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Assessment of new immobilized photocatalysts based on Rose Bengal for water and wastewater disinfection

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ABSTRACT

The aim of this study was to evaluate the water disinfection capability of new immobilized photocatalysts based on the organic photosensitizer Rose Bengal (RB) supported on glass wool and with two different photocatalyst load (RB_GW_0.22 and RB_GW_0.02). Tests were carried out under different operating conditions in a solar simulator at 30 W/m^2 average UV-A irradiation. Isotonic water (IW) (with 9 g/L NaCl), simulated tap water (STW) and simulated urban wastewater effluent (SUWWE) spiked with *Escherichia coli* and *Enterococcus faecalis* (10^6 CFU/mL initial concentrations) were used as water model microbial strains. The mere effect of only irradiance was also investigated as reference of the photocatalytic performance over both bacteria. Results showed that RB_GW_0.22 presented high inactivation for *E. faecalis*, reaching 6 Log Reduction Value (LRV) in 2 min in IW and STW at pH 5 and 6 in comparison with only irradiance (60 min). In SUWWE, the effect of RB_GW_0.22 was also better for *E. faecalis* inactivation (60 min), compared to that produced in *E. coli* (120 min) and without the presence of the photocatalyst (where total inactivation was not achieved after 120 min of treatment). Ecotoxicity (ISO 11348–3:2007) and phytotoxicity (EN ISO 18763:2020) tests of the treated water were also assessed and results showed any significant effect, concluding that the use of RB_GW_0.22 is a promising solar photocatalytic treatment for urban wastewater reclamation and reuse.

1. Introduction

The reclamation and reuse of secondary effluents from urban wastewater treatment plants (UWWTPs) is currently a very well-accepted alternative solution to increase the water availability around the world. Nevertheless, efficient tertiary treatments are needed to reach the reclaimed water quality criteria necessary to obtain a safety reuse. Despite the efficient water disinfection performance of the conventional tertiary treatments currently available in UWWTPs such as UV-C radiation, ozonation and chlorination, they still have several important drawbacks (regrowth of bacteria during water storage, high cost and potential generation of disinfection by products). Advanced Oxidation Processes (AOPs) have proven to be effective as tertiary treatments for the reduction or elimination of hazardous chemical compounds and for the inactivation of pathogenic microorganisms, due to their high oxidant capability [1]. Their effectiveness is based on the promotion of reactive oxygen species (ROS) generation, especially the hydroxyl radical ($HO^{\bullet}$) but also hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$). Among AOPs, photocatalytic processes driven by solar radiation have shown good promising results for water purification [2], promoting radicals' generation as a result of the radiation absorption by a photo-sensible compound (photosensitizer) or a photocatalyst.

Photosensitizers (organic colored compounds) have been demonstrated to be able to inactivate microbial pathogens in water [3], although their main application is as oxidative agents in antimicrobial photodynamic therapy, an alternative non-invasive medical treatment of microbial infections (including drug resistant bacteria) and widely used in dentistry and other related oral lesions or cancer treatment therapies. When photosensitizers are photo-excited with visible light (λ: 380–700 nm), two mechanisms of reaction can occur: Type I or electron transfer, where the reaction between the photosensitizer with organic compounds generate free radicals and radical ions (from the photosensitizer or organic substrates) that may latter react with oxygen and produce peroxides, $O_2^{\bullet-}$ and $HO^{\bullet}$, and/or Type II that consists on an

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energy transfer with dissolved oxygen resulting on the generation of $^1O_2$ as main ROS [3,4].

Different types of photosensitizers have been investigated for water disinfection including Rose Bengal (RB), methylene blue, eosin, riboflavin, fluorescein, chlorophyllin, hematoporphyrin, or Zn(II) phthalocyanine tetrasulfonic acid, among others [4–6]. The commercially available photosensitizer RB has been investigated for water disinfection as a non-toxic, cheap organic dye [7] photo-excited by visible light (maximum λ: 548 nm in water [8]). Upon irradiance and in the presence of oxygen, RB can generate $^1O_2$ in water with a quantum yield (Φ_{1O2}) of 0.75 [8]. The microbial inactivation mechanism is mainly attributed to the formation and accumulation of $^1O_2$ inside cells [4]. More recently, it has been reported that the inactivation of cells can be also attributed to the adsorption of RB onto the bacterial membranes, confirming this finding over *Enterococcus faecalis* and *Fusobacterium nucleatum* by fluorescence imaging [9]. Nevertheless, there is still scarce information about the mechanisms of RB over gram-positive bacteria. In literature, several authors have reported good results for microbial inactivation with very low concentrations of RB (range of μM). [10] reported *E. faecalis* inactivation (>8 LRV) by irradiating samples containing RB at several concentrations, ranging from 5 to 100 μmol/L. They observed best inactivation with 25 μmol/L (or 24.3 mg/L) under green laser at 1.1 W/cm^2 (5 min pre-irradiation time and 3 min irradiation time) in deionized water; while higher concentrations showed a clear toxic effect over the bacterium viability.

