Application of TiO_2 solar photocatalysis as tertiary treatment for wastewater disinfection

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ABBREVIATIONS

AOPs – Advanced Oxidation Processes

CBPs – Chlorination by-products

CFUs – Colony forming units

eV – Electron-volt

OH – Hydroxyl

TiO_2 – Titanium Dioxide

TOC – Total Organic Carbon

UV – Ultraviolet

WWTP – Wastewater Treatment Plant

BACHELOR'S THESIS

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ABSTRACT

Chlorination processes have been a method commonly used for wastewater disinfection. However, new methods without the formation of disinfection by-products have been recently investigated by researchers. Photocatalytic processes based on utilization of solar energy along with the addition of a catalyst are rapidly expanding technologies for wastewater treatment. During the last few years, these processes have demonstrated great potential in elimination of bacteria of low and high concentrations and have found versatile applications in treating contaminants of different wastewater types. One of the catalysts that has proved to be one of the most successful ones is TiO_2. In this project the process of TiO_2 heterogeneous photocatalysis was investigated as the tertiary method for water disinfection. The experimental work was aimed to examine at which parameters and conditions the bacterial and organic matter degradation will occur more effectively within a shorter period of time. With the data obtained in this work it can be estimated that the results of photocatalytic reactions were dependent not only on the experimental conditions, such as volume, light flux, and addition of TiO_2 catalyst, but also on the initial composition of the effluent used in reactions.

Language: English Key words: TiO_2 solar photocatalysis, total coliforms, tertiary wastewater treatment

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1. INTRODUCTION

The current world population is constantly growing. According to the estimates of scientists it is projected to reach 9.6 billion by year 2050 (United Nations, 2013). This implies an increasing demand for freshwater resources to sustain the livelihood of the people. Therefore, water reuse practices of domestic and industrial wastewater have become a significantly acute issue over the last decades.

The water supply still remains a very complex problem in some countries, accentuated by the lack of effective, accessible and environmentally-friendly techniques. Conventional methods of wastewater disinfection as chlorination and ozonation are widely known to promote the formation of chlorinated and brominated disinfection by-products that have been identified as potentially carcinogenic to human-beings (Ikehata et al., 2006). The growing concerns about such pathologies and more rigid legislations regarding public health make it key important to develop new alternative disinfection technologies that would provide reliable and efficient methods for simultaneous removal of microorganisms and traces of organic micropollutants.

Advanced Oxidation Processes (AOPs), based on the generation of highly oxidizing species such as hydroxyl radical (• OH) are considered to render in solving these problems. Among these processes, a special emphasis is placed on heterogeneous photocatalysis with TiO_2 due to its environmental conditions of operation, utilization of air as the oxidant and use of solar radiation as primary energy source (Benjamin et al., 2013, p. 469).

2. AIM AND OBJECTIVES

This thesis is based on the three months' research (February-April 2015) that was conducted in the wastewater treatment laboratory of Rey Juan Carlos University (URJC) in Madrid. The major aim of this study was the investigation of developing wastewater disinfection methods, known as *Advanced Oxidation Processes*, that have recently proved to be a worthy alternative to conventional methods. One of the methods that have been lately studied thoroughly by scientists in the lab of URJC is photocatalytic processes and a wide range of their application for the wastewater disinfection. Since the laboratory was engaged mostly with this area of research, which coincided with my studies specialty, I also became a part of this project.

This thesis work is only one part of a big project aimed to explore the possible application of solar energy for wastewater treatment. The major objective of the current research was to test the inactivation of total coliform group of bacteria by application of solar TiO_2 photocatalysis. Along with the bacterial degradation tests, the concentration of organic matter was also measured to examine its removal efficiency by this specific method. Therefore, the main research question was "under which conditions the elimination of bacteria and organic matter will occur more effectively within a shorter period of time". These experiments were performed using a solar simulator that was built in order to streamline the testing process.

The present work consists of six main chapters and several sub-chapters. The first two chapters give an overview on the current problems with the wastewater treatment, a description of the thesis work and its major objectives. The third chapter provides the information on various wastewater disinfection methods, with a deeper insight into heterogeneous photocatalysis and advanced oxidation processes. Chapter four tells about the investigation approach, as well as the methodology of experiments, the preparation and their realization. It also covers the experimental design of the reactor, used for the conduction of reactions. Finally, the summary of major results being obtained throughout the thesis work, followed by a critical assessment, is outlined in the last chapter.

3. BACKGROUND

Water scarcity and water pollution are regarded as one of the biggest challenges on our planet nowadays. There are a lot of countries that do not have sufficient amount of water suitable for livelihood maintenance, drinking, industrial and agricultural purposes. This growing problem increases the need for an effective management of water resources and an investigation of innovative wastewater treatment technologies. Thus, the main task of wastewater treatment companies and organizations, whether private or public, is to develop efficient methods for the removal of various organic and inorganic impurities from the wastewater that would be cost-effective and environmentally sound (FAO, 2007).

Natural waters used for drinking and industrial water supply, should have favorable organoleptic properties, be chemically harmless, and safe in sanitary and epidemiological terms. To achieve these properties the variety of wastewater disinfection methods are performed. According to Metcalf & Eddy (2003, p. 1220) the methods employed for wastewater disinfection could be divided into several generic groups, where the disinfection is carried out by the use of:

- Chemicals agents, including chlorine (Cl_2) and its compounds (hypochlorite salts), bromine (Br_2), ozone (O_3) and hydrogen peroxide (H_2O_2) among others;

- Physical agents, comprising such disinfectants as heat, UV light, and radiation (electromagnetic, acoustic and particle radiation as the commonly used ones), and by

- Mechanical means, involving various equipment such as screens, grit chambers, filters or settling basins.

Each country, depending on its economic development, resources availability and geographical location, has to use one or another method that is proved to be the most effective and affordable method of water purification in the area.

3.2. Conventional disinfection methods

3.2.1. Chlorination

Historically, chlorination has been the most popular disinfection method worldwide. This chemical agent is usually added in the water either in gaseous form (Cl_2), or as hypochlorite salts (HClO). The method is commonly used due to its high efficiency, low cost of reagents and relative ease of maintenance. However, one of the biggest drawbacks of chlorine disinfection is the formation of various chlorination by-products (CBPs) that might cause carcinogenic effects in mammals and human-beings, and be harmful for the environment (Sharma et al., 2009, p. 908). In addition, this method does not ensure total elimination of all undesirable microorganisms and there are still some bacterial species that remain resistive to it (Queensland Department of Environment and Heritage, 1993).

3.2.2. Ozonation

As for another alternative method, known as ozonation, the disinfection process is performed by means of ozone (O_3), which is a chemically active form of oxygen. This method is more effective than chlorine in removing viruses and bacteria, ensuring almost no regrowth of microorganisms after the treatment (Rakness et al., 1993). Nevertheless, ozone is chemically unstable, having a tendency to convert back quickly to oxygen after generation, thus it must be generated onsite (Metcalf & Eddy, 2003, p.1289). Due to high toxicity of the chemical, ozonation process could also pose health hazard.

