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Retention and Inactivation of Quality Indicator Bacteria Using a Photocatalytic Membrane Reactor

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Abstract: The development of effective disinfection treatment processes is crucial to help the water industry cope with the inevitable challenges resulting from the increase in human population and climate change. Climate change leads to heavy rainfall, flooding and hot weather events that are associated with waterborne diseases. Developing effective treatment technologies will improve our resilience to cope with these events and our capacity to safeguard public health. A submerged hybrid reactor was used to test the efficiency of membrane filtration, direct photolysis (using ultraviolet-C low-pressure mercury lamps, as well as ultraviolet-C and ultraviolet-A light-emitting diodes panels) and the combination of both treatment processes (membrane filtration and photolysis) to retain and inactivate water quality indicator bacteria. The developed photocatalytic membranes effectively retained the target microorganisms that were then successfully inactivated by photolysis and advanced oxidation processes. The new hybrid reactor could be a promising approach to treat drinking water, recreational water and wastewater produced by different industries in small-scale systems. Furthermore, the results obtained with membranes coated with titanium dioxide and copper combined with ultraviolet-A light sources show that the process may be a promising approach to guarantee water disinfection using natural sunlight.

Keywords: surface water; water quality indicators; disinfection; photolysis; photocatalytic membrane reactor

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1. Introduction

Water quality and safety, sanitation and hygiene are a basic human right crucial for human development and health. Unsafe drinking water is responsible for several illnesses. Every year, approximately 829,000 people are estimated to die from diarrhea because of unsafe drinking water, sanitation, and lack of hand hygiene. According to the Centers for Disease Control and Prevention, waterborne diseases affect over 7 million people in the United States every year with healthcare costs over 3.33 billion USD [1–3].

To protect consumers from waterborne diseases, drinking-water utilities must ensure that the distributed water is free from pathogenic organisms. Ultraviolet (UV) radiation is a reliable and economically viable technology for wastewater and drinking-water treatment [4,5]. UV treatment is effective against a wide range of waterborne pathogens such as bacteria, viruses, protozoan oocysts [4,6–8] and filamentous fungi [9–12]. UV light is absorbed by the DNA and RNA, causing several types of damage that can interfere with transcription and replication. UV can also damage the functional structure of proteins and affect the cell wall and the membrane structure of the microorganisms [11–15].

Low-pressure (LP) mercury vapor UV lamps that emit monochromatic light at 254 nm are widely used for wastewater and drinking-water disinfection [1].

In recent years, UV-light-emitting diodes (UV-LEDs) have been proposed as an alternative to LP mercury UV lamps for water disinfection. UV-LEDs do not contain mercury, guarantee a consistent intensity over a broad temperature range, are shock-resistant, use electricity more efficiently, produce little heat and have a longer lifetime [4,16]. Moreover, small-size UV-LED modules can be easily incorporated into various shapes of processing devices for the water industry [11,14].

Some studies reported the use of different LED wavelengths for the inactivation of microorganisms in water [4,8,9,11,17–19]. The choice of wavelength differs according to the purpose. Wavelengths in the UV-C range such as 254–255 nm, 265–269 nm and around 280 nm have been chosen to be compared with the conventional LP mercury lamps, due to the maximum absorption peak of DNA, and the maximum absorption peak of proteins, respectively. Most assays using UV LEDs were conducted using bacteria and bacteriophages spiked on laboratory-grade water or phosphate-buffered saline solution. Further work is needed to understand the scavenging effects of real water matrices.

Membrane filtration may ensure an effective rejection of a wide range of pollutants though size exclusion, adsorption and electrostatic repulsion. Any membrane filters with a molecular weight cut-off of microfiltration membranes (or lower) are expected to retain bacteria by size exclusion. Bacteria have negatively charged surfaces so they can also be rejected by negatively charged membrane surfaces [20]. Changes in pH may alter bacteria agglomeration and adsorption to the membrane materials [21].

When membrane filtration is coupled with photolysis and photocatalysis, the membrane retains the pollutants that can be then degraded or inactivated by direct photolysis and photocatalysis. Besides generating a high-quality permeate, these reactors also treat the concentrated retentate produced and degrade the pollutants retained by the membrane, which decreases fouling, therefore mitigating the two drawbacks associated with membrane-filtration processes.

Photocatalysis generates strong oxidizing radicals that degrade the pollutants. The photocatalyst may be dispersed in solution, which requires an additional separation step, or immobilized in the membrane. Immobilization of photocatalysts in membranes will reduce footprint, membrane fouling and photocatalyst loss [22]. The development of effective photocatalytic membranes can lead to advantages in terms of productivity and sustainability compared to traditional heterogeneous photocatalysts [23].

Photocatalytic membrane reactors have been described as emerging green technologies for removal of organic pollutants, photoreduction of heavy metals, photoinactivation of bacteria and resource recovery, with great potential to become "zero"-waste processes in the water and wastewater industries [22,24,25]. Chen et al. [22] describe these systems as extremely promising for the treatment and reuse of produced water due to their low cost, high efficiency, low energy consumption (using light irradiation) and small footprint.

However, further research is needed in terms of the development of photocatalytic membrane reactors and membranes with low costs that are stable in a wide range of operative conditions, resistant to fouling and show high and reproducible long-term performance [23].

Since the membranes chosen need to withstand UV light and the reactive oxygen species produced by the photocatalytic membranes, ceramic membranes should be used in photocatalytic membrane reactors. Ceramic membranes have several advantages compared to conventional polymeric membranes. They offer mechanical strength, resistance to harsh chemical conditions and can tolerate temperatures of up to 500 °C [26]. These advantages enable their specialized use in extreme operating conditions. For example, they allow for aggressive physical and chemical cleaning of the modules, which guarantees the removal of irreversible fouling, without the risk of damaging membrane integrity. Moreover, ceramic membranes exhibit outstanding corrosion resistance as well as inertness to microorganisms and organic media. The high reliability of ceramic membranes decreases the cleaning

requirements, reduces the replacement of membrane modules, and thus extends their operational life expectancy. However, ceramic membranes are often more prone to breakage than polymeric membranes; thus, they need to be handled carefully [27–30]. Due to their robustness and high water permeability, the use of silicon carbide membranes has expanded over the last decades in an increasing number of industrial sectors, including oil and gas, water purification, wastewater treatment and the processing of food and beverages [31].

