In this work, TiO₂ photocatalysis was used to disinfect domestic wastewaters previously treated by different biological treatment systems: Upward-flow Anaerobic Sludge Blanket (UASB), facultative pond, and duckweed pond. The microorganisms monitored were E. coli, total coliforms, Shigella species, and Salmonella species. Photocatalytic experiments were carried out using two light sources: a solar simulator (UV intensity: 68-70 W m⁻²) and black-light lamps (BLL UV intensity: 17-20 W m⁻²). Samples were taken after each treatment stage. Results indicate that bacterial photocatalytic inactivation is affected by characteristics of the effluent, including turbidity, concentration of organic matter, and bacterial concentration, which depend of the type of biological pretreatment previously used.

Keywords: photocatalysis; TiO₂ domestic wastewaters disinfection.

INTRODUCTION

In developing countries, re-use of domestic wastewater for agricultural and industrial activities has been proposed as a strategy to conserve limited water resources. However, the use of these effluents for crop irrigation must be carefully considered, as they could contain high levels of pathogenic agents and harmful chemical compounds.¹

The World Health Organization (WHO) has determined that the concentration of fecal coliforms must be <10³ MPN 100 mL⁻¹ and <10⁵ MPN 100 mL⁻¹ for unrestricted and restricted irrigation, respectively.² In order to achieve these targets, domestic wastewater must be adequately treated to eliminate chemical and biological hazards prior to reuse. The most widely used disinfection treatment is chlorination, which exhibits high bactericidal activity. However, even after previous biological treatment, domestic wastewaters contain high levels of organic materials; treatment of these waters by chlorination may produce chlorinated hydrocarbons, which have toxic, mutagenic, and carcinogenic properties.³

Previous work on the use of solar irradiation for photodynamic and photocatalytic disinfection,⁴ at batch levels illustrated the simplicity of this method, which does not use or produce toxic compounds.

When titanium dioxide (TiO₂) is illuminated with UV light, using either the sun or a UV lamp, it produces hydroxyl (•OH) and superoxide (•O₂⁻) radicals that are highly oxidative.⁵⁶ These radical oxygen species have the ability to degrade organic material and inactivate pathogenic microorganisms.

TiO₂ photocatalysis has been studied in the past 20 years for its role in the degradation of organic substances in water.⁷¹² Nevertheless, only recently has this technology been explored for water disinfection.⁶,⁷ Inactivation of microorganisms in distilled water using photocatalysis under different experimental conditions has been reported.⁸ Only a few studies have been published using wastewater.⁹,¹⁰

The effluents studied in this work are from domestic wastewater in the town of Ginebra (Valle del Cauca, Colombia), which was previously treated by various biological treatment systems. Photocatalytic disinfection was studied using two different kinds of light sources: a commercial UV A source (black-light lamp, BLL), and a Hanau Suntest AM-1 solar simulator. The effect of TiO₂-mediated inactivation on Escherichia coli, Salmonella species, Shigella species, and total coliforms was evaluated in effluents from three pretreatment systems.

EXPERIMENTAL

Materials and methods

Samples

The wastewaters in Ginebra are treated by nine different types of biological treatment. Three effluent types were chosen: upward-flow anaerobic sludge blanket (UASB), duckweed ponds fed with UASB, and facultative ponds. Each effluent was sampled in 500 mL volumes. The photocatalytic experiments were performed in triplicate, and the standard deviations of the replicates were <15%. The initial concentrations of the natural bacterial consortia present in each effluent are presented in Table 1.