Since ozonation has been used in water treatment over the years, it has not been considered as advanced oxidation process. However, due to ozone's ability to enter the reactions with OH^- ions and generate OH radical, it has some of the common features of AOPs (Benjamin et al., 2013, p. 469).

3.2.3. Ultraviolet disinfection

With the introduction of new regulations requiring low or non-detectable amounts of chlorine residuals in treated effluents, chlorination and ozonation systems have been replaced by alternative disinfection system such as ultraviolet (UV) radiation (Metcalf & Eddy, 2003, p. 17). The UV wastewater decontamination has been the safest method among others, as it does not require any use of chemicals. During this disinfection process, UV light disrupts the DNA of bacteria and viruses, which impairs the organism, leading to

the deactivation of the pathogens (Drinan et al., 2001, p. 97). This method is effective for the elimination of most of the bacteria. However, it does not provide the residual disinfection, requiring further treatment.

3.3. Advanced Oxidation Processes

Different kinds of wastewater can be generally processed efficiently by biological treatment plants or by conventional chemical treatments. However, sometimes these disinfection methods, as mentioned in chapter 3.2, are inadequate to achieve the required degree of purity, due to the presence of toxic substances in the wastewater that are not amenable to the biodegradation. Therefore, most of the industrialized countries resort to the use of so-called Advanced Oxidation Processes (AOPs). They are considered as innovative technological schemes that hold a great promise for more sustainable wastewater treatment in the future.

AOPs are characterized as the processes that enable the degradation and mineralization of organic compounds in aqueous medium due to their ability to generate hydroxyl radicals (•OH) (Parsons, 2004, p. 4). These radicals are powerful oxidizing species that can be generated by photochemical means or by other forms of energy (Litter, 2005). They produce fundamental changes in the chemical structure of pollutants under various conditions, leading to their degradation, or in case of microorganisms attack cell membranes, causing fatal damage (Malato et al., 2009).

AOPs could be divided into non-photochemical and photochemical processes. They can be used alone or in combination with other conventional wastewater disinfection methods. *Table 1* shows a list of the main AOPs (Litter, 2005):

Table 1. Advanced Oxidation Processes and other related processes.

Non-photochemical processes	**Photochemical processes**
• Alkaline ozonation (O_3/OH^-) • Ozonation with hydrogen peroxide (O_3/H_2O_2) • Fenton and related processes (Fe^{2+}/H_2O_2) • Electrochemical oxidation • *y*-Radiolysis and electron-beam treatment • Non-thermal plasma • Electrohydraulic discharge-ultrasound • Oxidation in sub/and supercritical water	• Water photolysis in vacuum ultraviolet (VUV) • UV/hydrogen perioxide • UV/O_3 • Photo-Fenton and related processes • UV/periodate • Heterogeneous photocatalysis

One of the AOPs that has drawn attention in recent years is purification of water by heterogeneous photocatalysis with TiO_2 (titanium dioxide) as catalyst. Since it is a non-selective technology, it can be used to treat complex mixtures of contaminants and it also allows the possibility of using solar radiation as primary energy source (Blanco et al., 1994; Ollis, 1994).

3.3.1. Heterogeneous photocatalysis

Heterogeneous photocatalysis is the process based on the absorption of radiant energy (visible light, UV or sunlight) by a suspended catalyst, whereby the cleavage process of organic compounds and elimination of bacteria occur (Verhoeven, 1996). In comparison to homogeneous photocatalysis, where initial reagents are present in the single phase (gas or liquid), the reaction in heterogeneous photocatalysis occurs in the surface layer at the interface between solid and liquid phases (Kumar et al., 2013).

The heterogeneous photocatalysis is a very complex process. In general, the oxidation of organic and inorganic compounds during the heterogeneous photocatalysis could be presented in several steps (Hermann, 1999; Park et al., 2009):

1. Transfer of reagents from the liquid phase to the catalyst surface
2. Adsorption of at least one of the reactants
3. Photocatalytic reactions in the adsorbed phase
4. Desorption of the products from the catalyst surface
5. Elimination of the products from the interfacial region

The only difference between the processes lies in the way in which the catalyst activation occurs, replacing the thermal activation with activation of photons (Hermann, 1999).

Of all the AOPs, heterogeneous photocatalysis has a number of advantages that differentiates it from the rest of photochemical processes. It has proved to be one of the most attractive disinfection methods in terms of its environmental benefits and economic viability, thanks to the use of non-toxic catalysts based on semiconductor materials and the use of renewable energy, such as sun. Heterogeneous photocatalysis also has favorable operating conditions, since it allows working at ambient pressure and temperature without the need for chemical oxidants apart from the air (Marugán et al., 2011). Moreover, this

photocatalytic process is a very effective method since it acts on all types of bacteria, as well as other microorganisms. Therefore, all of these advantages have aroused intense interest to the heterogeneous photocatalysis as to emerging sustainable technology for the treatment of wastewater nowadays.

This method has been a topic of this research, and will be described thoroughly later on in chapter 3.3.3.

3.3.2. Titanium dioxide as a catalyst

There are many different materials with suitable properties that can act as catalysts and perform photosensitized reactions, such as TiO_2, ZnO, CdS, iron oxides, WO_3, ZnS, etc. Among these various semiconducting materials, nano Titanium Dioxide (TiO_2, titania) has gained the most popularity as photocatalyst, due to its outstanding physical and chemical properties. It is relatively cheap and non-toxic material, which is a common ingredient in many commercial products such as paints, toothpaste, cosmetics as well as food color additives (Carp et al., 2004; Castellote et al., 2011). Apart from the large spectrum of application in these industries, it is also used for the wastewater disinfection and air purification since it is chemically stable compound that has a high photoactivity under UV light irradiation (Xiaobo et al., 2007; Benjamin et al., 2013, p. 482). In addition, it accelerates the rate of the photocatalytic reaction, without being consumed as a reactant and without becoming a part of the end products (Wubbels, 1983). These properties make it an attractive semiconducting material for the photocatalytic research.

Its wide application in photocatalytic processes stems from the electronic structure of the material that has a band gap of about 3.2 eV, suitable for performing many redox (oxidation-reduction) reactions. This results in long-lived state of excited electrons and exceptional resistance to the photocorrosion (Kumar et al., 2013).

TiO_2 occurs in nature in three different crystal forms: anatase, rutile and brookite. According to numerous photocatalytic studies, the anatase titania showed better photocatalytic properties than two other phases (Augugliaro et al., 1990; Sclafani et al., 1996; Kumar et al., 2013). Nevertheless, good results have also been obtained with mixture of crystalline anatase-rutile forms. It has been attributed to a greater adsorption capacity of molecular oxygen and slow hole-electron recombination rate (Kumar et al., 2013).

3.3.3. The operating principle of TiO_2 in heterogeneous photocatalytic process

As mentioned above, TiO_2 is a semiconductor compound that becomes a strong oxidant in the presence of the UV irradiation. This high oxidation ability of TiO_2 catalyst makes it suitable for the degradation of most organic and inorganic compounds even at very low concentrations of material (Castellote et al., 2011).

