in order to investigate the behaviour of the photocatalytic system under a wide range of experimental conditions.

### 3.2. Control tests

Control test was carried out to evaluate the contribution of UV radiation and adsorption mechanism, respectively, to bacteria inactivation as well as the natural decay of total coliforms. Beyond a natural decay (13%) of total coliform density after 60 min, UV disinfection as well as adsorption mechanism had a significant effect on total coliforms inactivation (27 and 74%, respectively, after 30 min); however, the best removal was observed during photocatalysis tests (96 and 99% after 30 and 60 min, respectively) (Fig. 1). The effective inactivation after 60 min can be estimated by subtracting the contribution of natural inactivation; accordingly we have 7, 52 and 86% inactivation by UV radiation alone, $TiO_2$ adsorption, and photocatalysis, respectively. Because of a so high contribution of adsorption mechanism to total coliform inactivation, which does not find any

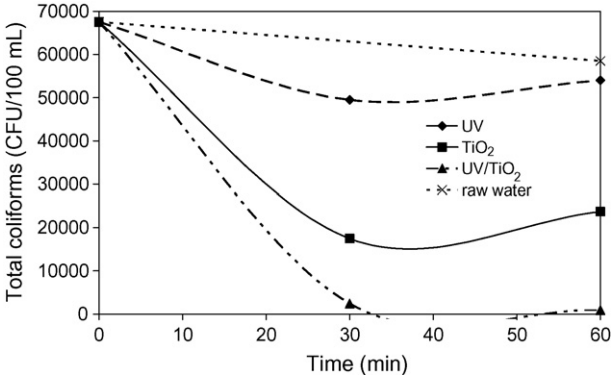


**Fig. 1.** Behaviour of total coliform density under different experimental conditions: (i) without any treatment (raw water), (ii) photolysis (UV), (iii) adsorption by 1.0 g $TiO_2$/L ($TiO_2$), and (iv) photocatalysis with 1.0 g $TiO_2$/L ($UV/TiO_2$).

**Table 1**
Characteristics of raw water samples.

| Parameter | Unit | Average | Min | Max |
|---|---|---|---|---|
| pH | – | 6.44 | 6.08 | 6.72 |
| $UV_{254}$ | 1/cm | 0.209 | 0.059 | 0.572 |
| Total coliforms (mEndo) | CFU/100 mL | 28,000 | 2,100 | 78,000 |
| Total coliforms (mT7) | CFU/100 mL | 89,000 | 13,000 | 330,000 |
| Nitrite | mg/L | 0.04 | 0.01 | 0.11 |
| Nitrate | mg/L | 8.7 | 1.8 | 50.1 |

confirmation in scientific literature, further adsorption tests with different $TiO_2$ loadings were carried out.

### 3.3. Effect of adsorption mechanism

Previous adsorption experiments carried out with *E. coli* suspensions showed that, after 120 min of stirring under dark conditions at 32 °C, all bacteria survived in presence or absence of $TiO_2$, indicating that adsorption of *E. coli* is not significant [25]. On the opposite, a significant effect of adsorption was observed in the experiments discussed in the present work (Fig. 1). In particular, the total coliform removal by adsorption was found to increase as $TiO_2$ loading and contact time were increased (Fig. 2). Such difference compared to data available in literature [5,6,25] can be explained by the characteristics of the model water used in this study: the biological denitrification reactor is characterized by biofilm detachment and organic particulate matter release from the support (cotton) used as carbon source [26]. The bacteria entrapped in these matrixes can be more easily adsorbed on to the photocatalyst.