On the other hand, the immobilization of photosensitizers in different supports such as glass, chitosan, silica gel, and polymers of styrene and acrylamide has been also investigated to solve limitations of real implementation, in which the photosensitizer is covalently linked, preventing leaching and facilitating recovery and subsequent reuse [11]. The main advantage is the reusability and easy separation or recovery of the catalyst after water treatment; nevertheless, lower disinfection kinetics and several difficulties to be implemented at higher scale are two of the main drawbacks of this type of application. Several studies in the literature report on the disinfection performance of RB immobilized in different support materials. Valkov et al., [4] investigated the capability of RB disodium salt (63% w/w), RB lactone (76% w/w), methylene blue (68% w/w) and hematoporphyrin (76% w/w) immobilized in polyethylene for the inactivation of gram-positive (*Staphylococcus aureus*) (~4 LRV) within 0.5–2 h and gram-negative (*Escherichia coli*) bacteria (~5 LRV) within 3–6 h (except hematoporphyrin immobilized in polyethylene that did not kill the cells even after 20 h) under illumination with a white luminescent lamp (1.25 mW/cm^2). Kim et al., [5] also reported good antibacterial effect in distilled water by RB and hematoporphyrin immobilized on a glass surface and under visible light radiation by LED lamps for two gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) and two gram-positive bacteria (*Rhodococcus qingshengii* and *Staphylococcus aureus*), concluding > 90% reduction in gram-positive bacterial cell numbers in 2 h. The potential of photosensitizers covalently attached to polymers attributed their good performances to the high (up to 0.91) quantum yields of singlet oxygen generated [12]; nevertheless, despite the potential application of photosensitizers using visible light (both in solution or immobilized), scarce literature is available for its combination with natural sunlight (UV and visible range).

The aim of this study was to evaluate the water disinfection capability of new immobilized photocatalysts based on the heterogenization of RB (as organic photocatalyst) on glass wool (GW), a very low cost material, available and manageable. The photocatalytic efficiency was evaluated against the simultaneous inactivation of gram-negative (*E. coli*) and gram-positive (*E. faecalis*) bacteria under simulated solar radiation. In addition, the influence of different parameters on the photocatalytic inactivation efficiency was assessed: (i) two immobilized photocatalysts with different quantity of RB (0.22% w/w and 0.02% w/w) in isotonic water, (ii) water pH (ranged from 5 to 8) and (iii) more complex water matrices (simulated tap water (STW) and simulated UWW (SUWW) with 12.6 ± 1.4 mg/L of dissolved organic matter content). Furthermore, the ecotoxicity and phytotoxicity of the treated SUWW were also assessed as a crucial factor for a safe UWW reuse in agriculture.

2. Materials and methods

2.1. Water matrices

Three types of water matrices were used to assess the photocatalytic performance: (i) isotonic water (IW) with 9 g/L of NaCl (to avoid bacterial osmotic stress) as model of simple matrix to determine the fundamental disinfection capability of the photocatalysts without the influence of chemicals, (ii) simulated tap water (STW), and (iii) simulated urban wastewater effluent (SUWWE) as model of a more complex water matrix avoiding common fluctuations on physicochemical contents of actual water matrices. The chemical composition was done according to Berruti et al., [13] and the main physicochemical characteristics measured of each water matrix are shown in detail in Table S1 (Supplementary material). Dissolved Organic Carbon (DOC) and HCO_3^- concentration were measured with a Shimadzu 138 TOC-VCN analyzer, inorganic cations and amines with a Metrohm ion chromatograph Model 850, turbidity with a turbidimeter (Hach model 2100AN), water pH using a pH-meter (110-K, Horiba Laqua act) and the conductivity using a conductometer GLP31 CRISON.

2.2. Synthesis and characterization of glass wool based-immobilized RB photocatalysts

Two new photocatalysts with different loads of immobilized RB on glass wool (GW) support were synthesized. Each photocatalyst was washed with pure water to avoid any residual contamination from the preparation phase before water disinfection tests. An absorbance spectrum of both photocatalysts (Fig. S1, Supplementary material) was performed in IW to demonstrate that there is no leaching of RB in the water. RB_GW_0.22 photocatalysts were synthesized in two steps:

1. Activated GW (10 g) was prepared upon overnight treatment with 3-aminopropyltriethoxysilane (APTES, 10 mL) in dry toluene (300 mL) at 150 °C. The excess of APTES was removed under vacuum.
2. The activated GW was stirred with 1-methylimidazole (0.2 mL), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.45 g) and RB (1.30 g) in 300 mL of dimethyl sulfoxide (DMSO) at room temperature, for further 24 h. Afterwards, the crude was washed with DMSO (3 ×300 mL), EtOH (3 ×300 mL), and water (3 ×300 mL), and finally dried under vacuum to get RB photocatalysts.

The synthesis of RB_GW_0.02 started with derivatization of RB with APTES and subsequent covalent anchoring to the activated GW as described elsewhere [14].