Most of the electrons in semiconducting compounds tend to stay at the bound state. To cause the excitation of these electrons, the energy of no less than 3.2 eV should be expended (Mills et al., 1997; Kumar et al., 2013). This amount of energy could be delivered by the photon (quantum of light, *hv*) with the wavelength equal or lower than $\lambda <$ 390 nm. When catalytic semiconductor (TiO_2) particles are illuminated with UV radiation, charge pairs of a free electron (e^-) and a hole (h^+) are generated, as it presented in *Figure1*.

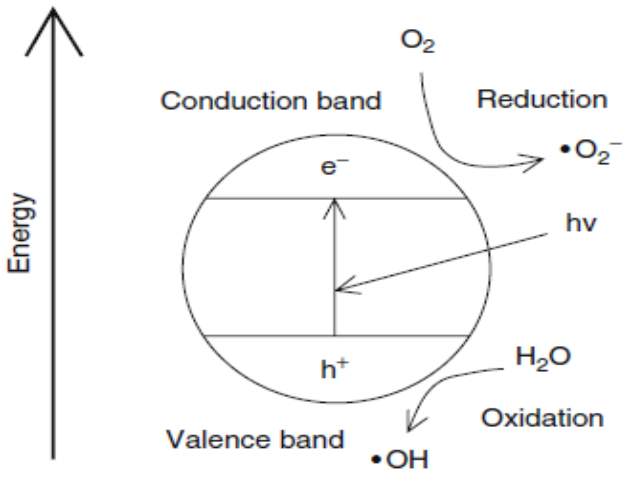

Figure 1. Schematic representation of the photocatalysis mechanism on the TiO_2 surface (Castellote et al., 2011).

During UV illumination, the electrons of the valence band (vb) of TiO_2 become exited and move to the conduction band (cb), leaving the positively charged "hole" at the valence band (vb) (Mills et al., 1993; Castellote et al., 2011; Benjamin et al., 2013, p. 481-482). This stage, known as the "photo-excitation" stage could be presented by the following reaction (1.1):

$$TiO_2 + h\nu \rightarrow TiO_2 + e^- + h^+ \quad (1.1)$$

Photogenerated e^-/h^+ pairs can recombine, resealing the absorbed light energy as heat, or react with molecules on the TiO_2 surface of semiconductor. Holes enter reactions with H_2O

to form hydroxyl radicals ($^{\cdot}OH$) and oxidize the organic compounds, whereas electrons react with O_2 to generate superoxide radicals (O_2^{-}) and perform decomposition and oxidation reactions (Venkatadri et al., 1993; Parsons, 2004; Thompson et al., 2006; Benjamin et al., 2013, p. 481).

Hydroxyl and superoxide radicals characterizing TiO_2 photocatalytic activity are formed by the following reactions (1.2, 1.3 and 1.4):

$$TiO_2\ (h^{+}_{vb}) + H_2O \rightarrow {}^{\cdot}OH + H^{+} \qquad (1.2)$$

$$TiO_2\ (h^{+}_{vb}) + OH^{-} \rightarrow {}^{\cdot}OH \qquad (1.3)$$

$$TiO_2\ (e^{-}_{cb}) + O_2 \rightarrow {}^{\cdot}O_2^{-} \qquad (1.4),$$

where *vb* stands for the valence band and *cb* for the conduction band respectively.

Further formed hydroxyl and superoxide radicals react with organic pollutants and oxidation of the latter occurs, decomposing the organic material into CO_2 and H_2O under soft conditions (Legrini et al., 1993; Benjamin et al, 2013, p. 472; Kumar et al., 2013).

These strong oxidizing agents generated during the TiO_2 heterogeneous photocatalysis make this process to be quite an effective method for elimination of bacteria and degradation of organic matter. TiO_2 suspension provides high performance for the desirable reduction of microorganisms, since it has higher surface area for the reaction. The only drawback of this process, increasing the time and complexity of this treatment, is the need for additional elimination of the TiO_2 particles from the water that could be achieved by filtration (Benjamin et al., 2013).

4. MATERIALS AND METHODS

4.1. Experimental set-up design

Experiments have been carried out in a compound parabolic collector (CPC) -based solar simulator that was built in the wastewater treatment plant at the Rey Juan Carlos University for investigation of AOPs and their application for water disinfection. As illustrated in *Figure 2*, the experimental set-up consisted of two main units: Xenolux XL-7000W cinema projector (Proyecson) and evacuated tube collector. The tube collector included four parallel rows of transparent glass tubes that were fused to the collector panel from both sides. The tubes were tilted by 90^0 from the horizontal with 200 ml of the total system volume and an illuminated area of 145 cm^2. Diameter of each tube was equal to 2.5 cm.

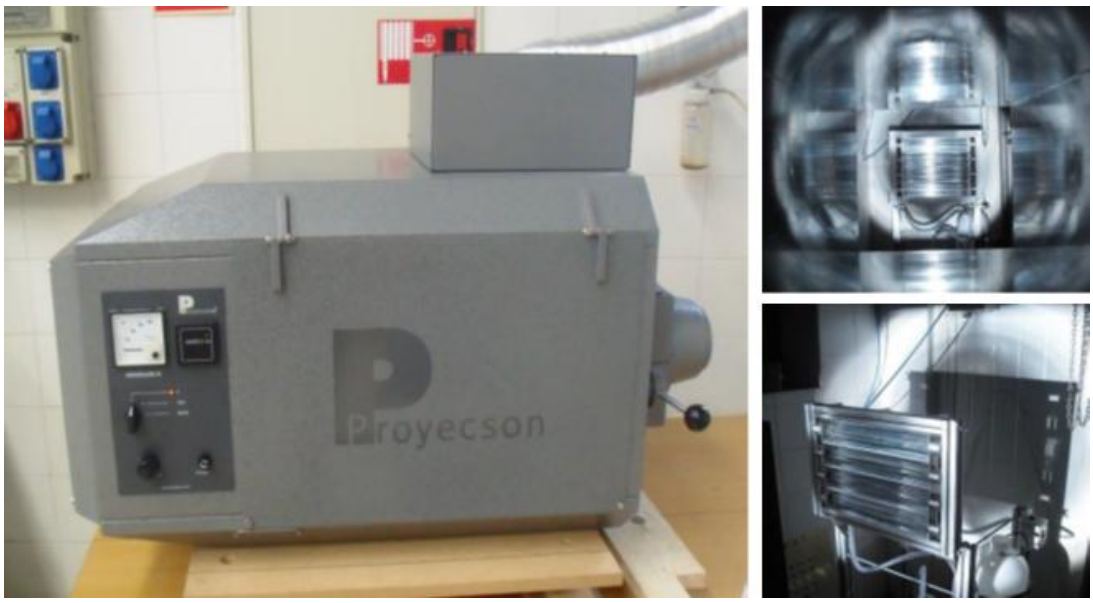

Figure 2. Experimental reactor system (1-Proyecson cinema projector; 2-Xenon lamp; 3- tube collector)
© Javier Marugán

The illumination was provided by XBO Xenon Short Arc Lamp (Fig. 2), installed inside the cinema projector. The lamp spectrum comprised all solar spectrum (infrared, UV light and visible light). However, the samples were taken at UV-A radiation, since the TiO_2 photocatalyst is activated only with this type of UV light. The wavelengths covered a range from 300 to 400 nm, depending on the parameters of reaction. Due to heterogeneity of the radiation, two units of the system were equidistant from each other 120 m apart to ensure effective exposure of the reactor to irradiation. To have relatively the same rate of radiation, only one tube was used to run the experiments (Fig.3).