The combination of light-emitting diodes that emit at different wavelengths, with unmodified and modified ceramic silicon carbide membranes was tested at laboratory scale (using small circular membrane coupons and three single LEDs) and found to be extremely promising for disinfection purposes, since it ensures not only the production of a high-quality permeate but also an effective inactivation of the microorganisms retained by the membrane [32]. Since the small unmodified and modified ceramic silicon carbide membrane coupons were found to be extremely promising, commercial high-flux submerged flat sheet silicon carbide ceramic membranes that filter from outside to inside were acquired and tested in this study to evaluate the possibility of their use in scale-up studies. Photocatalytic membranes were modified by dip coating with silicon dioxide (SiO_2) and titanium dioxide (TiO_2) degussa nanoparticles using a solvent-free modification procedure [29,32]. To increase the optical electronic properties of TiO_2 and extend its light-absorbing capacity to the visible range, in this study, flat sheet silicon carbide ceramic membranes that filter from the outside to the inside were also coated using TiO_2 mixed with copper, a nonprecious Earth-abundant metal. The combination of copper with TiO_2 has been described to improve the visible-light photocatalytic efficiency of other support materials [30,33].

This study aims to evaluate the implementation of the combined treatment using larger membranes and test a novel modification procedure combining titanium dioxide and copper in a hybrid reactor that can be easily scaled up [34] and upgraded with novel custom-built LED panels that emit at 265 nm (UV-C) and 385 nm (UV-A).

The treatment processes were tested in terms of their ability to retain and inactivate water quality indicator bacteria (total coliforms, *E. coli* and enterococci) present, at occurrence levels, in surface water collected at the Tagus River (Algés, Portugal).

2. Results and Discussion

2.1. Characterization of the Photocatalytic Membranes Modified with TiO_2 and Copper

In this work, only the silicon carbide membranes modified with TiO_2 and copper MMCu) were characterized, since the characterization of the microfiltration membrane modified with TiO_2 and SiO_2 (MMSi) was previously described [32,34].

The surface morphology of the membranes (unmodified and modified membranes) was analysed by scanning electron microscopy (SEM) (Figure 1).

The Image J software was used to estimate the porous features of the membranes using the SEM images acquired of the top surface in two different membrane zones (Z1 and Z2; Table 1).

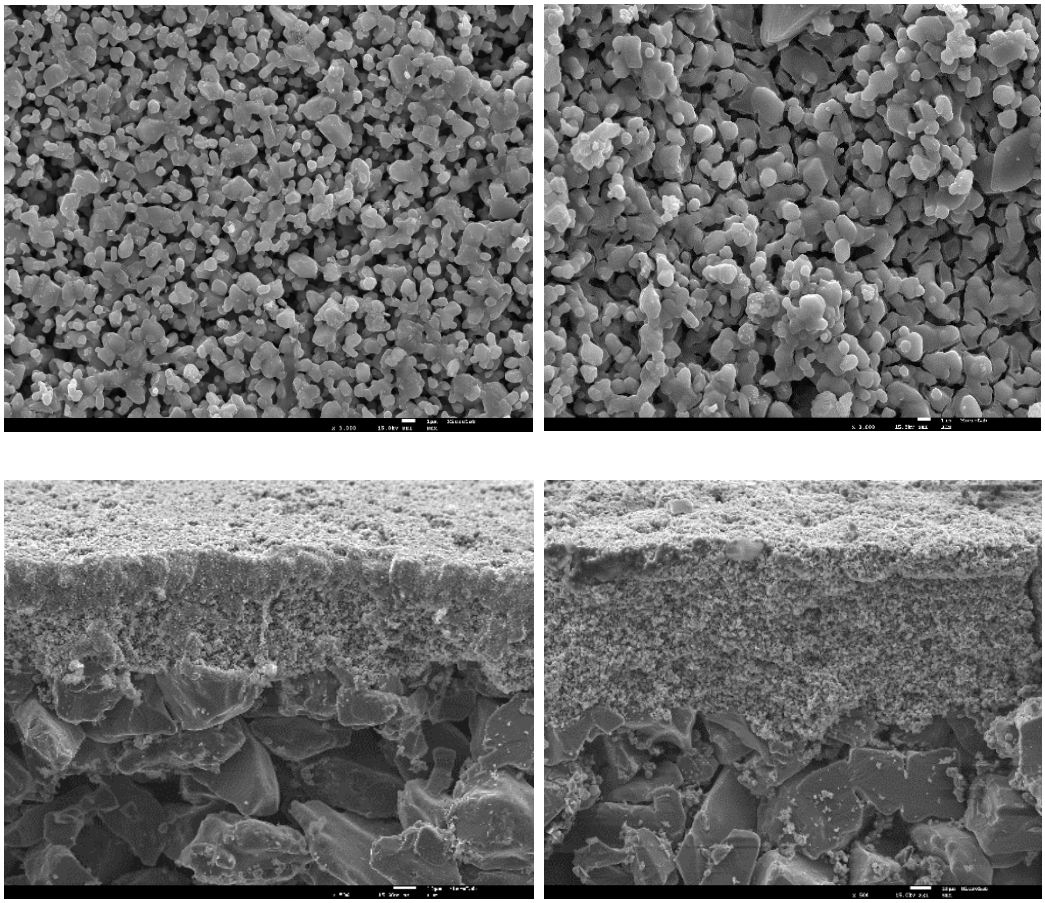


Figure 1. Scanning electron microscopy for the unmodified (UM) and the modified membrane (MMCu): top surface (**up**) and cross section (**down**) analysed at ×3000 and ×500 magnification, respectively.

Table 1. Porous properties estimated in two zones (Z1 and Z2), for the membranes described in this work (magnification of ×3000).