The characterization of the immobilized RB photocatalysts (Fig. 1) was done according to the following procedures:

2.2.1. Quantification of RB

It was carried out by an Inductively Coupled Plasma Spectrometer with Agilent 7900 Mass Detector (ICP-MS). The samples were subjected to digestion in a high pressure microwave oven, under basic medium at 220 °C and the % of RB was determined based on the concentration of iodine: RB (0.22% w/w) on RB_GW_0.22. To determine the content of RB in the case of RB_GW_0.02, an accurately weighted amount of RB_GW_0.02 (ca. 20 mg) was added to a 10 mL volumetric flask and filled more than half with milli-Q water. Next, it was autoclaved at 110 °C for 15 min (x2), followed by sonication for 20 min at 40 °C to ensure the release of the hydrolyzed RB into water, and finally, it was made up to volume with water. The absorbance was recorded in the UV–vis spectrophotometer Cary 50 (Varian) and the exact concentration

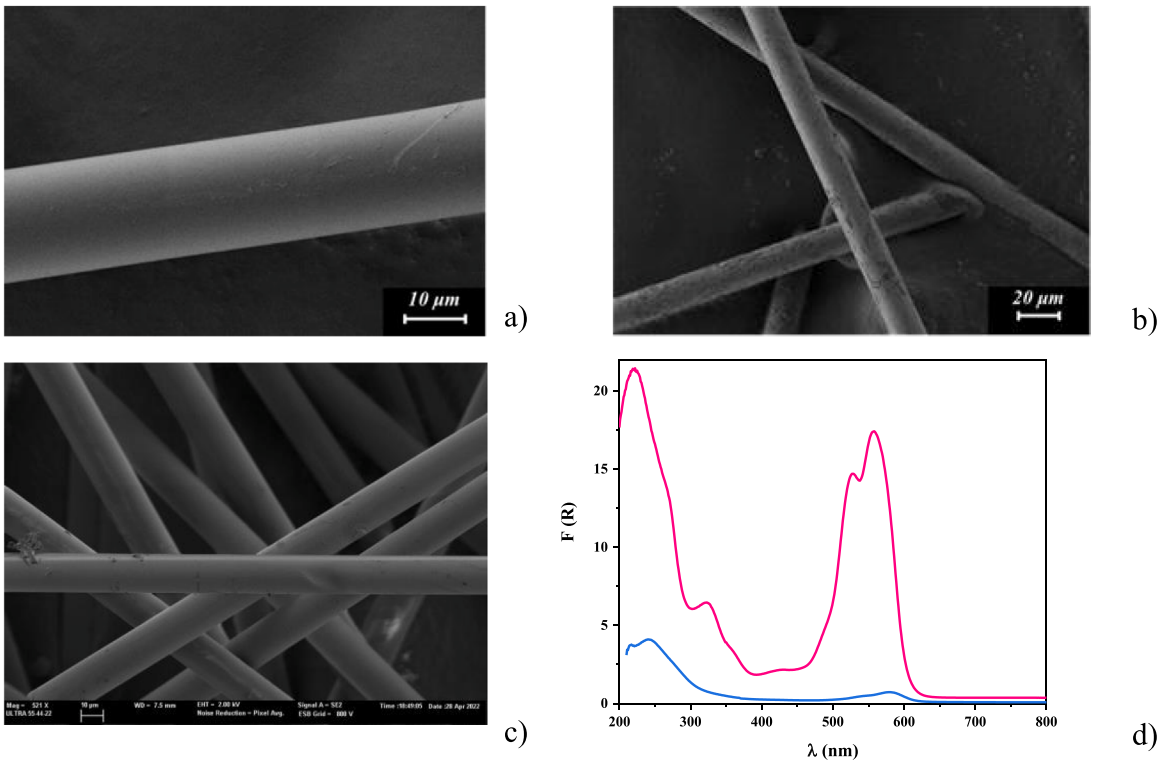

Fig. 1. Microscopy images of (a) commercial non-functionalized GW, (HRFESEM, 10 μm), (b) RB_GW_0.22 (HRFESEM, 20 μm) and (c) RB_GW_0.02 (FESEM, 10 μm). D) Diffuse reflectance spectra of RB_GW_0.22 (pink) and RB_GW_0.02 (blue).

of RB was determined from a calibration curve of RB in Milli-Q water. All the spectra were recorded at room temperature, using quartz cells of 1 cm optical path length.

2.2.2. Diffuse reflectance

Diffuse reflectance was recorded using a Cary 5000 from Agilent Technologies.

2.2.3. Field emission scanning electron microscope (HRFESEM)

The images of GW and RB_GW_0.22 were taken on a GeminiSEM500 (ZEISS OXFORD INSTRUMENTS) HRFESEM. The samples were prepared in their solid form in a 1 cm diameter disk.

2.2.4. Field emission scanning electron microscopy (FESEM)

High resolution images of RB_GW_0.02 were obtained using a FESEM (model ULTRA 55, ZEISS). The samples were prepared by covering them with a layer of palladium using a Sputter Coater and were analyzed with an acceleration voltage of 1.5 kV, followed by and appropriate magnification.