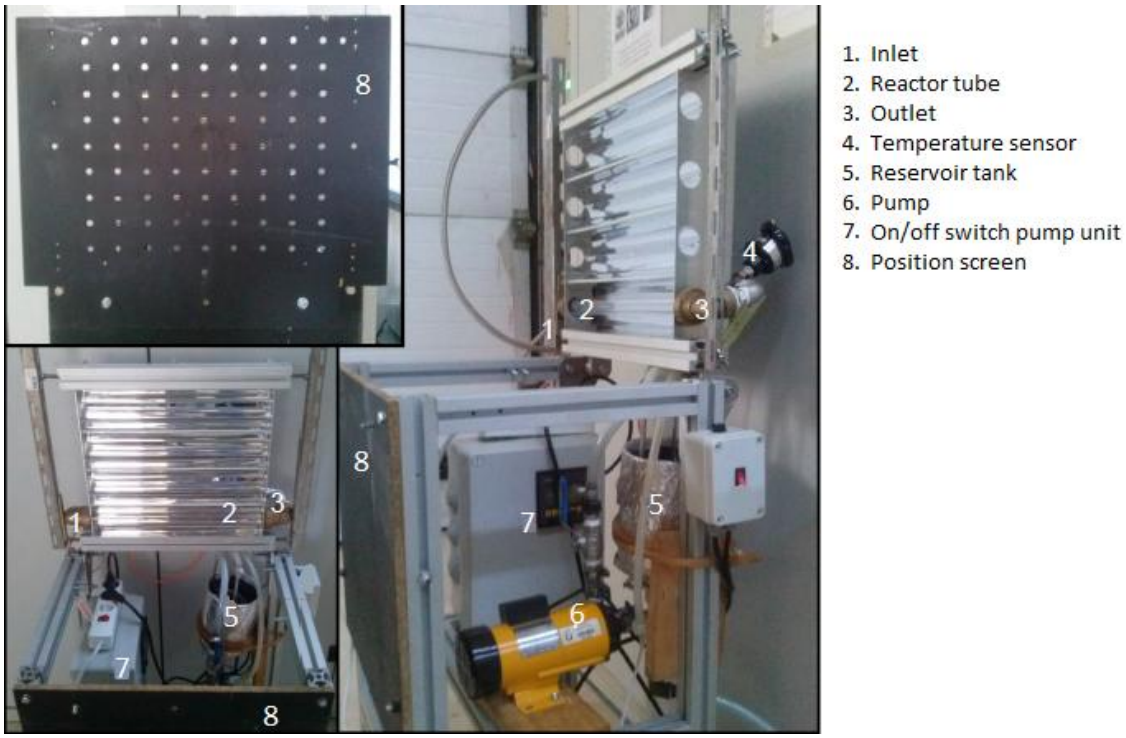

Figure 3. Large scale CPC-based solar simulator © Javier Marugán

This annular photoreactor was operating in recirculation mode with a stirred reservoir tank (shown in Fig.3), where the wastewater effluent was added. The work of the evacuated tube collector was controlled by a small data log unit attached at the bottom of the construction. This unit was equipped with two switches that allowed pump up the effluent with bacteria from the tank inside the tube. Once, the tube was filled with solution, the actual experiments commenced.

4.2. Experimental conditions

The main variables measured throughout the research were the bacterial colony-forming units (CFUs) and the level of organic matter degradation from the treated biological effluent. These variables were measured regarding two variables: (1) treated volume of effluent and (2) light flux (irradiation power). The concentration of TiO_2 used in all the reactions was the same.

Three different volumes, that were tested separately, were 1, 1.5 and 2L of secondary biologically treated effluent. Each working volume was mixed with corresponding amount of TiO_2 catalyst to enhance the action of light. The catalyst, Degussa P25 TiO_2, was used in suspension under corresponding concentration of 0.1, 0.15 and 0.2 g/L. Samples were

then exposed to the UV-A light at three radiation flux of 20, 40 and 60 W/m^2.

Prior to the analysis of photocatalytic results, two control experiments, such as TiO_2 dark absorption (without irradiation) and UV direct photolysis (without TiO_2) were carried out to determine the contribution of each factor to the efficiency of the inactivation process.

4.3. Process

To stabilize the behavior of bacteria, three manipulations were applied at the beginning of each reaction:

1. The biological effluent was mixed up with TiO_2 on the stirrer preliminarily to pouring the mixture to the reservoir tank.

2. The effluent samples and the catalyst were added to the reservoir tank and pumped into the tube for 5 minutes for the total dispersion of TiO_2 all over the tube to reach equilibrium of the system.

3. The reactor lamp was switched on outside the reactor for 15-20 min to stabilize its emission power before the actual reaction.

Once these three manipulations were completed, the photocatalytic wastewater treatment commenced. Each of the experiment was carried out by undertaking the following steps:

- The wastewater was exposed to three different light fluxes of 20, 40 and 60 W/m^2. During each sampling time, water samples were collected from the tank at different irradiation times, and placed to the Eppendorf tube for the further processing.

- Further on the evolution of the reaction was examined by quantifying the concentration of visible bacteria after the treatment. It was performed by the serial dilution method using the TYE agar plates as the media for growing the bacteria.

- Along with the bacterial samples, concentration of TOC was also measured four times during each reaction to examine the degradation of organic matter. To get rid of TiO_2 from the TOC measurements, each sample was passed through a filter of 0.25 μm and, then placed to another glass vial to be stored in refrigerator until the test realization.

- To identify the number of CFUs, eight replicates of each dilution were then incubated at 37ºC for 24 h before the counting. Concentration of total coliforms was calculated the following day from the dishes to determine the inactivation rate of bacteria.

- Reactions at radiation flux of 60 W/m^2 were run at longer irradiation times to see a clear bacterial degradation.

- Some experiments were repeated to test the consistency of the disinfection results.

Due to initial concentration instability, the wastewater effluent with same characteristics was used in all the experiments to be able to compare the results from different reactions.

4.4. Test preparation and procedures

4.4.1. Wastewater effluent

In order to successfully evaluate the effectiveness of solar photocatalytic water treatment, the water used in the reaction process must be wastewater to provide an excellent environment for bacterial growth. The wastewater effluent, used for the reactions in this study, was taken from the miniature biological reactor system that was built in URJC laboratory (Fig. 4).

