

Article

Water Disinfection by Immobilized Photosensitizers

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Abstract: Fresh water shortage has become a global problem. A partial solution for this problem is the use of treated and disinfected wastewater for irrigation. However, most existing wastewater disinfection methods are based on the use of aggressive chemicals or power-consuming physical processes. Photodynamic eradication of waterborne bacteria by immobilized photosensitizers may be a good alternative to conventional methods. In the present work, the photosensitizers Rose Bengal sodium salt, Rose Bengal lactone, methylene blue, and hematoporphyrin were immobilized in polyethylene or polypropylene using a “green” method of co-extrusion, without addition of any chemicals, yielding polymeric strips and beads containing the photosensitizers. The antibacterial efficiency of these immobilized photosensitizers was tested against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* in batch and continuous regimes upon illumination with a white luminescent lamp. All examined photosensitizers demonstrated a good ability to decrease the bacterial concentration, up to their total eradication. Immobilized photosensitizers are proposed for batch or continuous disinfection of wastewater after secondary treatment.

Keywords: photosensitizers; PACT; Rose Bengal; methylene blue; hematoporphyrin; water disinfection

1. Introduction

Use of treated and disinfected wastewater for irrigation can be a good solution for conserving drinking water in areas with limited natural water resources. Existing methods of bacterial eradication in wastewater involve the use of aggressive chemicals, such as chlorine-based compounds and ozone, or power-consuming physical methods, such as UV radiation. Photodynamic treatment with the help of photosensitizers may comprise an alternative approach to wastewater disinfection [1,2].

Photosensitizers (PSs) are colored compounds which are excited under illumination by visible light and can either transfer their excitation energy or exchange an electron with other substances [3]. Upon excitation, PSs can respectively follow two pathways, named Type I and Type II reactions. In the Type I mechanism, PS molecules react with bio-organic molecules, producing active free radicals and radical ions of the PS or another organic substrate which further react with oxygen producing peroxides, superoxide ions and hydroxyl radicals [4]. The Type II reaction is accompanied by energy transfer to molecular oxygen dissolved in an aqueous phase [5]. The photosensitizers typically interact with triplet oxygen species to produce reactive oxygen species (ROS), such as singlet oxygen, superoxide anion, hydroperoxyl radical, hydrogen peroxide, and hydroxyl radical [6,7]. In nature, singlet oxygen is generated in neutrophils and macrophages for killing microorganisms. Microorganism cells produce superoxide dismutases, catalases and peroxidases in order to defend themselves against radical- and reduced-oxygen species. However, these enzymes are not effective against singlet oxygen [6]. Contrary to antimicrobials, acquired resistance to singlet oxygen by a bacterium, fungus, or virus has

never been reported. Gram-positive bacteria are more sensitive to singlet oxygen than Gram-negative bacteria [8,9]. In the case of viruses, enveloped species are inactivated by singlet oxygen more easily than non-enveloped viruses [10,11].

PSs can be applied for eradication of microorganisms in a free form, encapsulated into liposomes or immobilized onto solid supports. Application of free PS for wastewater disinfection was examined by Jemli et al. [1,12] who showed that free MB, RB, and meso-substituted cationic porphyrin were effective for inactivating faecal bacteria under illumination and proposed treating wastewater by PSs in order to reuse the treated wastes. Introducing water-soluble PSs into the aqueous phase necessitates further extraction of the former, whereas using PSs immobilized onto a solid phase makes this additional stage redundant. Application of immobilized PSs for water disinfection affords several additional advantages over the use of free disinfectants: 1. Easy separation between the disinfecting agent and the treated water; 2. The possibility of designing a continuous process; 3. The possibility of reusing the immobilized PSs in batch schemes; and 4. Increased resistance of immobilized PSs to bleaching by light and oxygen over free PSs.

Immobilization of PSs can be performed by covalent bonding, by formation of ionic bonds between ion-exchange resins and PSs, by adsorption onto a solid support or by incorporation into polymers [13–21].

Covalent attachment of a large number of PSs, such as Rose Bengal (RB), eosin, fluorescein, chlorophyllin, hematoporphyrin, and Zn(II) phthalocyanine tetrasulfonic acid to various supports, including silica gel, poly(styrene-co-vinylbenzyl chloride), poly[(*N*-isopropylacrylamide)-co-(vinylbenzyl chloride)], poly[(sodium *p*-styrenesulfonate)-co-(4-vinylbenzyl chloride)], and chitosan, has been reported [13,22–25]. PSs covalently attached to polymers demonstrated high (up to 0.91) quantum yields of singlet oxygen [23].