	UM		**MMCu**	
	Z1 (×3000)	**Z2 (×3000)**	**Z1 (×3000)**	**Z2 (×3000)**
Porosity (%)	6.2	6.6	6.5	6.3
Pore density (μm^{-2})	2.14	3.10	1.89	2.06
Mean Pore Area (μm^2)	0.029 ± 0.122	0.021 ± 0.097	0.034 ± 0.158	0.031 ± 0.147
Minimum Pore Area (μm^2)	0.0003	0.0002	0.0003	0.0003
Maximum Pore Area (μm^2)	1.9160	2.2040	2.8450	2.5390
Average circularity	0.836 ± 0.267	0.830 ± 0.267	0.823 ± 0.273	0.807 ± 0.278
Average Feret diameter (μm)	0.15 ± 0.34	0.13 ± 0.29	0.17 ± 0.40	0.16 ± 0.39
Maximum Feret diameter (μm)	3.381	3.256	3.819	5.464
Minimum Feret diameter (μm)	0.025	0.021	0.024	0.024

Figure 1 and Table 1 show that the top surface of the membranes is extremely similar with small deposits noticeable in the membranes modified with copper (Figure 1) that were not enough to considerably alter the porous morphology of the silicon carbide support used. Compared with these membranes, the previously characterized membranes modified with titanium dioxide and silicon dioxide presented a predicted lower mean pore area that

may be indicative of a lower-molecular-weight cut-off and might consequently lead to a higher pollutant rejection [34].

The image analysis estimations should be verified in future studies by physisorption analysis, since it only characterizes the top surface of the membrane based on a threshold that is defined by the analyst, not taking into account the tortuosity, interconnection and the nonuniform cross section of the pores.

It was impossible to measure a static contact angle value for the unmodified membranes (UM) and the membranes modified with titanium dioxide and copper (MMCu), since the water passed through the membrane very quickly. Previous studies conducted with the unmodified membranes and the membranes modified with titanium dioxide and with silicon dioxide showed the same behaviour [29,35]. After irradiation with the LED panel that emits light at 385 nm, the modified membranes with titanium dioxide and copper (characterized in this study) seem to exhibit a slight decrease in contact angle (Supplementary Material, Figure S1). The increase in hydrophilicity of SiO_2/TiO_2 nanocomposite films have been attributed to the light-induced hydroxyl groups formed on TiO_2 films [22].

2.2. Surface-Water Treatment Using a Submerged Hybrid Reactor

Membranes that filter from the outside to the inside were used in the hybrid reactor since they allow the retention of microorganisms in the outside of the membrane. The retained microorganisms will then be inactivated by direct photolysis or direct and indirect photolysis through the production of hydroxyl radicals when the modified membranes are used.

Eleven water treatment experiments (detailed in the Materials and Methods section) were performed during 1 h in a 10 L submerged hybrid reactor.

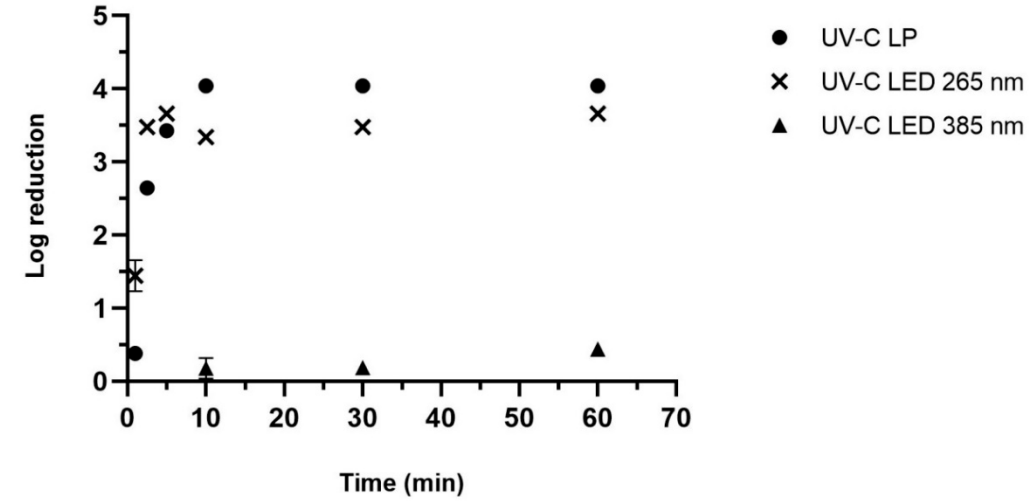
The presence of total coliforms, *Escherichia coli* and enterococci in drinking water indicates that there could be a problem with treatment systems, contamination of the source water or a breach in the distribution system.

The enumeration of total coliforms, *E. coli* and enterococci was performed to evaluate the efficiency of surface water treatment using filtration (unmodified membranes, modified membranes with TiO_2 and SiO_2 and modified membranes with TiO_2 and copper), direct photolysis (LP mercury lamps or UV LEDs panels that emit light at two wavelengths, 265 and 385 nm) and filtration combined with photolysis.

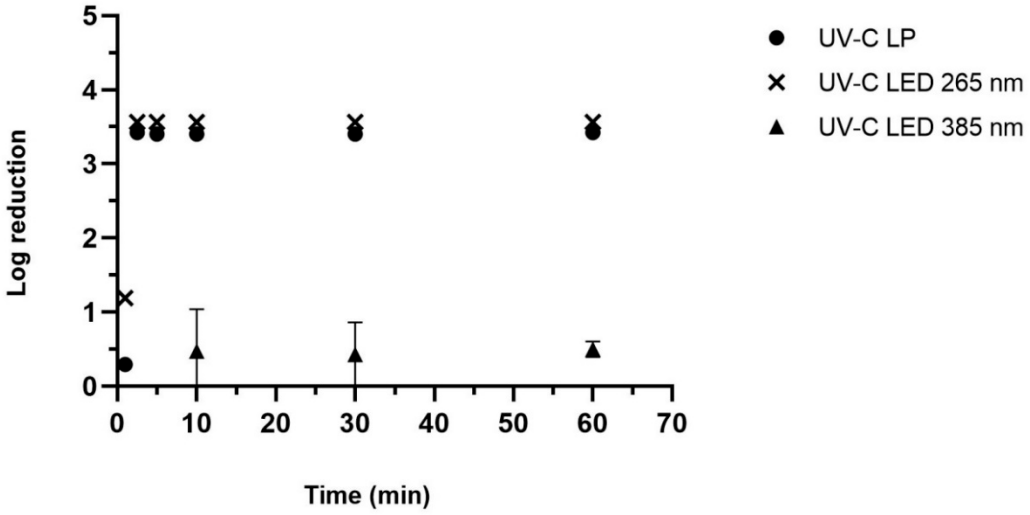
2.2.1. Direct Photolysis

The effect of direct photolysis was analyzed during the inactivation experiments using two LP UV-C mercury lamps (254 nm) or two UV-LED panels that emit light at two different wavelengths (265 nm and 385 nm). The log reductions of total coliforms and *E. coli* obtained on the inactivation experiments are shown in Figure 2. Log reduction of enterococci was only determined at the end of the experiments (after 60 min of treatment).