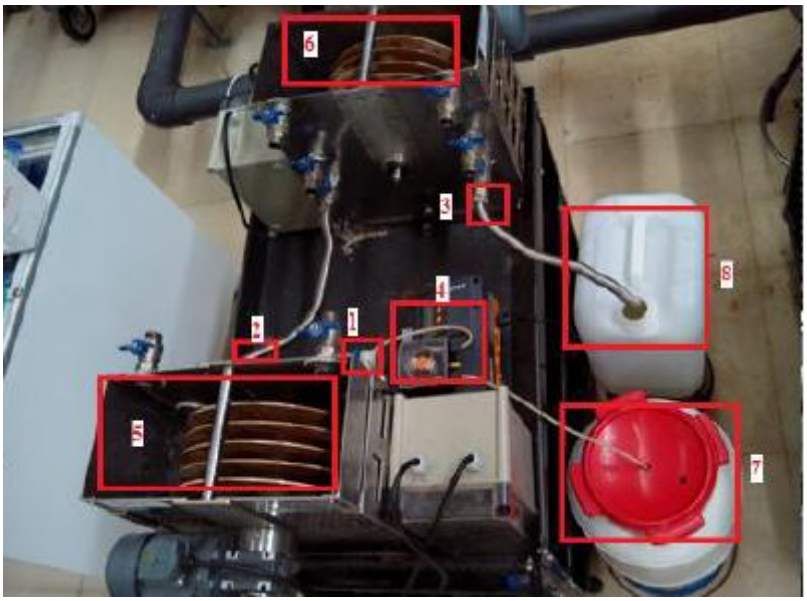

Figure 4. Top view of the biological rotating system : (1) corresponds to the nutrition entry in the first basin; (2) corresponds to the intermediate stream; (3) corresponds to the output current from the second basin; (4) corresponds to the peristaltic pump; (5) corresponds to the first biological reactor (RBC 1); (6) corresponds to the second biological reactor (RBC 2); (7) corresponds to the food grade plastic drum; (8) corresponds to the container with the final effluent. © María Baquero

This system was constructed for the researching purposes on biological elimination of pharmaceutical residues in water. The composition of compounds that were used to stimulate the wastewater for the biological treatment is listed in the *table 2*. Due to the different objectives of the current research and the lack of time, the characteristics of bacteria and exact nature of organic substances treated with the photocatalytic process, has not been fully obtained. The most important thing was to observe the effectiveness of studied process for the elimination and degradation of bacteria and organic matter.

Table 2. Composition of stimulated wastewater for the biological treatment

Compounds	Concentration (g/L)
Sucrose	0.418
Glucose	0.418
Sodium acetate	0.512
Monopotassium phosphate	0.057
Calcium chloride	0.081
Urea	0.018
Ammonium chloride	0.149
Sodium bicarbonate	0.138
Yeast Extract	0.003
Sodium chloride	0.163
Magnesium chloride hexahydrate	0.195
Ferric sulfate (II)	0.007
Manganese(II) sulfate hydrate	0.004
Zinc sulfate heptahydrate	0.007
Copper sulfate (II) pentahydrate	0.003
Boric acid	0.00003

Prior to every reaction, this biological effluent was taken from containers with capacity of 25L, where it was stored after preliminary biological treatment, and then charged into beakers of three examined volumes: 1, 1.5 and 2L. They were then left overnight to allow floating particles to settle down, since the water composition and its state can have a significant impact on the removal efficiency of organic materials under the photocatalytic process. The following day the wastewater concentrate was added to the reservoir tank prior to testing and mixed with certain amount of TiO_2.

4.4.1. Agar dish preparation

For the preparation of agar dishes, TYE nutrient agar (Tryptone yeast extract agar, Scharlau) was used, serving as a solid nutrient media for growing total coliform bacteria. This cultivation process was performed to be able to follow the total inactivation and simplify the counting of bacterial colonies.

One bottle of 2L containing 80 g of agar was prepared once a week to provide a necessary amount of agar plates to run the experiments. The bottle with solid media was steamed in an autoclave at 120 °C for 180 min to melt the agar. Melted agar was then left to solidify and dry at room temperature prior to filling the Petri dishes with 16 ml of medium culture.

4.4.2. Serial dilution method

To be able to determine bacterial concentration of the samples and count colony-forming units (CFUs), such method as serial dilution was performed. Once the effluent samples were collected in the Eppendorf tubes, they were serially diluted through the following scheme: d^0 (1/1), d^1 (1/10), d^2 (1/100) and d^3 (1/1000). The original sample solution (d^0) was diluted to three times to be able to count total coliform bacteria in 10^x CFUs, having 10^6 CFU initial bacterial concentration. *Figure 5* illustrates schematic representation of the serial dilution method.

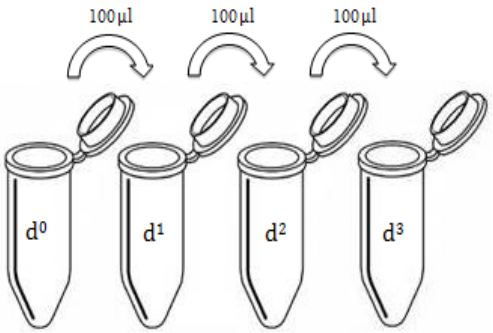

Figure 5. Serial dilution process

First dilution was done by adding 100 µL of original sample to the second tube refilled with 900µL of Milli-Q water (ultrapure water). Therefore, this sample was diluted to a maximum concentration of 10^5 CFU / ml (identifying that tube as d^1). Gained bacterial suspension was then mixed thoroughly, using the vortexer, to evenly distribute the bacteria in the water sample. This procedure was repeated until reaching the concentration of 10^3 CFU / ml (d^3).

4.4.3. Spread plating of serially diluted cells

After completing appropriate dilutions, spread plating of diluted cells commenced. Each diluted sample was stirred, and placed onto a plate, containing agar medium with the use of electronic micropipette. This pipette was taking 80 µL of diluted sample at once, applying 8 droplets in amount of 10 µL at every agar plate. The plates were air dried and then

incubated for 24 h at 37 ºC until colonies were visible. Prepared plates were placed in the oven upside down to prevent condensation from dripping down onto the agar surface and interfering with developing microbes. After 24 h of incubation in the oven, colonies were most readily counted using a magnifying glass and electronic plate counter (Fig.6).

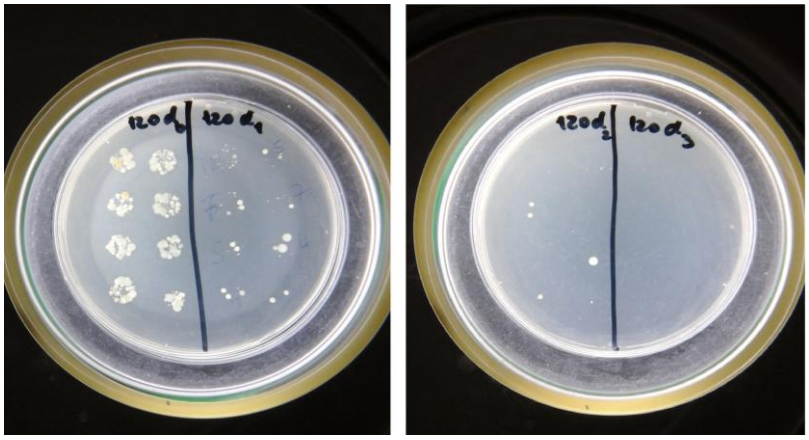

Figure 6. Colony counting of the total coliform bacteria. Counting concentration d^0, d^1, d^2, d^3 after 120 min of UV-A exposure.

4.4.4. Pre-test sterilization

All the material used throughout the experiments, as well as Milli-Q dilution water, was sterilized at 120°C for 180 min by means of sterilizing machine, called autoclave. This material was then placed in a sterile environment until the further testing phase. All the bacterial dilutions and other related tests were carried out under a laminar airflow hood using the taken samples and Eppendorf tubes. Performance of these methods was essential to prevent any outside contamination that would be visible on the Agar plates after incubation.