Using immobilized PSs for water disinfection was tested by Bonnet et al. [13], where PSs were immobilized on chitosan. The group of García-Fresnadillo [26,27] proposed immobilizing PSs from a polyazaheterocyclic Ru(II) group onto porous silicone in order to apply them for water disinfection. The main topic in García-Fresnadillo's [7] review on photoinactivation of microorganisms in water is dedicated to water treatment in photoreactors with the help of PSs based on fullerenes and Ru(II) complexes immobilized in polymeric supports, where sunlight is used as a light source. In all of the above cases, the immobilized PSs demonstrated high efficacy in bacterial eradication.

We have previously reported inclusion of PSs into a polymeric film by mixing solutions of PSs in chloroform with solutions of polystyrene, polycarbonate or poly(methyl methacrylate) in the same solvent, with subsequent air evaporation of the latter. The polymeric films obtained via this procedure were effective in eradication of Gram-positive and Gram-negative bacteria under moderate white light illumination [16,17,28].

Most PS immobilization methods include complicated chemical schemes using toxic and expensive reagents and/or organic solvents. Although the resulting immobilized PSs exhibit high cytotoxic activity, the high ecological "price" of these processes is not compensated by the environmental and economic profits gained from using disinfected wastewater instead of drinking water for irrigation.

The aim of the current work was to develop a green reagent-less PS-immobilization method for disinfection of contaminated water.

2. Materials and Methods

2.1. Materials

Low-density polyethylene (PE) beads (5 mm diameter) and polypropylene (PP) beads (3 mm diameter) were purchased from Carmel Olefins Ltd., Haifa, Israel.

Rose Bengal disodium salt (RB), purity 95%, Rose Bengal lactone (RBL), purity 95%, methylene blue chloride·3H₂O (MB), purity 97%, and hematoporphyrin (HP), purity 97%, were purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Thermogravimetric Analysis

PS were tested for thermostability by TGA using a TGA-DSC instrument (Mettler Toledo International Inc., Greifensee, Switzerland). PS samples of 5–10 mg were placed into standard aluminum crucibles and heated from 25 to 300 °C at the rate of 10 °C min^{−1} in the flow of nitrogen supplied at a flow of 50 mL min^{−1}.

2.3. Immobilization of PSs

Immobilization of the PSs in polymers was performed by co-extrusion using an extruder (Allspeeds Ltd., Accrington, England) under an inlet temperature of 80–90 °C and an outlet temperature of 150–200 °C. For this purpose, a mixture of polymer beads and PS powder were placed in a feed and the extruder was activated to melt the mixture at 43 rpm. The resulting fluid composition was then pushed through a die with a 5 mm round section or a flat 1 × 19.6 mm section. This procedure yielded polymeric rods with the incorporated PS. The rods were chopped into 3 mm beads or used as is.

2.4. Evaluation of PS Inclusion into Polymers

The inclusion yield of PSs into polymers was determined after the immobilization of RB, RBL, and MB, taking the amounts of PS applied for immobilization and the non-incorporated amounts into account. The latter were evaluated by washing the inner chamber of the extruder with water after the extrusion and measuring the absorbance of the washings at an appropriate wavelength (544 nm for RB, 557 nm for RBL and 665 nm for MB) using a Cary 100-Bio UV-VIS spectrophotometer (Varian, Sydney, Australia). The amount of PS not incorporated into the polymeric matrix in the measured volume of washings was calculated using calibration curves. Since HP has poor solubility in water, its inclusion was determined gravimetrically by weighting the HP and the polymer before the extrusion and the immobilized HP after the extrusion.

2.5. Testing PS Leakage from the Polymers

Leakage of PSs from the polymeric matrices was evaluated by soaking a known amount of co-extruded polymer-PS pellets in a bath with a known volume of tap water at ambient temperature for washing from non-entrapped PS. The pellets did not undergo preliminary washing before the experiment. Water was changed twice a day for five days, where all washings were monitored using a spectrophotometer at the appropriate wavelength. The amount of leaked PS was calculated after measuring the absorbance in the washings using calibration curves at the wavelengths mentioned above for RB, RBL, and MB, and at 615 nm for HP. In the case of RB immobilized in PE, RB leakage was also tested for 260 g polymer placed in a bath with 1.5 L of tap water using continuous washing by tap water for three weeks under illumination at a flow rate of 2 mL/min provided by a multi-channel peristaltic pump (Ecoline, Ismatec, Glattbrugg, Switzerland). The washings at the outlet were tested by HPLC analysis with a Jasco LC model (JASCO International Co., Tokyo, Japan) on a RP-18 column YMC-Triart C18, 75 × 3.0 mm, bead size 1.9 µm, in the isocratic regime using an eluent composed of 10:40:50 v/v of 20 mM ammonium acetate: acetonitrile:methanol. The RB concentration in the washings was calculated using calibration curves.