Table 1. Initial concentration of bacterial consortia in the effluents used

<table>
<thead>
<tr>
<th>Effluent Bacteria type</th>
<th>UASB Concentration in CFU mL⁻¹</th>
<th>Facultative pond Concentration in CFU mL⁻¹</th>
<th>Duckweed ponds Concentration in CFU mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>3.1 x 10⁶</td>
<td>7.6 x 10⁶</td>
<td>3.8 x 10⁶</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>3.0 x 10⁴</td>
<td>5.8 x 10⁴</td>
<td>8.9 x 10⁴</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>5.1 x 10⁵</td>
<td>7.2 x 10⁵</td>
<td>5.0 x 10⁵</td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>4.3 x 10⁵</td>
<td>9.0 x 10⁵</td>
<td>6.5 x 10⁵</td>
</tr>
</tbody>
</table>

Photocatalytic experiments

The photocatalytic experiments were performed using two different illumination systems: A Hanau Suntest solar simulator (Xenon lamp Intensity UV: 68-70 W m⁻²), and an illumination box using five UV black-light lamps Phillips TLD 18 W (17-20 W m⁻²). The Hanau Suntest (AM-1) solar simulator lamp had a spectral distribution as follows: about 0.5% of photons were emitted at wavelengths shorter than 300 nm (UV-C range); about 4% between 300-400 nm (UV-B and UV-A range); and the distribution of photons emitted between 400-800 nm corresponded to the solar spectrum (Figure 1). On the other hand, UV black light lamps (BLL) have a spectral distribution between 340-400 nm (mainly UV-A) with an emission maximum...
at 360 nm. The UV intensity was measured with a radiometer UV
UV-Tex a+b idm Optix Tech.

Figure 1. Emission spectra of the Suntest lamp and Black Light Lamps (BLL)

The effluent samples were processed immediately after collection. 
TiO₂ (Degussa P-25) was added to a 50 mL aliquot of the sample, to 
a final concentration of 0.5 g L⁻¹. Experiments were conducted in a 
Pyrex glass reactor that was illuminated for 3 h with either the solar si-
mulator or BLL. The temperature during experiments was below 38 °C.

Samples were taken at different time intervals during the expe-
riments. Serial dilutions were performed in peptone-buffered water. 
The samples were spotted and spread using standard techniques onto 
Cromocult (Merck) plates (selective for Escherichia coli and total 
coliforms), and SS (Merck) plates (selective for Shigella and Salmon-
ella). The plates were incubated at 37 °C for 24 h prior to enume-
ration. Control experiments were carried out in the absence of TiO₂.

Re-growth experiments

After the illumination stage, the Pyrex glass reactors were covered 
and maintained in dark conditions for 24 h with continuous stirring.

RESULTS AND DISCUSSION

Bacterial inactivation in absence of TiO₂

Salmonella species were inactivated in the absence of TiO₂ using 
both illumination systems, except in UASB effluent under BLL UV 
light (Figures 2a-2c). In previous work,²⁰ it was reported that UASB effluents contain higher levels of turbidity and phosphates than other 
types of effluents (Table 2). Under BLL UV irradiation (17-20 W m⁻²), 
the lower light intensity combined with the chemical nature of the 
UASB effluent probably has a protective effect, prohibiting inactiva-
tion of Salmonella. This effect has also been reported by other authors 
using UASB effluents and UV disinfection.²¹ In facultative pond and 
duckweed pond effluents, Salmonella was completely inactivated in 
the absence of TiO₂ using both BLL and the solar simulator. This 
could be due to the low turbidity and low concentrations of organic 
and inorganic matter in these effluents, as well as the low initial 
bacterial concentrations.

It is well known that UV-B light can induce bacterial death via 
the formation of dimeric pyrimidine photoproducts, which cause 
inhibition of DNA replication and increase bacterial mutations.²²

In contrast to the black light lamps, the solar simulator emits not 
only UV light (Figure 1) but also visible light. Photosensitizers such 
as humic substances, which absorb visible light, produce reactive 
y oxygen species (ROS) such as OH and singlet oxygen (¹O₂).²³,²⁴ 
Therefore, the observed responses in the facultative and duckweed 
pond effluents could also be due to the presence of photosensitizers.