### 3.4. Inactivation by photocatalysis: effect of $TiO_2$ loading and initial total coliform density

Although the fundamental mechanism has not yet been elucidated, it is generally accepted that the inactivation of microorganisms by $TiO_2$ photocatalysis is mainly due to oxidative radicals (mainly •OH) produced by $TiO_2$ irradiation [5]. Accordingly, the bacteria inactivation is expected to increase as photocatalyst loading increases because an higher amount of adsorption sites are available and the formation of radicals species increase as well. Since the turbidity increases according to higher $TiO_2$ powder loadings, a diminished light absorption due to the opacity of the solution is expected too. In order to evaluate the effect of photocatalyst loading a set of photocatalysis experiments were carried out on the same water sample using 0.5, 0.75, 1.0, 1.5 and 2.0 g $TiO_2$/L and 10 min irradiation time. According to Fig. 3, the inactivation efficiency increased as photocatalyst loading increased until to reach

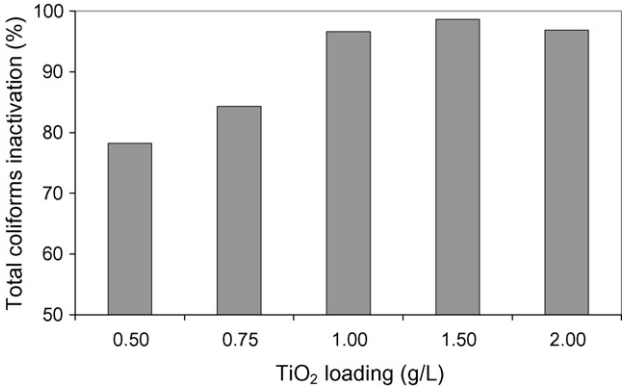


**Fig. 3.** Effect of $TiO_2$ loading in total coliform inactivation by photocatalysis.

the optimum condition at 1.5 g $TiO_2$/L, after which inactivation started to decrease.

Initial total coliform density effected the disinfection efficiency of the photocatalytic process too. Fig. 4 shows the effect of $TiO_2$ loading on two samples different in terms of initial total coliforms density. For the lower density sample (33,000 CFU/100 mL) the total coliforms removal was higher under all $TiO_2$ loadings investigated; moreover, only a small increase was observed (2.2%) as photocatalyst loading increased from 0.5 to 1.5 g $TiO_2$/L. On the other side, the total coliforms removal for the higher initial density sample (78,000 CFU/100 mL) was significantly lower (76.5%) at the lower photocatalyst loading (0.5 g $TiO_2$/L) as compared to the best removal (89.3%) detected at the higher investigated loading (1.5 g $TiO_2$/L). The higher disinfection efficiency for the lower density sample finds confirmation in scientific literature; Bekbölet [5] investigated the photocatalytic bactericidal activity of $TiO_2$ in aqueous suspensions of *Escherichia coli* and she found that inactivation rate decreased as initial cell concentration was increased. We may postulate that, for a given photocatalyst loading and irradiation time, the ratio •OH/bacteria is lower in the suspension with

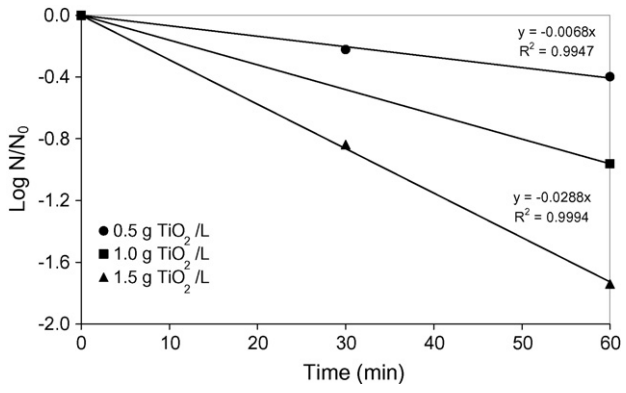


**Fig. 2.** Effect of adsorption mechanism in the removal of total coliforms: comparison among different $TiO_2$ loadings.