2.6. Bacterial Growth

Cultures of *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228) and *Escherichia coli* (ATCC 10798) were grown on brain-heart agar (BHA, Acumedia, Lansing, MI, USA) for 24 h, after which they were transferred into brain-heart broth (BH, Acumedia, Lansing, MI, USA) and

grown at 37 °C and shaking at 170 rpm to $OD_{600} = 0.3$. Cells were harvested by centrifugation, washed twice with sterile 0.05 M phosphate-buffered saline (PBS), pH 6.5, diluted with PBS to $OD_{600} = 0.1$ which corresponded to a final concentration of 10^8 cells mL^{-1} and then serially diluted in two to four 10-fold dilutions.

2.7. Antibacterial Activity Assay

The antibacterial activity of the polymer-PS compositions was studied in batch experiments as follows: 25 mL portions of a *S. aureus*, *S. epidermidis*, or *E. coli* suspension at a concentration of 10^5 cells mL^{-1} in sterile PBS were dispensed into Petri dishes with 0.1–10 g of immobilized PS beads. In all the experiments the beads were thoroughly pre-washed before a use. The plates were illuminated from the top for periods of 30 min to 24 h with a white luminescent lamp emitting in the range of 400–700 nm with a fluence rate of 1.25 mW cm^{-2} (light doses of 4–194 $J \text{ cm}^{-2}$). The light intensity was measured with a LX-102 Light-meter (Lutron, Taiwan). The distance between the lamp and Petri dishes was 40 cm. Control experiments were carried out in the dark in the presence of the immobilized PS and under illumination with bacterial suspensions in the presence of polymeric pellets not containing PS as well as in the absence of any beads.

Antibacterial activity in a continuous regime was studied by flowing a suspension of bacteria in saline solution from the top down through a vertical column ($1 \times 50 \text{ cm}$) packed with 14 g of pre-washed RB/PE beads at flows of 0.2–0.9 mL/min using the multi-channel peristaltic pump. A control column, packed by PE beads not containing RB, was connected in a parallel mode to the same bacterial source. The columns were permanently illuminated from the side by two fluorescent lamps installed in a vertical mode parallelly to the columns at the fluence rate of 1.25 mW cm^{-2} . Sampling was performed at the inlet and the outlet of the column and the bacterial concentration was estimated as the number of colony forming units (CFU) per mL determined by the live count method. The suspension of the source bacteria was replaced daily by a fresh one.

2.8. Photostability

Photostability experiments were performed by placing 14 g of preliminary washed 1% RB immobilized in PP in a 3-liter bath with tap water into which suspensions of *S. epidermidis* bacteria were added daily. The control bath contained no polymeric material or PS. Both baths were permanently illuminated by a luminescent lamp. Samples from both baths were taken daily before addition of fresh portions of bacteria and the bacterial concentration was tested using the live count method.

2.9. Statistical Data Processing

The results obtained from at least three independent experiments carried out in duplicates were statistically analysed by single-factor or two-factor ANOVA analyses. The difference between results was considered significant when the *p*-value was less than 0.05.

3. Results

3.1. Thermal Stability of PS

Thermal stability of RBL, RB, and MB was tested by the TGA-DSC method and decomposition temperatures were determined from TGA thermal curves and data on HP was obtained from literature [29]. Decomposition temperature of RBL was 265 °C, of RB—300 °C, of MB—250 °C, and of HP—250 °C. These data indicate that all the used PS possess good thermal stability and do not decompose at the extrusion conditions of maximal heating up to 200 °C.

3.2. Immobilization of PS into Polymers

Photosensitizers were immobilized in polyethylene or polypropylene polymeric matrices at a weight ratio of 1:100 by means of extrusion, yielding coloured polymeric beads. Figure 1

presents examples of such beads containing RB (Figure 1a), RBL (Figure 1b), MB (Figure 1c), and HP (Figure 1d) in polyethylene, where these samples are designated RB/PE, RBL/PE, MB/PE, and HP/PE, respectively.