Figure 2. Effect of UV light on different microorganisms in various effluents 
using a solar simulator and Black light lamps (BLL): (a) UASB effluent; (b) 
facultative pond effluent; (c) duckweed pond effluent. Intensity of the UV irra-
diation: solar simulator, 68-70 W m⁻²; Black light lamps, 17-20 W m⁻². pH = 7.1

Table 2. Chemical characteristic of effluents (Castillo, 2006)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UASB Concentration</th>
<th>Facultative pond Concentration</th>
<th>Duckweed ponds Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>[PO₄]²⁻</td>
<td>5.7 mg L⁻¹</td>
<td>4.2 mg L⁻¹</td>
<td>3.5 mg L⁻¹</td>
</tr>
<tr>
<td>Turbidity</td>
<td>45.4 NTU</td>
<td>34 NTU</td>
<td>28 NTU</td>
</tr>
</tbody>
</table>

The fact that the Salmonella species showed the fastest inactivation 
rate under simulated solar light does not necessarily mean that this 
microorganism is generally more sensitive to UV irradiation or ROS. 
On the contrary, Berney et al.²⁹ found that in mineral water, Salmo-
rella typhimurium was highly resistant to UV-A light exposure. The 
contrasting results obtained in our work are probably due to: the matrix 
effect of treated domestic wastewaters; the selective agar used to culture 
Salmonella and Shigella; and the low initial concentration of Salmonella 
in the effluent consortia.
Bacterial inactivation in the presence of TiO$_2$

In the UASB effluents (Figure 3a), TiO$_2$ photocatalysis had a remarkable effect on bacterial cultivability only when the Suntest solar simulator was used. The experiments conducted using BLL light did not show any bacterial inactivation (data not shown). The Shigella strains showed a 2-log inactivation after 3 h of illumination. Escherichia coli counts decreased by 3 logs, and the total coliforms did not show any change in concentration. Salmonella was completely inactivated in the presence of TiO$_2$ only under the solar simulator.

In the facultative pond effluents (Figure 3b), Salmonella lost its cultivability after less than 1.5 h of irradiation under both light conditions, whereas E. coli and Shigella were affected only when the solar simulator was used. The total coliforms from the facultative pond did not show any inactivation during the photocatalytic treatment. In water from the duckweed pond (Figure 3c), TiO$_2$ photocatalysis had a strong inactivating effect on all the studied microorganisms only when the solar simulator was used. Salmonella, E. coli, and Shigella were inactivated after less than 2 h of illumination, while total coliform concentrations decreased by almost 1 log in 3 h. This inactivating effect via TiO$_2$ photocatalysis is known to be due to the action of •OH radicals on the bacterial cell membrane, leading to perturbation of various cellular processes.  

The difference between UASB and the other effluents is most likely due to its higher concentration of organic and mineral compounds. An inhibitory effect of organic matter and inorganic ions on photocatalytic disinfection has been previously reported. These chemical substances (particularly organic matter and HCO$_3^-$) compete for the photogenerated radicals, thereby protecting the bacteria against oxidative attack. In addition, other ions such as HPO$_4^{2-}$ can alter the photocatalyst surface.

The initial concentration of bacteria (Table 1) present in the wastewater also plays an important role in photocatalytic disinfection. Higher bacterial concentrations require longer time to total inactivation. Table 1 shows that in the UASB effluent, the initial concentration of E. coli is around 10$^5$ CFU mL$^{-1}$, while in other effluents the concentration is 10$^4$ CFU mL$^{-1}$. Shigella and total coliforms showed similar behavior as E. coli in the UASB effluent. Total coliforms never reached total inactivation after 3 h of treatment.

The type of lamp used plays also an important role. The solar simulator emits almost 4 times more UV-A radiation (68-70 W m$^{-2}$) than BLL (17-20 W m$^{-2}$). In certain cases, bacterial inactivation was only observed when the solar simulator was used. Previous works reported that light intensity exerts an important role in the photocatalytic inactivation of E. coli. In addition, Figure 1 shows that the solar simulator can emit UV-B radiation, and as was mentioned before, this radiation can induce bacterial inactivation. Total coliforms presented the highest resistance to photocatalytic inactivation, whereas E. coli and Shigella inactivation depended on their initial concentrations and the light source used.