Figure 2 shows that the log reduction obtained for total coliforms and *E. coli* in surface water after different UV exposures times was higher using UV-C light sources than when the UV-A light source was used. These results were expected and agree with results obtained by Bernardo et al. [32], due to the low capacity of absorption of light by the DNA at this wavelength. In this study, after 60 min of treatment, extremely low log reductions of total coliforms (0.5) and *E. coli*. (0.4) were observed using the UV-A LED panels. On the other hand, after only 2.5 min of UV-C LP exposure, log reductions of 2.6 for total coliforms and 3.4 for *E. coli* were observed. Moreover, after 2.5 min of treatment, log reductions of 3.5 for total coliforms and 3.6 for *E. coli* were observed using the custom-built UV-C LED panels that emit light at 265 nm.



(**A**)



(**B**)

Figure 2. Log reduction of total coliforms (**A**) and *E. coli* (**B**) as a function of irradiation time on surface water. Error bars represent duplicate results.

For enterococci, after 60 min of UV-C LP, UV-C LED, and UV-A LED exposure, log reductions of 2.9, 2.7 and 0.2 were observed, respectively. Both the UV-C LP and UV-C LED 265 nm systems were very efficient on enterococci inactivation (higher than 99.8% inactivation). The UV-A LED 385 nm system presented a low inactivation efficiency (34.0%) for enterococci.

UV-C LP and UV-C LEDs 265 nm presented high inactivation percentages for total coliforms, *E. coli* and enterococci.

Previous studies reported results concordant with our work. Vilhunen et al. [36] showed that UV LEDs were efficient for *E. coli* inactivation. The effect of using different wavelengths (269 and 276 nm) was noticeable, but the test medium did not have much impact on inactivation. Chatterley and Linden [37] developed and evaluated a UV LED prototype as a proof-of concept of this technology for a point-of-use disinfection option. Oguma et al. [38] revealed that fluence-based inactivation efficiency was high for the 265 nm UV-LED. Li et al. [39] also demonstrated that 265 nm LEDs were more effective for *E. coli* disinfection than 280 nm LEDs and LP UV lamps.

2.2.2. Membrane Filtration

The results shown in Figure 3 clearly reveal an extremely high retention by filtration, regardless of the membrane used (>99.0%), of total coliforms and *E. coli* present on the river water. For enterococci, identical rejection percentages were obtained (>99.9%, >98.8% and >98.5% for UM, MMSi and MMCu, respectively). Our results are consonant with previous experiments conducted with small flat membrane coupons (with 4.5 cm diameter) [32] which show that the molecular-weight cut-off of the commercially available honeycomb membranes used is enough to guarantee an effective removal of the target microorganisms from real water sources.