2.3. Bacterial enumeration and quantification

The bacterial strains *E. coli* K12 (CECT 4624) and *E. faecalis* (CECT 5143) were selected as common indicators of fecal contamination in water. Both strains were obtained from the Spanish type culture collection and the enumeration and quantification procedures were done according to a previously published work [13]. Briefly, suspensions of 10^9 CFU/mL per bacteria were obtained using liquid media Luria-Bertani (LB, Sigma-Aldrich) by incubation in rotary shaking incubator for 20 h at 37 °C and 100 rpm. After that, each suspension was centrifuged 15 min at 3000 rpm, the pellets were re-suspended in phosphate buffer solution (PBS) and directly diluted in the sample to obtain an initial concentration of 10^6 CFU/mL. Quantification of microbial concentration from all water samples was carried out by serial dilution of samples in PBS and the standard plate counting method. 50–500 μL of sample (diluted or not) was spread on specific medium, ChromoCult® Coliform Agar (Merck KGaA, Germany) and Slanetz Bartley Agar (Scharlau®, Spain), and incubated 24 h for *E. coli* and 48 h for *E. faecalis* (both at 37 °C), respectively. Detection limit (DL) of this technique was found to be 2 CFU/mL.

2.4. Experimental procedure

The photocatalytic tests were carried out in a solar simulator SUNTEST XLS+ (Atlas Material Testing Solutions) with 30 W/m^2 average UV-A irradiation. The experimental set-up was composed of a 145 mm diameter and 30 mm height UV-transparent beaker (simulating same tube diameter employed in Compound Parabolic Collectors for heterogeneous photocatalysis) and magnetically stirred at 200 rpm. Water temperature was monitored with a portable thermometer model CRISON TM 65 (CRISON INSTRUMENTS S.A., Spain). Maximum water temperature was below 37.5 °C, discarding therefore thermal effects over the bacterial viability. The incident radiation was monitored by a portable UV-A radiometer, model PMA2111 (Solar Light Co., Inc, Philadelphia), ensuring an average radiation of 30 W/m^2 (range 320–400 nm, with a high resolution (0.01 W/m^2)).

All tests were done following the same experimental scheme: after filling the beaker with the corresponding water matrix, and for tests carried out at different pHs, it was adjusted with sulfuric acid or sodium hydroxide and monitored by using a pH-meter. After that, an appropriate volume from the prepared microbial stock suspension was added to reach an initial concentration of 10^6 CFU/mL per bacteria, and 1 g/L of immobilized RB. The mere effect of solar only irradiance and only the photocatalyst in the dark, were also investigated as blanks of the photocatalytic performance over both bacteria abatement. After a few minutes of homogenization in the dark, the solar simulator was switched on, starting the irradiation exposure for 2 h. Along this time, samples at regular intervals were taken out and analyzed for each target. All tests were carried out in triplicate, and results of each target removal were plotted in graphs as the average value of all replicates with the standard

deviation as error bars.

2.5. Toxicity analysis

Phytotoxicity assessment was performed by root and stem length measurement of *Sinapis alba* (mustard), *Lepidium sativum* (garden cress) and *Sorghum saccharatum* (sorgho) seeds provided by a commercial kit (Phytotoxkit liquid samples, Microbiotests Gent, Belgium) exposed to the water samples following standard procedure (EN ISO 18763:2020) [15]. Briefly, 10 seeds were placed on esterilized Petri dishes lined with white cellulose wattle filter paper with 20 mL of sample (SUWWE as negative control and treated SUWWE samples) and incubated during 72 h at 25 ± 1 °C in the dark. Toxicity is classified according to the relative growth index value (RGI, sample/negative control radicle length ratio): stimulation (benefit) for RGI > 1.2; no significant effect for 0.8 ≤ RGI ≤ 1.2 and inhibition (toxic effect) for 0 < RGI < 0.8 [16].

Eco-toxicity test assessment was performed with a psychrophilic marine bacterium, *Aliivibrio fischeri* (NRRL B-11177), by using the commercial kit BioFix Lumi-10. The determination of the inhibitory effect of water samples on bioluminescence emission of *A. fischeri* was evaluated according to standard procedure (ISO 11348–3:2007) [17]. All the samples (including control test) were tested in triplicate. The bioluminescence emission was measured with a BioFix® Lumi-10 luminometer (Macherey-Nagel GmbH & Co. KG, Duren, Germany) after 15 and 30 min of sample exposure. Toxicity results were expressed as bioluminescence inhibition percentage (BI %), in comparison to an uninhibited control. BI < 50% indicates that the water sample does not pose a harmful effect to the receiving aquatic environment [17].

3. Results and discussion

3.1. Synthesis and characterization of the immobilized RB photocatalysts

The synthesis of RB_GW_0.22 and RB_GW_0.02 was carried out by two different synthetic protocols, in order to obtain two materials with different RB loading on their surface, but both based on the formation of the amide bond between RB and APTES to ensure that inactivation of bacteria was in all cases heterogeneous. HRFESEM and FESEM images showed that the morphologies of the GW fibers remained intact upon derivatization (Fig. 1a-c). Tests to determine the amount of RB revealed that RB_GW_0.22 has a higher percentage of RB than RB_GW_0.02, 0.22% vs 0.02% (w/w), respectively. This difference in the degree of functionalization was also observed on the diffuse-reflectance spectra of the two materials (Fig. 1d).