5. RESULTS

5.1. Control tests

As it was methioned in chapter 4.2., two control tests were performed prior to the study of the photocatalytic process. They were carried out in the stirred photoreactor to evaluate the contribution of UV radiation and catalyst absorption mechanisms to the reduction in colony-forming units of the total coliform bacteria. *Figure 7* shows the degree of bacterial removal from wastewater by means of photolysis phenomenon in the photoreactor. Along

with photolytic effect, the same *Figure 7* exhibits the adsorption level of the emergent contaminant molecules onto the TiO2 surface.

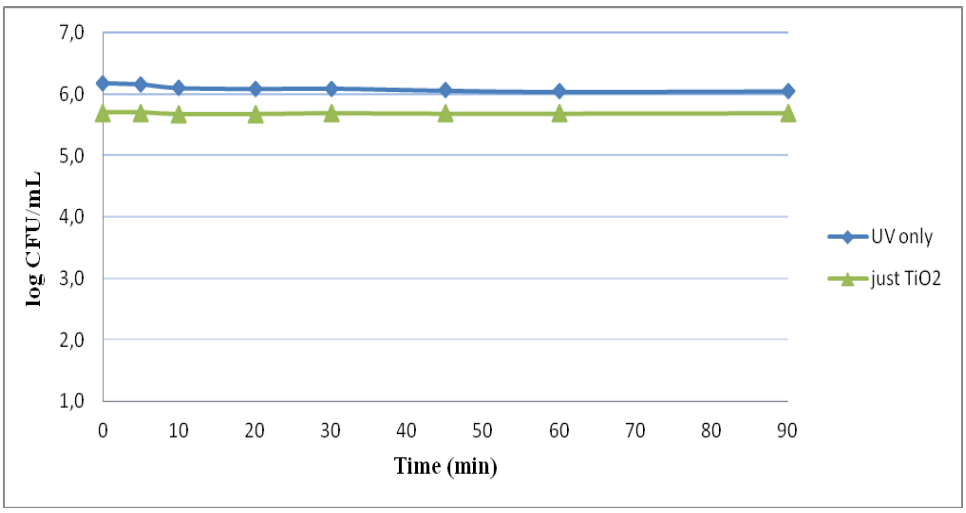

Figure 7. Behavior of total coliform density under the UV direct photolysis and TiO_2 dark absorption control tests.

Considering plotted results, direct photolytic process as well as TiO_2 dark absorption had relatively low effect on inactivation of the total coliform in wastewater, demonstrating the best bacterial removal rate during photocatalysis tests after 60 and 90 min, respectively. Bacteria were not inactivated in the absence of TiO_2 as well as without the light.

5.2. Inactivation by photocatalysis

5.2.1. Effect of TiO_2 loading and initial total coliform density

As it was expected before the performance of all the experiments, the reactions with lower total volume of 1L demonstrated the higher bacterial inactivation rate with all three light sources (20, 40 and 60 W/m^2). Following the processes outlined in chapter 4.3., certain amount of TiO_2 corresponding to the volume of the reaction was added as the slurry in the treated effluent and exposed to continuous illumination. *Figure 8* shows the degree of bacterial removal from the wastewater by means of UV-A light and suspended TiO_2.

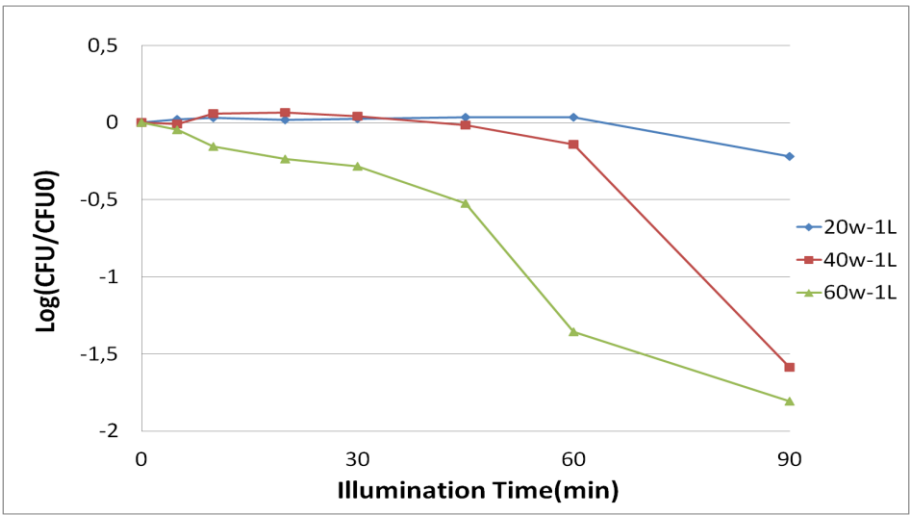

Figure 8. Inactivation of total coliform bacteria at three various light fluxes in the effluent of 1L.

As observed in *Figure 8*, the degradation rate during the photocatalytic treatment at light flux of 20 W/m^2 remained relatively unchanged and did not show any significant reduction in the number of CFUs within tested illumination time. In contrast, starting with the lowest volume concentration and the same TiO_2 catalyst concentration, photocatalysis at 40 and 60 W/m^2 managed to lower the bacterial counts (CFUs/ml) by approximately 1.5 and 2 orders of magnitude, respectively, by 90 min, as presented above.

In case of effluent treatment of 1.5 L, the degree of bacterial degradation notably decreased since the increase of total volume, as well as organic and inorganic impurities presence in it. Analyzing all the reactions at different light sources, photocatalysis at 40 and 60 W/m^2 again had better performance of bactericidal activity of total coliforms, in comparison to the relatively low effect for the coliforms removal during reaction with irradiation power of 20 W/m^2 (Fig. 9).

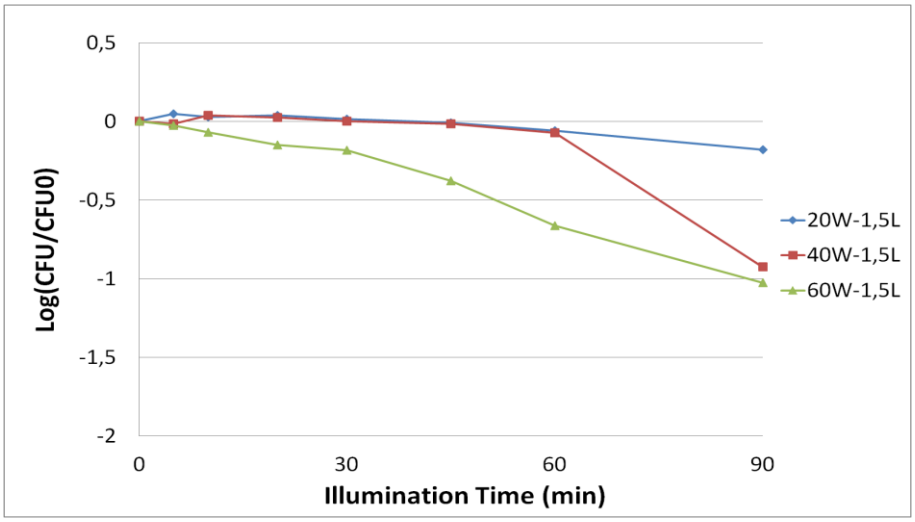

Figure 9. Inactivation of total coliform bacteria at three various light fluxes in the effluent of 1.5 L.