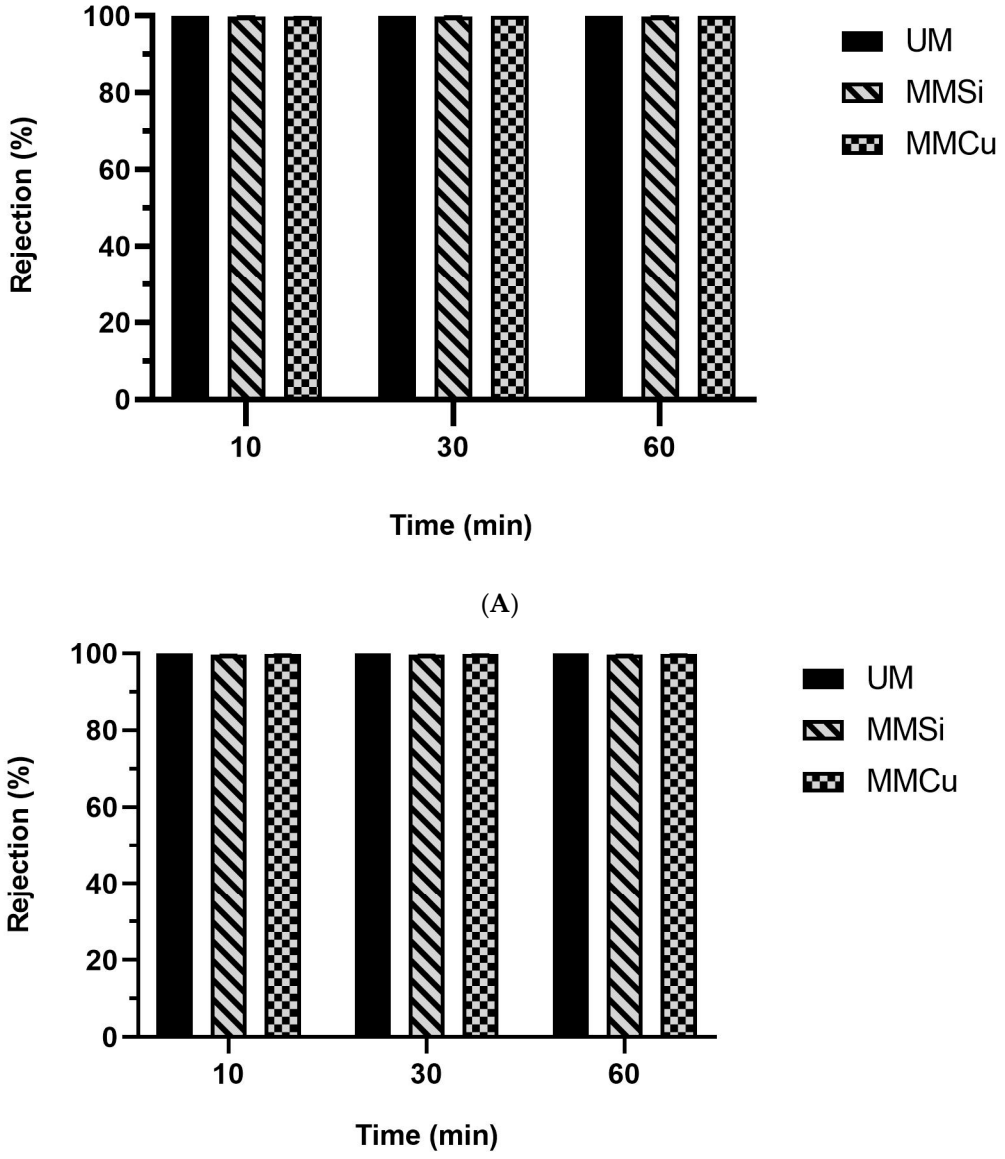


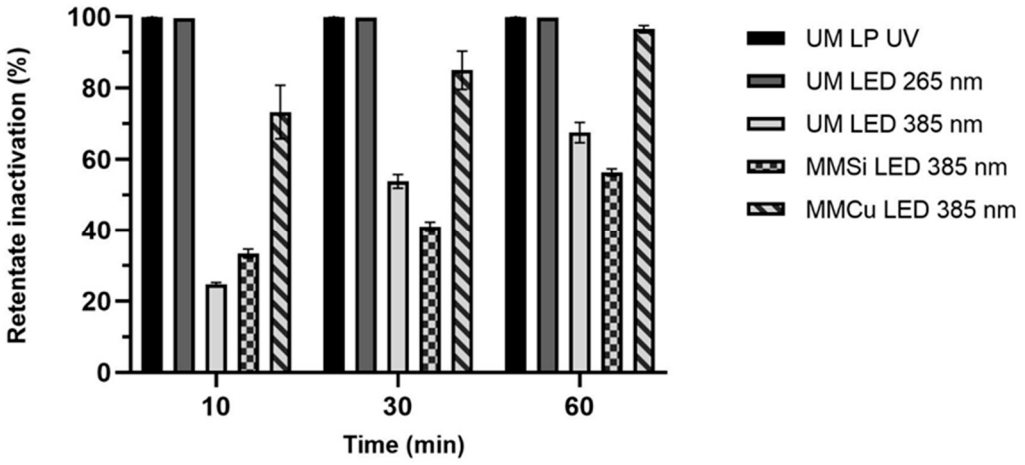
Figure 3. Rejection percentages of total coliforms (**A**) and *E. coli* (**B**) after filtration of surface water along different experimental times using unmodified (UM) and modified membranes (MM). Error bars represent duplicate results.

Silicon carbide ceramic membranes were chosen because they are extremely durable ceramics with high water permeability, which contributes to increased membrane lifetime and reduced downtime for cleaning of the membranes when fouled. Besides the high rejection

of bacteria reported in this study conducted with surface water, these ceramic membranes offered consistent and continuous rejection of suspended solids at high-throughput rates regardless of the feed conditions in studies using wastewaters with higher turbidity [34]. Besides their effectiveness as membrane filters, they are therefore considered good alternatives to use as support with photocatalytic activity, due to their expected resistance to the hydroxyl radicals produced.

2.2.3. Membrane Filtration Combined with Photolysis

One major disadvantage of filtration processes is the production of a concentrated retentate. Coupling UV photolysis with membrane filtration using unmodified or modified membranes could allow the treatment of the retentate through the inactivation of microorganisms. Figure 4 shows the results of the percentage of inactivation in the retentate obtained when the combined treatment by filtration with the unmodified membrane and photolysis (using the UV-C LP, UV-C LEDs 265 nm and UV-A LEDs 385 nm) were tested. The possibility of using UV-A light sources to treat the retentate using the modified photocatalytic membranes was also tested.



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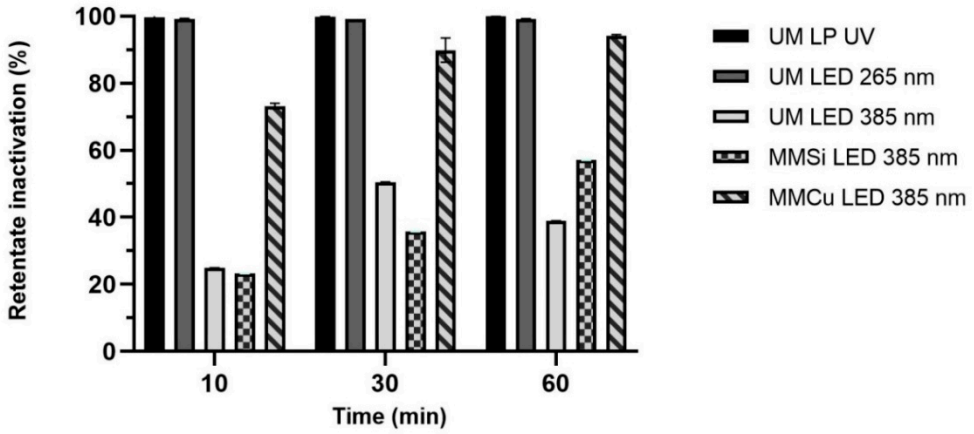

(**B**)

Figure 4. Retentate percent inactivation of total coliforms (**A**) and *E. coli* (**B**) present in surface water when filtration with the unmodified membrane (UM) was coupled with LP-UV, UV-C LEDs 265 nm and UV-A LEDs 385 nm as well as when the modified (MM) membranes were coupled with UV-A LED panels that emit light at 385 nm. Error bars represent duplicate results.

From Figure 4 we can see that the combination of an unmodified membrane with LP-UV (UM LP UV) or UV-C LEDs 265 nm (UM LED 265 nm) achieved extremely high levels of inactivation in the retentate (>99.0%).

Results presented on Figure 4 also show that after 60 min of exposure to UV-A LED 385 nm, the membrane modified with TiO_2 and copper (MMCu LED 385 nm) achieved a higher percentage of inactivation in the retentate for total coliforms and *E. coli* (95.8 and 93.8%, respectively) than the modified membrane with TiO_2 and silicon dioxide (MMSi LED 385 nm) (55.6 and 57.1%, respectively) and the unmodified membrane (UM LED 385 nm) (64.6 and 37.3%, respectively). The higher capacity of the membranes modified with copper to absorb light in the visible range (Supplementary Material, Figure S2) may lead to a higher production of hydroxyl radicals, higher photocatalytic activity and consequent higher inactivation efficiency. On the other hand, copper is also known to affect the cell-protein surface and the nucleic acid of cells [40], so a synergistic effect might occur when the modifications include copper.