3.2. Photocatalytic disinfection in isotonic water

Fig. 2 shows the photocatalytic inactivation profiles of *E. coli* and *E. faecalis* obtained in IW by RB_GW_0.22 and RB_GW_0.02 including also the inactivation by solar only disinfection and dark exposure.

Firstly, the viability of both bacteria in the presence of 1 g/L of each heterogeneous photocatalyst was assessed in the dark. Results showed no effect over *E. coli* viability as the initial concentration remained constant along the treatment time. Nevertheless, for *E. faecalis* a significant inactivation (5 LRV in 120 min of treatment) was obtained but only with RB_GW_0.22 photocatalyst. The higher susceptibility of gram-positive bacteria to photosensitizers has been widely reported in the literature, and it is attributed to their different cell-wall architecture. Gram-positive bacteria have been reported to be more easily photoinactivated due to the high porosity of the outer membrane (to allow transfer of nutrients, glycopeptides, polysaccharides, and also photosensitizers) [18]; while in gram-negative bacteria, the transfer of low molecular weight molecules occurs through specific channels (porins), limiting, therefore, the internalization of the photosensitizers [19]. In addition, the high negatively surface charge of gram-negative bacteria due to the lipopolysaccharide layer, limits or prevent the adsorption of

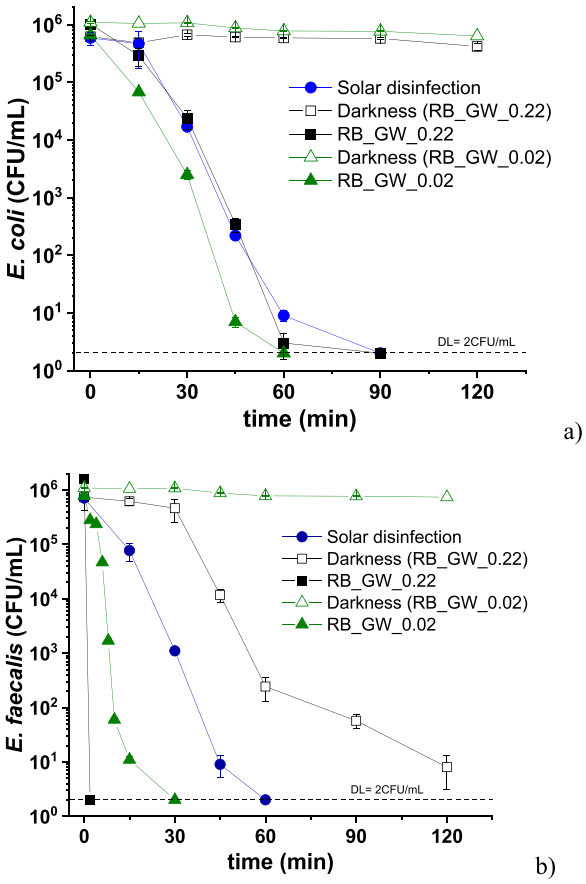

Fig. 2. Inactivation profiles of *E. coli* (a) and *E. faecalis* (b) with RB_GW_0.22 and RB_GW_0.02 in IW, under irradiation and in the dark.

anionic dyes [20,21] such as RB, which perfectly explains the results obtained in our study.

On the other hand, the results obtained in IW shown that the load of the catalyst is key factor on the different inactivation behavior for *E. faecalis*. The leaching of RB was evaluated at the end of each experiment with both photocatalysts by measuring an absorbance spectrum of treated water, and said leaching was discarded as it is shown in Fig. S1. Therefore, it is possible to conclude that attraction/repulsion forces between *E. faecalis* and the photocatalysts may be responsible of the different inactivation observed in the dark, being the higher the RB concentration, the faster the attraction and therefore the bacterium inactivation. Nevertheless, this specific aspect is not the objective of this study, and other research are needed in the future to clarify this behavior.

When irradiating the sample, the catalyst load is also evidencing the key role. For *E. coli*, RB_GW_0.22 photocatalyst did not improve the inactivation kinetics in comparison with the mere effect of solar irradiance (Fig. 2a), reaching > 5 LRV in 90 min. Nevertheless, with the RB_GW_0.02 photocatalyst, the inactivation of *E. coli* was 30 min faster than only solar irradiance. On the contrary, the inactivation kinetic of *E. faecalis* by both immobilized RB was significantly improved in comparison with only solar irradiance (6 LRV in 60 min). The inactivation reactivity order found was in good agreement with the load of immobilized RB, and therefore with the previous effect observed in the dark: RB_GW_0.22 (6 LRV in 2 min) > RB_GW_0.02 (6 LRV in 30 min). This results agree with other studies found in the literature. Cooper et al., [22] reported, using low concentrations of RB (at 0.1 and 1 mg/L) in deionized water at pH 7, a total inactivation of *E. coli* from 10^3 to 10^4 CFU/mL of initial concentration in 60 min. Nakonechny et al., [23] studied the antibacterial activity of free RB and immobilized RB at

50 mg/L in both cases, against *S. aureus* at 10^5 CFU/mL of initial concentration, obtaining total killing of bacteria in 1 min and 10 min for free RB and immobilized RB, respectively.