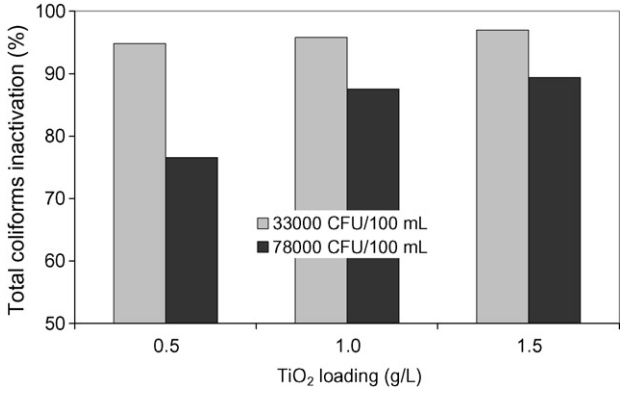


**Fig. 4.** Effect of initial total coliform density on disinfection efficiency by photocatalysis according to different $TiO_2$ loadings.

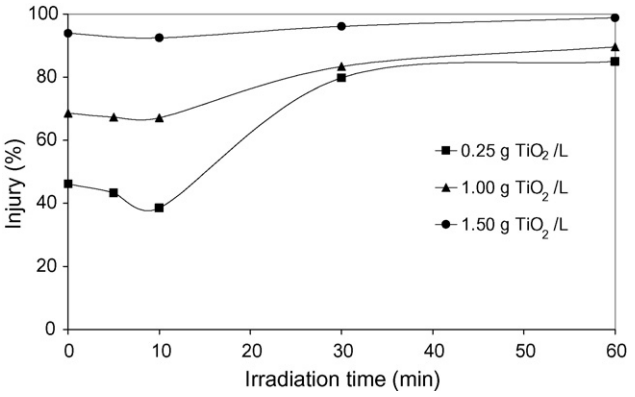


**Fig. 5.** Characterization of injury of $TiO_2$ photocatalysis on total coliforms.

the higher total coliform density, thus resulting in a less efficient disinfection process.

### 3.5. Injury characterization

The disinfection efficiency in bacteria inactivation depends on several factors (disinfectant type and dosage, bacteria specie and density, contact time between bacteria and disinfectant, pH, temperature) among these the injury due to stress factors occurred before disinfection process is important too. The samples taken from the denitrification reactor to be used in photocatalysis tests were characterized by different levels of injury (in the range of 46–94%); this injury may have effected the efficiency of subsequent disinfection process. Two different behaviours of injury curves depending on initial injury ($t = 0$) and catalyst loading were observed (Fig. 5): (i) until to 10 min irradiation the photocatalytic process did not result in any significant injury to bacteria, on the opposite, for the lower catalyst loading investigated (0.25 g $TiO_2$/L) a decrease was observed (probably photocatalytic process needs some time to effectively injure bacteria); (ii) from 10 to 60 min the injury increased as irradiation time increased depending on initial bacteria density and injury as well as catalyst loading.

Anyway, although several conditions were investigated in terms of photocatalyst loading as well as initial injury, photocatalytic process did not result in any irreversible injury (98.8% being the higher injury) until to 60 min irradiation time.

## 4. Conclusions

The potential application of $TiO_2$ photocatalysis as primary disinfection system of drinking water was investigated. The main results can be summarized as follows: (i) photocatalysis was effective in coliform bacteria inactivation (>91% after 60 min irradiation time, depending on both catalyst loading and initial density of coliform bacteria detected by mEndo) in water with both high organic matter concentration and bacterial density, although no total removal was observed after 60 min irradiation time; (ii) due to the nature of source water rich in particulate organic matter and biofilm, the contribution of adsorption mechanism was significant (60–98% after 60 min, depending on catalyst loading) compared to previous investigations; (iii) photocatalysis process did not result in any irreversible injury (98.8% being the higher injury) under investigated conditions, thus a bacteria regrowth may take place after disinfection if both no final disinfection process (e.g., chlorine or chlorine dioxide) is not used and optimum environmental conditions for bacteria regrowth occur in the distribution network.

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