For enterococci, after 60 min of filtration and exposure to LP UV, LED 265 nm and LED 385 nm using the unmodified membrane, as well as LED 385 nm using the photocatalytic membranes modified with TiO_2 and silicon dioxide as well as TiO_2 and copper, percentages of inactivation in the retentate of >99.9%, >99.0%, 70.2%, 74.9% and 94.2% were observed, respectively. The unmodified membrane in combination with UV-C LP or UV-C LED 265 nm and the modified membrane with TiO_2 and copper in combination with UV-A LED 385 nm were very efficient.

In sum, if UV-C light is used, the unmodified silicon carbide membranes can be used to retain the bacteria that will then be effectively inactivated by the UV-C light. If UV-A light is used, the best treatment of retentate is ensured using membranes modified with copper. Given the higher visible light absorption of this membrane (Supplementary Material, Figure S2) it will also be a good candidate to test with real solar light radiation.

Our results agree with other studies. Xiong and Hu [41] demonstrated the improvement of the inactivation of *E. coli* with the increase in UV light intensity using a UV-A LED 365 nm/TiO_2 water treatment. Claro et al. [42] proved that the combination of UV-A LED 385 nm with different photocatalysts (TiO_2, SiZnO, N-SiZnO, and F-N-SiZnO) achieved a high inactivation efficiency of river-water matrices. Biancullo et al. [43] assessed the proficiency of UV-A LED 381 nm/TiO_2 treatment on urban wastewater and reported a decrease in the bacterial load. Bernardo et al. [32] showed that the combination of photocatalytic ceramic membranes modified using a solvent-free procedure with LEDs that emit light at higher wavelengths improved the retentate treatment due to the production of highly reactive oxygen species. Oliveira et al. [10] developed a new photocatalytic-membrane reactor combining filtration with ceramic-modified membranes and UV photolysis/photocatalysis under a LP-UV lamp to treat filtered surface water inoculated with *Aspergillus fumigatus*. The study showed that photocatalysis originated spores' deformation and changes in membrane permeability and enzymatic activity.

As described by Zhang et al. [24], membrane fouling is a result of interactions between the feed composition and membrane. Fraga et al. [34] used the same photocatalytic membrane reactor to treat a complex and challenging olive mill wastewater matrix (characterized by its high solids content and black color). After 20 min of operation, cake formation on the membrane surface due to high concentration of particulates in the effluent prevented the light from reaching the photocatalytic layer of the membrane with a consequent reduction in the permeate quality produced. In this study, since the matrix used has much lower levels of solids, turbidity and total organic carbon, the quality of the permeate was maintained and the retentate treatment increased during the 60 min of operation that was tested (Figure 4). In longer studies, when fouling becomes an issue, the application of strategies to minimize fouling, such as backwashing and backpulsing, should be considered [34,44]. The best fouling-control strategies that are able to improve both the photocatalytic rate and conduct to fouling alleviation [24] should be defined.

3. Materials and Methods

3.1. Water Sampling

Several untreated surface-water (UTSW) samples were collected at the Tagus River (Algés, Portugal) between January and November of 2020. The samples were stored at 4 °C until the experiments that took place up to 24 h later, since previous studies showed that the concentration of microorganisms significantly decreases over time, even when samples are stored at 4 °C. The surface-water sample used was characterized in a previous study [9]: pH 7.3; TOC-3.9 mg/L C; turbidity-14.6 NTU; total suspended solids-70.5 mg/L.

3.2. Submerged Hybrid Reactor

A submerged hybrid photocatalytic membrane reactor (Figures S3, S4a, S5a and S6a) previously developed, described and tested for the treatment of olive mill wastewaters [34] was used in this study. The reactor contains two diaphragm pumps (12 V 3.0 A, 5.5 bar; SZY-4155, Shui Zhi Yuan), a pressure sensor and controller set (Aplisens, PCE 28, Poland). Sample mixing was achieved through aeration at a rate of 0.33 L of air per litter of liquid per minute (aeration rate of 0.33 vvm). Aeration can also prevent the cake layer formation in the membranes and thus improve the photocatalytic activity of the modified membranes [24].

Commercial high-flux submerged flat sheet silicon carbide ceramic membranes (LiqTech International, Hobro, Denmark) (Figure S4b) that filter from outside to inside (17 cm × 10 cm × 0.6 cm; 145 cm^2 of active filtration area in each side) were used unmodified and as substrate to develop photocatalytic membranes.

The inactivation of the retained microorganisms and activation of the photocatalytic layer of the membrane (when the modified membranes were used) was achieved through the following:

- two UV-C low-pressure mercury lamps (Puro TAP, UVC, 11 W, type GPH212T5L, New Zealand) placed submerged at 1.6 cm from each side of the membrane (Figure S5a); low-pressure mercury lamps are widely used in drinking and wastewater facilities since they are known to be extremely effective to achieve microbial inactivation.
- two novel custom-made UV-C LED panels that emit light at 265 nm placed submerged at 2 cm from each side of the membrane (Figure S6b); these panels were built following previous studies that proved UV-C LEDs at 265 nm are extremely effective to achieve inactivation of different *Aspergillus* species and water-quality-indicator bacteria [9,32].
- two novel custom-made UV-A LED panels that emit light at 385 nm placed submerged at 2 cm from each side of the membrane (Figure S6c); these panels were built following a study by Bernardo et al. [32] that showed that UV-A light sources could be used to activate photocatalytic surfaces and achieve inactivation through indirect photolysis.

The new custom-made UV LED panels are waterproof.

3.2.1. Modification of the Membranes

Before being modified, the membranes were cleaned several times using a solution of citric acid (2 *w/v*) and distilled water, followed by a drying step at 80 °C overnight. Both outer sides of the membrane were modified.

Two different modification procedures were followed by dip coating the silicon-carbide membranes:

(a) a previously detailed solvent-free procedure [29] with silicon dioxide (SiO_2) and TiO_2 degussa nanoparticles. These photocatalytic microfiltration membranes were tested for treatment of olive mill wastewaters [34], and recently, small circular coupons were combined with three small ultraviolet A light-emitting diodes to ensure the retention and effective inactivation of the concentrated membrane retentate [32].