As for the photocatalytic activity in the biggest treated volume of 2L (Fig. 10), the degradation rate was observed only under 60 W/m^2 input solar radiation, whereas at 20 and 40 W/m^2 it stayed almost identical to the initial concentration of bacteria.

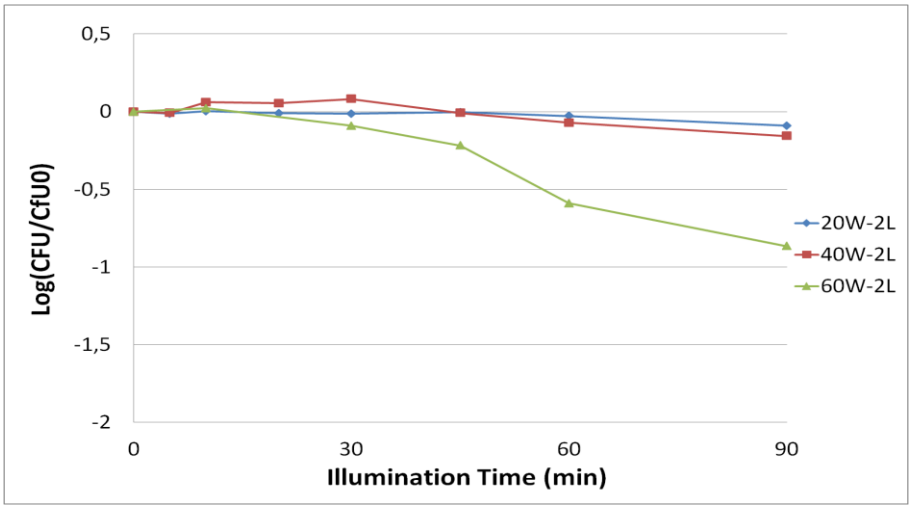

Figure 10. Inactivation of total coliform bacteria at three various light fluxes in the effluent of 2L.

The overall set of experiments conducted in this research is plotted in *Figure 11*.

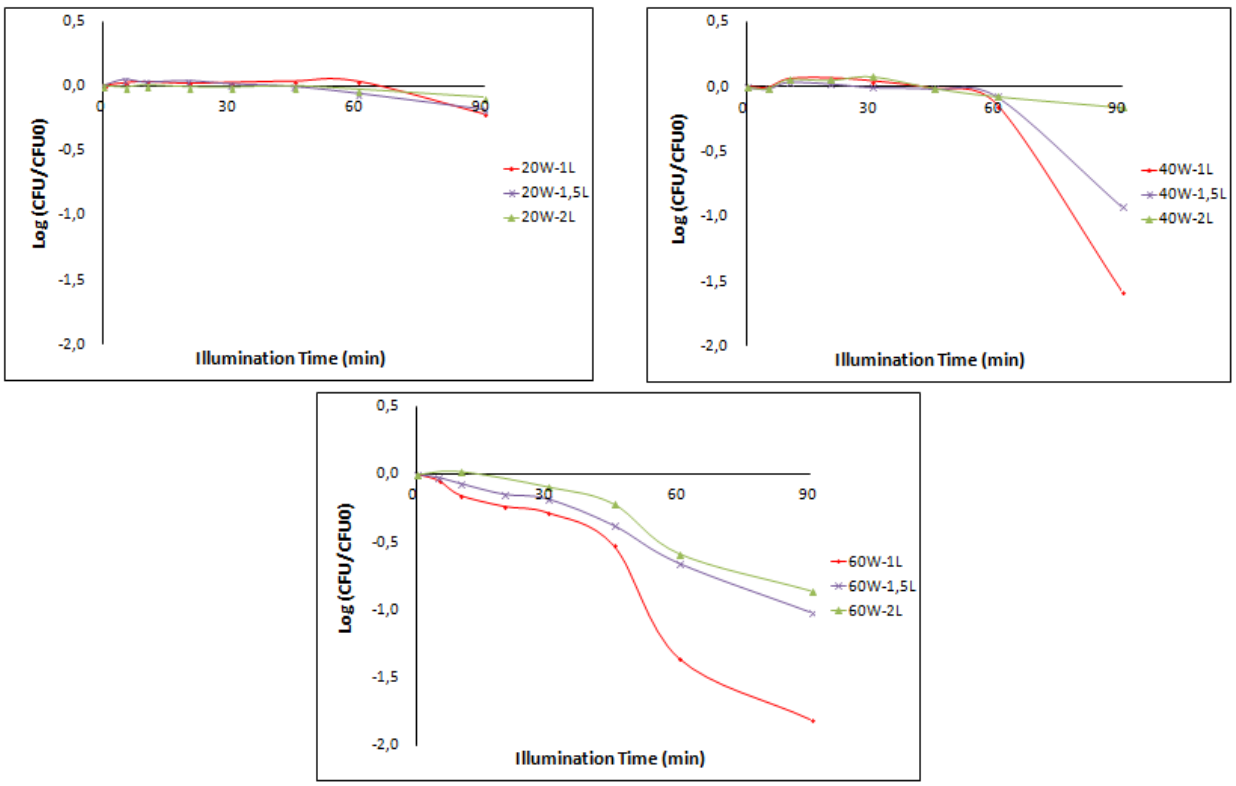

Figure 11. Behavior of total coliform density under three experimental light fluxes of 20, 40 and 60 W/m^2.

When comparing figures 8, 9, 10 and 11, it becomes clear that the reactions conducted with the irradiation power of 60 W/m^2 had the biggest rate of bacterial inactivation at three treated volumes. To see the bigger inactivation rate of bacteria under this irradiation power, each reaction was repeated several times for a longer period of 180 min (Fig.12).

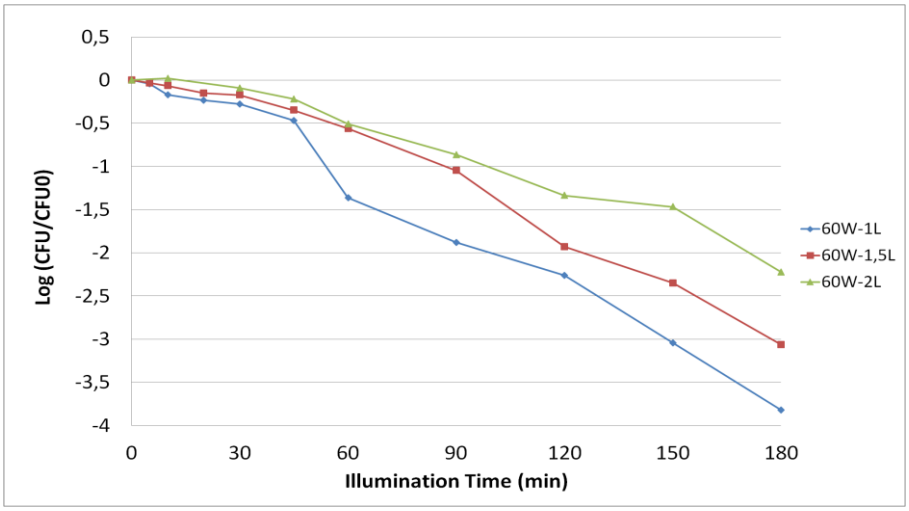

Figure 12. Inactivation of total coliform bacteria at light flux of 60 W/m^2 at three different volume of effluent.