Post irradiation events after the photocatalytic treatment

In effluents from the UASB reactor (Figure 3a), E. coli and Shigella showed slight recovery after photocatalytic treatment during the subsequent 24 h under dark conditions. Salmonella from all effluents and E. coli from facultative (Figure 3b) and duckweed (Figure 3c) ponds suffered a complete loss of viability; no recovery was observed after 24 h in the dark. In a previous work using the same wastewater samples, it was found that Shigella and Salmonella were the most resistant microorganisms to photocatalytic treatment using a non-selective agar for these both microorganisms. The contrasting results presented here could be explained by the selective media used in this study for Shigella and Salmonella; this discriminative agar was used in order to distinguish between the two microorganisms.

Bacterial re-growth after the photocatalytic treatment of wastewaters has been mentioned in previous works. Recovery of the total coliform and Shigella populations could be explained by the fact that, under oxidative stress, their cells can enter a viable but non cultivable (VBNC) state. UV irradiation and attacks by oxidative species generate changes in the permeability of the lipid membrane and/or modify the bacterial DNA, leading the loss of cultivability while viability remains unaltered. When the oxidative stress ends (dark conditions), the microorganisms recover their cultivability. In addition, the growth state of bacteria (exponential or stationary) in wastewater varies in time. Depending on this parameter, some bacteria could persist under photocatalytic conditions and consequently, their recovery rate in the dark could also be influenced. It has been proposed that Shigella can form cellular aggregates, thereby protecting the cells from possible lethal injuries. It is important to note that the result of zero culturable
microorganisms obtained after photocatalytic treatment does not always represent total bacterial death. The ROS produced during the photocatalytic process can induce oxidative stress on the microorganisms, causing the cells to enter a viable but non culturable (VBNC) state.

Bacterial re-growth is also aided by the oxidative destruction of organic matter, which generates highly oxidized and bio-available organic by-products.2-33

Bacterial recovery during the dark period after exposure to commercial black light lamps is not shown, because little change in concentration was observed after 3 h of illumination. In this case, only *Salmonella* from facultative and duckweed ponds suffered complete loss of viability and cultivability; other types of bacteria were not affected. The detrimental screening effect of TiO$_2$ on UV light with BLL lamps is probably more important than the generation of oxidative species on the photocatalyst surface.

Results presented in this paper indicate that factors linked to the wastewater composition (such as concentration and nature of organic and inorganic matter and turbidity) could affect the photocatalytic disinfection of domestic wastewaters. However, the physiological characteristics of the different bacteria present in the consortia cannot be neglected. Monitoring of other pathogenic microorganisms is crucial to assess whether domestic wastewater effluents treated by photocatalysis can be re-used in agricultural applications.

**CONCLUSIONS**

The time required for complete bacterial inactivation by photocatalysis, without re-growth after 24 h in the dark, depends of the type of microorganism, the chemical characteristics of the effluent, the illumination intensity, the type of illumination source used, and the initial bacterial concentration. This is especially important considering the low number of studies carried out with natural waters in real conditions. Heterogeneous photocatalysis could be a promising technology for eliminating bacterial contamination from domestic wastewaters only in effluents with low initial bacterial concentration and low concentration of inorganic and organic matter. However, it is also necessary to further evaluate the response of different microorganisms to photocatalytic conditions in a specific water matrix, taking into consideration their physiological features, their cell densities and their relationship with the different components of the treated water.

Regarding treated domestic wastewater reuse, WHO regulates only the presence of *E. coli*. From this perspective, the domestic wastewaters treated here by photocatalysis could be used for irrigation. However, we showed that after photocatalytic treatment there are still pathogenic microorganisms present, such as *Shigella* and *Salmonella* species, indicating that these effluents are not suitable for crop irrigation. Taking in account the results reported here, it is suggested revise the using of *E. coli* as reference for microbiological water quality.

Finally, in spite of the promising results obtained with heterogeneous photocatalysis to inactivate microorganisms in real waters, there is yet a main technological obstacle remaining: the photocatalyst separation after the process. So, the next efforts must be addressed to this issue either looking for novel separation processes or fixing the TiO$_2$ in supports.

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**REFERENCES**