The fast inactivation of *E. faecalis* compared with *E. coli* in the presence of RB was already clearly demonstrated at the end of 80's. Dahl et al., [24] reported 200% faster inactivation kinetics for gram-positive bacteria (*Streptococcus salivarius, S. faecalis, S. aureus*) in comparison with a gram-negative bacterium (*Salmonella typhimurium*) by illuminated RB. Recently, Gurianov et al., [6] demonstrated higher antibacterial activity of RB immobilized on silica using visible white light, against gram-positive (*S. aureus*) compared to gram-negative (*E. coli*) bacteria. Nevertheless, other effects apply in the case of gram-negative bacteria in which the less charged RB_GW_0.02 performs slightly better than RB_GW_0.22. This result could be due to the more positive surface of the former due to the less charge with the negative RB [25].

3.3. Photocatalytic disinfection in simulated tap water

With the main objective of investigating the photocatalytic performance of both RB photocatalysts in more complex water matrices, Fig. 3 shows their inactivation capability in STW. In addition, the influence of the pH (ranged from 5 to 8) against *E. coli* and *E. faecalis* inactivation has been also evaluated as a potential key factor on the photocatalytic performance. The water pH, once adjusted to the desired value, remained constant along the entire experimental time. As it has been previously well reported [26,27], target bacteria do not show any detrimental effect in their viability varying the pH in such range, therefore the inactivation differences observed in our study could be only attributed to the water pH effect on the photocatalytic capability of both new immobilized RB photocatalysts.

For *E. coli,* any significant effect of pH on inactivation rates was observed, achieving DL between 45 and 60 min in all cases for both new immobilized photocatalysts. Nevertheless, for *E. faecalis*, best inactivation results were obtained with RB_GW_0.22 at pH 5, achieving DL after 2 min with an inactivation kinetic constant k of (3.30 ± 0.00) min^{-1}, being 18.3 times higher than RB_GW_0.02 (30 min). Moreover, the inactivation rate followed the same pH order for both photocatalysts and an increase of pH led to a decrease of k values, obtaining 4.3 and 2 times lower inactivation rates at pH 8, compared to pH 5, reaching DL after 15 min and 60 min for RB_GW_0.22 and RB_GW_0.02, respectively.

3.4. Photocatalytic disinfection in simulated urban wastewater effluent

The inactivation profiles of both bacteria with and without the presence of 1 g/L of RB_GW_0.22, selected as the one that showed better performance in previous experiments, was assessed in SUWWE (Fig. 4). Prior to the experiments under simulated solar radiation, the mere effect of 1 g/L of RB_GW_0.22 over both bacteria viability (*E. coli* and *E. faecalis*) was assessed in the dark. Any of the bacteria suffered a significant viability reduction (< 0.5 LRV) in 120 min of contact time (data not shown). The different behaviour obtained in the dark regarding *E. faecalis* in comparison with IW (5 LRV in 120 min) can be attributed to the higher complexity of SUWWE, where the presence of organic matter makes the bacteria less susceptible to oxidative stress [28].

Photocatalytic results clearly show a significant accelerated inactivation of both bacteria in comparison with the mere effect of solar only radiation. The inactivation kinetics of *E. faecalis* was again higher than in the case of *E. coli* (Table S4), reaching in both cases the DL after 60 and 120 min, respectively. The wastewater disinfection in the presence of RB has been poorly reported in literature. Jemli et al., [29] described a higher susceptibility of natural occurring gram-positive bacteria (fecal streptococci) than gram-negative (fecal coliforms) in wastewater in the