The results indicated that total coliform bacteria were almost degraded in ca. 180 min in almost all cases at light flux of 60 W/m^2, but total coliforms never reached the total inactivation of 3 hours of treatment. At the end of reactions, the residual cell counts for the bacteria were detectable, but in very little amounts, being eliminated by almost 4 orders of magnitude in case of 1L, and by 3 and 2 cell numbers in 1.5 and 2L, respectively.

5.2.2. Effect of TiO_2 loading and organic matter degradation

TOC measurements showed that the total organic content did not decrease in the same extent as the bacterial colonies throughout the entire set of experiments, demonstrating the values with a great level of variability. For example, the initial values obtained under all irradiation powers were characterized as low at the beginning of each experiment. However, throughout the photocatalytic disinfection they increased by 30 min of irradiation and after 60 min of treatment started to degrade.

6. DISCUSSION

6.1. Control tests

Control experiments (Fig.7) demonstrated that behavior of total coliform density under the UV direct photolysis and TiO_2 dark absorption tests did not differ. This could have occurred due to the high turbidity rate and high concentration of organic and inorganic impurities in the effluent, as well as the high initial concentration of bacteria. These factors might have affected the performance of the two control tests and lowered the inactivation rate of the bacteria. Suspended impurities can absorb the UV radiation, therefore decreasing the effectiveness of UV wastewater disinfection. These particles can also form colloids that can serve as the shelter for various microorganisms from the irradiation and disinfectants (Burch et al., 1998). Perhaps, a low level of turbidity could have been reached through filtration, and then the degradation process would be more visible in this analysis.

In contrast to the present results, numerous photocatalytic studies demonstrated that UV solar simulators have been proved working and having destructive effect on microorganisms, specifically bacteria, both in the presence and absence of a catalyst due to synergistic effects of the UV radiation (Burch et al., 1998; Rincón, 2003). This could depend on the type of bacteria and their resistance to the radiation. This was consistent with the results in this thesis, for example, in figures 8, 9 and 10, where the stronger radiation levels reduced significantly the concentration of bacteria.

6.2. The effect of initial bacterial concentration

The photocatalytic method tested in this study was proved to be effective in reducing the total coliform bacteria. Nevertheless, high variations of initial chemical and bacterial concentrations present in wastewater effluent could have driven the disinfection process and, thus played an important role in the performance of the method (Rengifo-Herrera et al., 2010). At the beginning of every photocatalytic experiment, different water composition could have affected the efficiency of the water treatment. In this sense, the variation observed in organic composition was more likely to be caused by instability in the range of parameters in micro-experimental WWTP project as well as by a variable nature of wastewater composition itself (USEPA, 1990; Darby et al., 1993; NYSERDA, 2004; Rengifo-Herrera et al., 2010). The main variables that could have had a significant impact on the composition of treated water are pH, temperature, flow rate and reactor's

intrinsic noise (Thampi, 1990; USEPA, 1990; NYSERDA, 2004). These factors might have contributed a lot to the presence of bacterial feeding rates leading to unstable bacterial composition in the effluent. Therefore, any changes of the biological variables could have affected the results of current photocatalytic experiment, making its interpretation complex. However, after application of several manipulations to stabilize the behavior of bacteria, outlined in the chapter 4.3., relatively even distribution of bacteria was achieved, simplifying the comparison of the results.

6.3. Inactivation process of bacteria and organic matter degradation

As initially expected, the highest bacterial inactivation rates were observed in the reactions with irradiation power of 60 W/m^2. This is attributed to velocity increase of redox (oxidation-reduction) reactions occurring at the surface of the catalyst under higher UV photon flux. Along with the type of irradiation power, the treated volume of wastewater also had a significant impact on the performance of the photocatalytic process. The oxidation of total coliforms under the light flux of 60 W/m^2 in a volume of 1L (Fig.8) was proved to be the most effective photocatalytic disinfection wastewater method in this research. Even though the total inactivation of coliform bacteria was not achieved after 180 min of treatment, the present results indicated the feasibility of using photocatalysis as post-biological treatment to eliminate bacteria in the water (Fig.12).

Considering the results obtained at irradiation power of 20 and 40 W/m^2, elimination efficiency also depended on the treated volume. Although a higher degradation could be achieved in longer irradiation times under these two light fluxes, results demonstrated that the treatment could be too time-consuming to be economically convenient at a bigger scale.

Concerning the organic matter degradation, no firm conclusions could be done. Statistically there are variations that cannot be easily controlled and that might affect the results obtained in the experiments. One of the assumptions was that an initial increase in UV illumination between 30-60 min would decrease the degradation rate of TOC, nevertheless this was not observed, and instead the increase in TOC occurred. This suggested a regrowth of bacterial population after the photocatalytic treatment, supported by similar results obtained from numerous studies (Parra et al., 2002; Rengifo-Herrera et al., 2010). These studies showed the recovery of total coliforms after 24 h photocatalytic disinfection, however, the rate of that bacterial regrowth depended on the type of bacteria

presented in the wastewater samples. Consequently, the effectiveness of organic matter degradation should be evaluated taking into consideration the total time from water treatment as well as bacterial characteristics.

6.4. Critical assessment

Considering all the factors that might have had direct effects on the final results of the photocatalytic wastewater treatment, two main lessons can be learnt:

Firstly, the behavior of the biologically treated effluent should be thoroughly examined prior to experiments. The chemical composition of the wastewater had a great impact on the time required for the degradation, so the conduction of additional experiments is essential to investigate the effluent characteristics.

Secondly, to be able to analyze the behavior of total coliform bacteria under present photocatalytic disinfection process, it would be necessary to examine the bacterial composition of the effluent as well. This would help to understand in more detail the bacterial activity for the two tested variables which were treated volume of secondary effluent and light flux.

Although the total inactivation of bacteria and contaminants was not achieved by the end of tested irradiation time, this research demonstrated positive results that pointed out a potential application of this photocatalytic technique for effective wastewater disinfection. This is supported with the results of additional experiments conducted at the radiation level of 60 W/m^2 within 180 min interval. With the data obtained in these experiments, it can be estimated that effluent should be exposed to more prolong photocatalytic treatment to reach the desirable results due to its abundant organic content.

In conclusion, this thesis research confirms the utility of photocatalysis as an efficient method to disinfect wastewater. It provides the data and observations that can be useful for the further investigations. For municipal level, this research could be useful in terms of providing the ground to achieve a large scale plan of wastewater disinfection, but also should be important to adapt this methodology in rural areas. This method will significantly reduce the cost for wastewater treatment, guarantee ease implementation process and ensure sufficient disinfection of water supply in developing areas.

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