(b) a solvent-free procedure with TiO_2 and copper described below.

The membranes were modified using Titanium (IV) isopropoxide (TTiP) (Sigma-Aldrich, 97%) as TiO_2 precursor, Copper (II) nitrate hemi(pentahydrate) (ACS reagent purity >99.99%) as Cu-dopant and acetic acid (analysis grade, Carlo Erba), as catalyst. The

modification procedure was based on Kumar et al. [45] and Fisher et al. [46]. In brief, 70 mL of titanium isopropoxide (TTiP) and 120 mL of acetic acid were magnetically stirred during 30 min and further mixed slowly with 360 mL of deionized water [45]. The solution was mixed during 1 h. In parallel, a solution including 0.408 g of copper-nitrate reagent in 300 mL of deionized water was prepared [46], and after its complete homogenization was added to the TTiP solution described above, resulting in 0.75% molar ratio of Cu compared to Si. Similarly, Fisher et al. [46], reported a rapid inactivation of bacteria using a molar ratio of 1%, under solar light. After two weeks the solution seemed stable, obtaining a blue-white homogeneous turbidity. At this point, membranes were sealed with silicon (sealant) in the holes for avoiding the filtration of the sol-gel solution and modified twice in both outer sides using a ND-DC dip coater (Nadetech Innovations, Spain), with the following conditions: immersion and withdrawal speed of 150 mm/s; immersion time of 5 s. The membranes were then left drying for 3 h at room temperature in a chamber and at 80 °C overnight. After removing the sealant, the membranes were subject to a final thermal protocol in a programmable muffle to promote the formation of the inorganic network. The following thermal protocol was applied: initial rate of 1 °C/min up to 450 °C, maintaining this temperature for 2 h, followed by a second heating stage at 3 °C/min up to 600 °C that were kept during 30 min. The membranes were cleaned with isopropanol [46] and dried at 85 °C (overnight). The modified membrane was labeled as MMCu and compared with the unmodified membrane (UM, Control).

All reagents were used without further purification.

3.2.2. Characterization of the Membranes

Membrane Morphology

The top surface and cross section of the unmodified membranes (control) and the membranes modified with titanium dioxide and copper were morphologically analyzed by SEM in a JSM 7001F microscope after sputtering the samples with a Au/Pd thin film using a Quorum Technologies Q150T ES model device.

To determine parameters related with the porous properties of the membranes, the SEM images of the top surfaces were analyzed using an open-source image-processing program, ImageJ software (University of Wisconsin, Madison, WI, USA) [47,48].

Membrane Hydrophilicity

The water contact angle of a sessile drop was determined using DSA25B equipment (KRUSS technology, Germany), a fully computer-controlled instrument based on video capture of images and automatic image analysis. A piece of flat sample was used after drying in a desiccator overnight. A sessile drop of distilled water with a volume of 3 µL (syringe diameter of 0.6 mm) was imaged over time (consecutive frames). Twenty frames were attained for each measurement, with a frame interval of 100 ms. The contact angle was measured at least in three different locations on both sides of the membranes, and the mean values were calculated.

3.3. Experimental Procedure

Before each experiment, the hybrid reactor was cleaned with ethanol 70% followed by sterile distilled water with recirculation for 30 min. To verify the baseline contamination of the reactor, 200 mL of water was collected after this cleaning procedure and analyzed in terms of the target microorganisms—total coliforms, *E. coli* and enterococci—using the methods described below in Section 3.4. All the samples collected after the cleaning procedures were found to be free of contamination with the target microorganisms.

Eleven experiments were performed to test the efficiency of membrane filtration (using unmodified and modified membranes), direct photolysis (using LP-UV lamps, LED panels that emit at 265 nm and LED panels that emit at 385 nm) and membrane filtration coupled to photolysis to retain and inactivate microorganisms present at occurrence levels in river water. For each experiment, 10 L of river water were treated during one hour in the

submerged hybrid reactor. During treatment, 300 mL of sample was collected at different experimental times (Table 2).

Table 2. Description of the eleven experiments performed in the submerged hybrid reactor.

Experiment ID		Sampling Time (min)	Membrane Type	UV Light	Filtration	Objective
1	LP-UV (254 nm)	1, 2.5, 5, 10, 30, 60	No	Yes	No	Evaluate direct photolysis using low-pressure mercury UV lamps
2	UV-C LED 265 nm	1, 2.5, 5, 10, 30, 60	No	Yes	No	Evaluate direct photolysis using light-emitting diode panels that emit light at 265 nm
3	UV-A LED 385 nm	10, 30, 60	No	Yes	No	Evaluate direct photolysis using light-emitting diode panels that emit light at 385 nm
4	UM	10, 30, 60	Unmodified (UM)	No	Yes	Evaluate the filtration performance of the unmodified silicon-carbide membrane
5	UM + LP-UV (254 nm)	10, 30, 60	Unmodified (UM)	Yes	Yes	Evaluate the combined effect (retention and inactivation) of the unmodified membrane and low-pressure mercury UV lamps
6	UM + UV-C LED 265 nm	10, 30, 60	Unmodified (UM)	Yes	Yes	Evaluate the combined effect of the unmodified membrane and light-emitting diode panels that emit light at 265 nm
7	UM + UV-A LED 385 nm	10, 30, 60	Unmodified (UM)	Yes	Yes	Evaluate the combined effect of the unmodified membrane and light-emitting diode panels that emit light at 385 nm
8	MM TiO_2 + SiO_2	10, 30, 60	Modified (MM)	No	Yes	Evaluate the filtration performance of the membrane modified with TiO_2 and SiO_2
9	MM TiO_2 + SiO_2 + UV-A LED 385 nm	10, 30, 60	Modified (MM)	Yes	Yes	Evaluate the combined effect of the membrane modified with TiO_2 and SiO_2 and light-emitting diode panels that emit light at 385 nm
10	MM TiO_2 + Copper	10, 30, 60	Modified (MM)	No	Yes	Evaluate the filtration performance of the membrane modified with TiO_2 and copper
11	MM TiO_2 + Copper + UV-A LED 385 nm	10, 30, 60	Modified (MM)	Yes	Yes	Evaluate the combined effect of the membrane modified with TiO_2 and copper and light-emitting diode panels that emit light at 385 nm

For the inactivation experiments, different light sources, LP UV mercury lamps (that emit monochromatic light at 254 nm) and two different UV LED panel systems that emit monochromatic light at two different wavelengths (265 nm and 385 nm), were submerged in the hybrid reactor and used to evaluate direct photolysis. The LED panels that emit at 385 nm were not expected to achieve inactivation by direct photolysis, but their effectiveness was evaluated since these panels were tested in later photocatalytic experiments in combination with modified membranes.