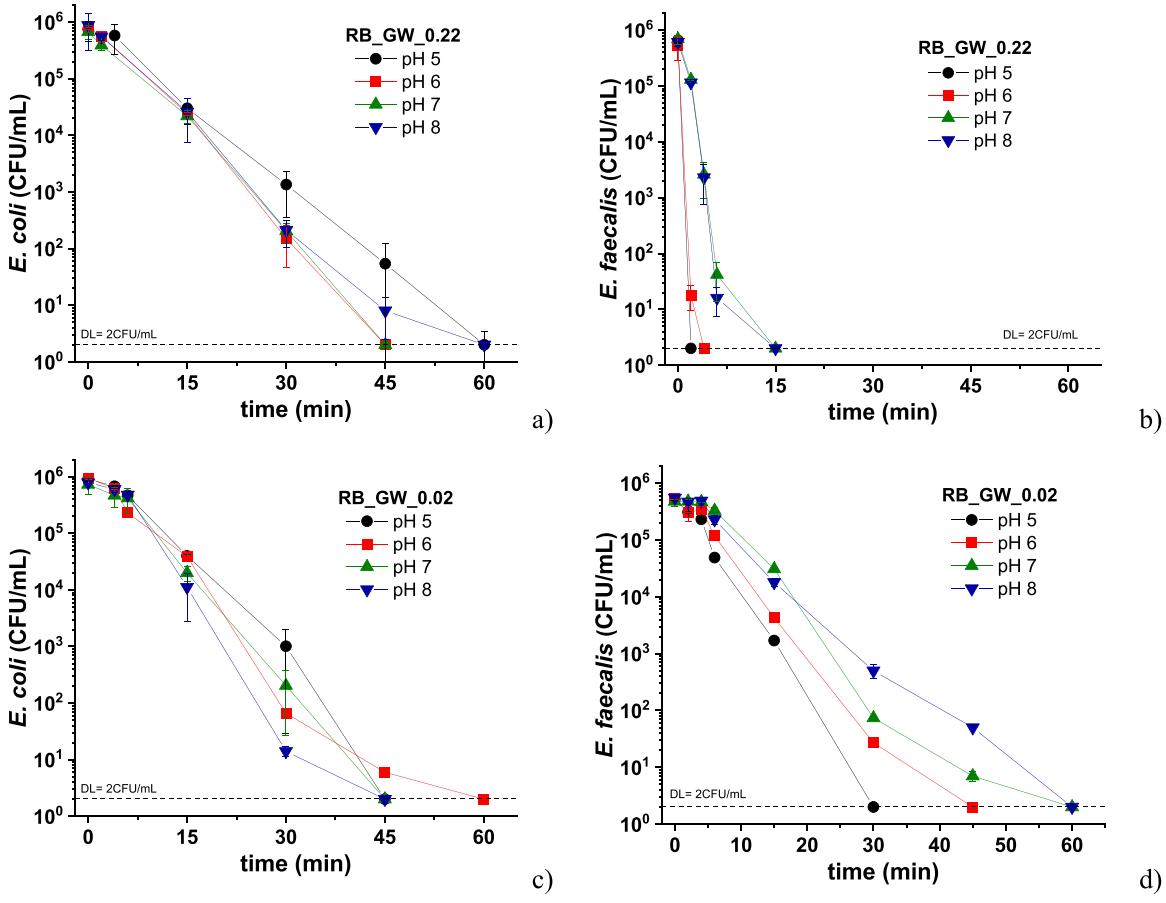

Fig. 3. Inactivation profiles of *E. coli* and *E. faecalis* with RB_GW_0.22 and RB_GW_0.02 under irradiation in STW at different pHs.

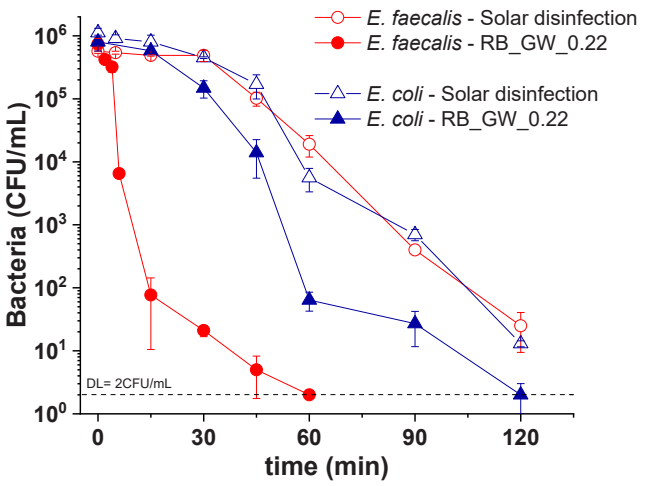

Fig. 4. Inactivation profiles of *E. coli* and *E. faecalis* with RB_GW_0.22 immobilized photocatalyst under irradiation in SUWW.

presence of several photosensitizers, and regarding pure RB, 10 μM of concentration showed best results (~3 LRV), reaching coliform count below 1 CFU/mL in 120 min of irradiation time. Therefore, the good results obtained with the new immobilized photocatalyst shows a very promising disinfection capability to be used for treating actual wastewater.

3.5. Toxicity assays

Phytotoxicity results from the root and stem growth of *S. saccharatum*, *S. alba* and *L. sativum* seeds are shown in Table 1. Two conditions were simultaneously analysed: (*i*) a negative control (with distillated water) and (*ii*) treated SUWWE with RB_GW_0.22 photocatalysts at 1 mg/L initial concentration combined with artificial solar radiation (30 W/m^2). Root and stem growth results showed a non-significant effect for all seeds after the addition of RB_GW_0.22 to the SUWWE (t_0). After 60 min of treatment time, stem growth results also showed a non-significant effect for *S. alba* and *L. sativum* (RGI between 0.8 and 1.2), though a reduction on the RGI (below 0.8) was observed for *S. saccharatum*. Moreover, root growth results showed slight phytotoxicity for *S. alba* and *S. saccharatum* (RGI below 0.8). Finally, root and stem growth results showed slight toxicity (RGI average 0.6 for shoot and 0.7 for root) after 120 min of solar treatment with RB_GW_0.22 photocatalyst. In most cases, a significant non-toxic effect was observed (RGI values between 0.8 and 1.2). Therefore, it can be concluded that the RB_GW_0.22 photocatalyst is not a toxic material to treat UWW for further purposes of reusing in agriculture.

Table 1
Phytotoxicity and ecotoxicity results obtained for treated SUWW by GW_RB_0.22 photocatalyst.

PHYTOTOXICITY						
	L. sativum		***S. alba***		***S. saccharatum***	
	cm	**RGI**	**cm**	**RGI**	**cm**	**RGI**
Stem Growth						
(-)	1.87 ± 1.05		2.30 ± 0.46		0.62 ± 0.36	
t_0	2.16 ± 0.44	1.50 (S)	2.50 ± 0.81	1.05 (NS)	0.28 ± 0.18	0.43 (I)
t_{60}	2.28 ± 0.38	1.62 (S)	2.05 ± 0.54	0.88 (NS)	0.58 ± 0.43	0.80 (NS)
t_{120}	1.77 ± 0.53	1.15 (NS)	1.64 ± 0.46	0.70 (NS)	0.48 ± 0.33	0.70 (I)
Root growth						
(-)	5.02 ± 1.52		6.20 ± 0.96		2.20 ± 0.74	
t_0	5.38 ± 0.58	1.14 (NS)	5.74 ± 1.49	0.91 (NS)	1.39 ± 0.41	0.64 (I)
t_{60}	5.52 ± 0.69	1.16 (NS)	3.99 ± 1.58	0.62 (I)	2.48 ± 0.82	1.13 (NS)
t_{120}	4.21 ± 1.53	0.82 (NS)	3.66 ± 1.34	0.57 (I)	1.79 ± 0.83	0.78 (I)

ECOTOXICITY		
	***A. fischeri* (BI %)**	***A. fischeri* (BI %)**
	Sample after 15 min	**Sample after 30 min**
t_0	Estimulation (4%)	9.5 ± 3.5
t_{60}	17 ± 3	29.5 ± 3.5
t_{90}	11 ± 0	21.0 ± 2.8
t_{120}	16.5 ± 4.9	30.0 ± 2.8

(-): Negative control; (I): Inhibition (0 < RGI < 0.8); (NS): No significant effect (0.8 ≤ RGI ≤ 1.2); (S): Stimulation (RGI > 1.2)

Ecotoxicity of the samples against *A. fischeri* (BI %) was also analyzed and results are summarized in Table 1. Results showed that the mere effect of RB_GW_0.22 photocatalyst addition (t_0) led to a slight inhibition percentage after 30 min of contact (9.5%), though a stimulation effect (4%) was observed for the same sample after only 15 min of contact. By increasing the treatment time of SUWWE with RB_GW_0.22 (60, 90 and 120 min), any significant inhibitory effect on *A. fischeri* after 15 and 30 min of contact was observed (<50% BI). However, an expected increase from around 10–17% till 20–30% was detected after increasing the contact time with *A. fischeri* to 30 min.

4. Conclusions

New immobilized photocatalysts based on Rose Bengal supported on glass wool at two loads of catalyst (RB_GW_0.22 and RB_GW_0.02) have been successfully tested for the inactivation of bacteria in different water matrices (IW, STW and SUWWE). The presence of both photocatalysts had a strong inactivation effect on both target bacteria (> 5LRV), being more significant for *E. faecalis* (gram-positive) than for *E. coli* (gram-negative) in all cases and in comparison with the mere effect of solar irradiance. The RB_GW_0.22 photocatalyst showed better inactivation performance for both bacteria than RB_GW_0.02, demonstrating that even under an immobilized system, the load of catalyst plays a significant role.

The impact of the water chemical composition on the RB_GW_0.22 photocatalyst disinfection performance showed a non-significant effect over *E. coli* kinetic rates; while a significant *k* reduction was observed for *E. faecalis* as increasing the water complexity: IW (2.95 ± 0.00 min^{-1}) > STW (pH of 6, 1.35 ± 0.51 min^{-1}) > SUWWE (0.09 ± 0.02 min^{-1}). These results clearly indicate that the susceptibility of the *E. faecalis* to the RB is greatly influenced by the chemical composition of the water, as well as it was evidenced by the effect of pH observed only for this bacterium.

Finally, RB_GW_0.22 photocatalyst showed very promising results on SUWWE, reaching > 5 LRV of *E. coli* and *E. faecalis* in 120 and 60 min of irradiation time. Also, the reclaimed water showed slightly toxic effect for *A. fischeri* while phythotoxicity results demonstrated null or non-significant toxicity for plants. Nevertheless, more research in this line is required to give insights into the mechanisms operating during the investigated photocatalytic inactivation with RB to ensure the total safety of actual treated secondary effluents to be reused for crop irrigation.

CRediT authorship contribution statement

Alba Hernández-Zanoletty: Formal analysis; Investigation; Writing – original draft. **Isabel Oller:** Supervision; Project administration; Writing – review & editing. **Maria Inmaculada Polo-López:** Supervision, Writing – review & editing. **Alberto Blazquez-Moraleja**: Formal analysis, Writing – original draft. **Jenny Flores:** Formal analysis, Investigation. **Francisco Boscá:** Supervision, Writing – review & editing. **M. Luisa Marin**: Conceptualization, Supervision, Writing – review & editing. **Sixto Malato:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cattod.2022.11.002.

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