The membrane filtration experiments were conducted with the unmodified and modified membranes in the absence of UV light to evaluate the retention (rejection) of the microorganisms due to size exclusion and adsorption. The modification of the membranes

may lead to a pore size reduction and the different material of the top layer may affect the membrane adsorption capabilities.

Finally, experiments combining both processes, membrane filtration and UV photolysis, were carried out to evaluate the maximum potential of the hybrid treatment combination. The unmodified membranes were tested with low-pressure mercury lamps, UV-C and UV-A LED panels that emit light at 265 nm and 385 nm. The membranes modified following two different procedures (TiO_2 with SiO_2 and TiO_2 with Cu) were tested in combination with LED panels that emit at 385 nm to evaluate the effect of the photocatalytic layers of the membranes. Both sides of each immersed membrane were subject to the UV light sources. Using this reactor, it is possible to irradiate the feed/retentate and decrease fouling.

A chiller was used to maintain the samples at a constant temperature (18 °C).

A control sample was also tested to verify the stability of the target microorganisms in a dark environment during the experimental time.

3.4. Bacteria Identification and Enumeration

The river water samples were characterized, before and after treatment, in terms of the presence of total coliforms, *E. coli* and enterococci, bacteria frequently used as fecal-contamination indicators of water quality. The bacteria enumeration was performed as described below.

3.4.1. Total Coliforms and *E. coli*

Water samples were analyzed in terms of total coliforms and *E. coli* using the Colilert-18 kit (IDEXX, Maine, USA) described by Warden et al., Yakub et al. [49,50] and on the standard ISO 9308-2:2012 [51]. Briefly, 100 mL of each water sample not diluted and decimal serial dilutions were added to sterile flasks; the substrate (DST nutrient-indicator) was added and shacked until a homogenized solution was obtained. The liquid was then transferred to a Quanti-Tray and incubated at 35 ± 0.5 °C for 18 h. Yellow wells were counted to determine the most probable number (MPN) of total coliforms and the yellow/fluorescent (observed using an UV lamp 360 nm) wells were counted to determine the MPN of *E. coli*.

3.4.2. Enterococci

Water samples were analyzed for intestinal enterococci, such as *Enterococcus faecium* and *E. faecalis* using the Enterolert-E Kit (IDEXX, Maine, USA) as described by Yakub et al. [50], Standard Methods for Examination of Water and Wastewater (APHA 9230 Section C Membrane Filtration Techniques) and ISO 7899-1:1998/COR1:2000 [52]. Briefly, 100 mL of each water sample not diluted and decimal serial dilutions were added to sterile flasks and mixed with the substrate (DST nutrient-indicator) until a homogenized solution was obtained. The liquid was transferred for a Quanti-Tray and incubated at 41 ± 0.5 °C for 24 h. Fluorescent wells were counted (using an UV lamp 360 nm) to determine the most probable number (MPN).

3.5. Data Analysis

Log reduction of the target bacteria (total coliforms, *E. coli* and enterococci) was determined as log (C_0/C) for the experiments with direct photolysis, where C is the most probable number (MPN) of the UV irradiated sample, and C_0 is the MPN of the sample before UV irradiation.

The apparent rejection percentage of bacteria (total coliforms, *E. coli* and enterococci) was determined for the filtration experiments using the following equation:

$$\text{Rejection (\%)} = 100 \times [1 - (C_p/C_f)] \quad (1)$$

where C_p and C_f are the concentrations of bacteria in the permeate and feed, respectively.

The retentate inactivation percentage of bacteria (total coliforms, *E. coli* and enterococci) was determined for the filtration and photolysis combination experiments using the following equation:

$$\text{Retentate inactivation (\%)} = 100 \times [1 - (C_f/C_{fi})] \quad (2)$$

where C_f and C_{fi} are the most probable number of bacteria in the feed/retentate at a certain time and the initial feed, respectively.

4. Conclusions

A solvent-free procedure was used to modify by dip coating commercially available silicon carbide membranes using titanium dioxide and copper. The addition of the Earth-abundant metal was proposed to extend the light-absorbing capacity of titanium dioxide to the visible range. These and other modified membranes previously developed and characterized were used in a hybrid reactor that can be easily scaled up to test the effectiveness of the combined treatment. The results obtained showed that the photocatalytic membranes in combination with UV-A light sources achieved a high percentage of inactivation in the retentate.

Novel custom-made UV-C LED panels built were found to be extremely effective to inactivate water-indicator bacteria.

Two effective treatment and disinfection solutions can be envisioned to ensure an effluent with high quality and inactivation of the retentate: (a) the use of unmodified membranes and UV-C light sources; (b) the use of modified membranes with UV-A light sources.

These hybrid treatment approaches were found to effectively retain microorganisms that can then be successfully inactivated by photolysis and advanced oxidation processes. The effective treatment results obtained with the combined system using the UVA light sources show that the process may be a promising approach to guarantee water disinfection using natural sunlight. These hybrid reactors could therefore be a promising approach to treat drinking water and wastewater in high- and low-income countries.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal12070680/s1, Figure S1: Dynamic water contact angle for unmodified membrane (UM, control) before being irradiated and modified membrane before and after being irradiated (MMCu and MMCu + LED 385 nm, respectively). Figure S2: Absorbance of unmodified membrane (UM) and membrane modified with titanium dioxide and copper (MMCu). Figure S3: Schematic representation of the hybrid reactor. Figure S4: Filtration treatment. Figure S5: Combined treatment (membrane filtration and LP UV photolysis). Figure S6: Combined treatment (membrane filtration and UV LED photolysis).

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