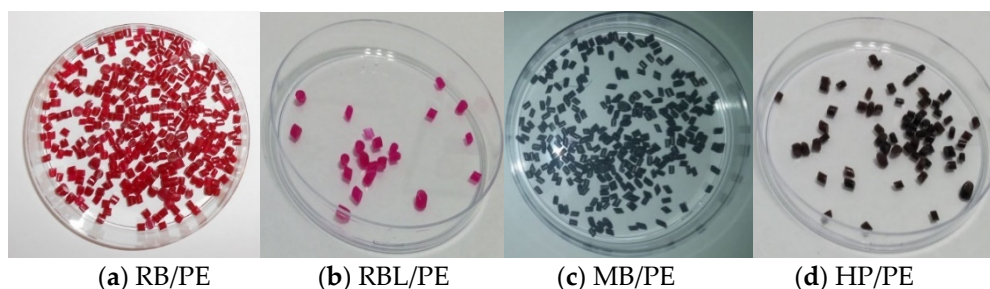


Figure 1. Polyethylene beads containing 1% (*w/w*) PS.

Yields of PS inclusion into polyethylene were 63% for RB, 68% for MB and 76% for both HP and RBL as calculated relative to the amount of PS fed to the extruder. Low inclusion yields can probably be explained by electrostatic sticking of fine PS powders to the parts of the inner extruder chamber. Leakage of PSs from the polymeric matrices was very low: 3% for RB/PE and MB/PE, 7% for RBL/PE, and zero for HP/PE.

Thus, most of the immobilized PS remained within the polymer structure and was not released into the aqueous phase. After washing, the polymeric pellets were free from non-entrapped PS and no further visible leakage of PS into the aqueous phase was detected. Nevertheless, the issue of possible leakage of PS from polymers was examined as well. In this experiment, non-washed RB/PE beads were placed in a tank with water, through which water was flown in a continuous mode. The water at the tank outlet was sampled and tested for RB using HPLC. The results of this experiment, presented in Figure 2, show that only washings on day 1 and day 2 contained detectable concentrations of RB and that from day 3, the concentration of RB in the aqueous phase was below the instrument's detection limit of 0.01 ppm.

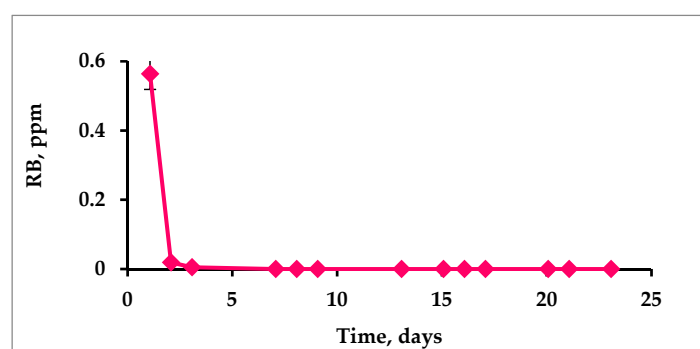


Figure 2. Leakage of RB in continuous washing of RB/PE by tap water. A total of 260 g of RB/PE as loaded in a bath with 1.5 L of tap water and washed by it at a flow rate of 2 mL/min under illumination with 1.25 mW/cm² white light.

3.3. Antibacterial Activity of Immobilized PS

The immobilized PSs were tested for antibacterial activity in batch experiments when the samples of preliminary washed polymers with incorporated PS were placed in illuminated dishes at various loadings with suspensions of bacteria at various concentrations. Samples of all immobilized PSs were found to be active against Gram-positive and Gram-negative bacteria under illumination, whereas the polymers themselves, before inclusion of the PSs, showed no antibacterial activity when illuminated. It should be mentioned that the immobilized PSs were inactive in the dark. Figure 3 illustrates the activity of immobilized PSs against Gram-positive *S. aureus*. It can be seen that the immobilized

PSs caused total eradication of the bacterial cells within 0.5–2 h. RBL/PE showed good antibacterial activity and all bacteria were killed within an hour (Figure 3a), whereas RB/PP and RB/PE inactivated *S. aureus* after 1 and 2 h, respectively (Figure 3b,c), but since these composites were tested under different loadings, they can be considered as exhibiting similar antibacterial properties. MB/PE eradicated the bacteria within half an hour (Figure 3d) and HP/PE within 1 h (Figure 3e).

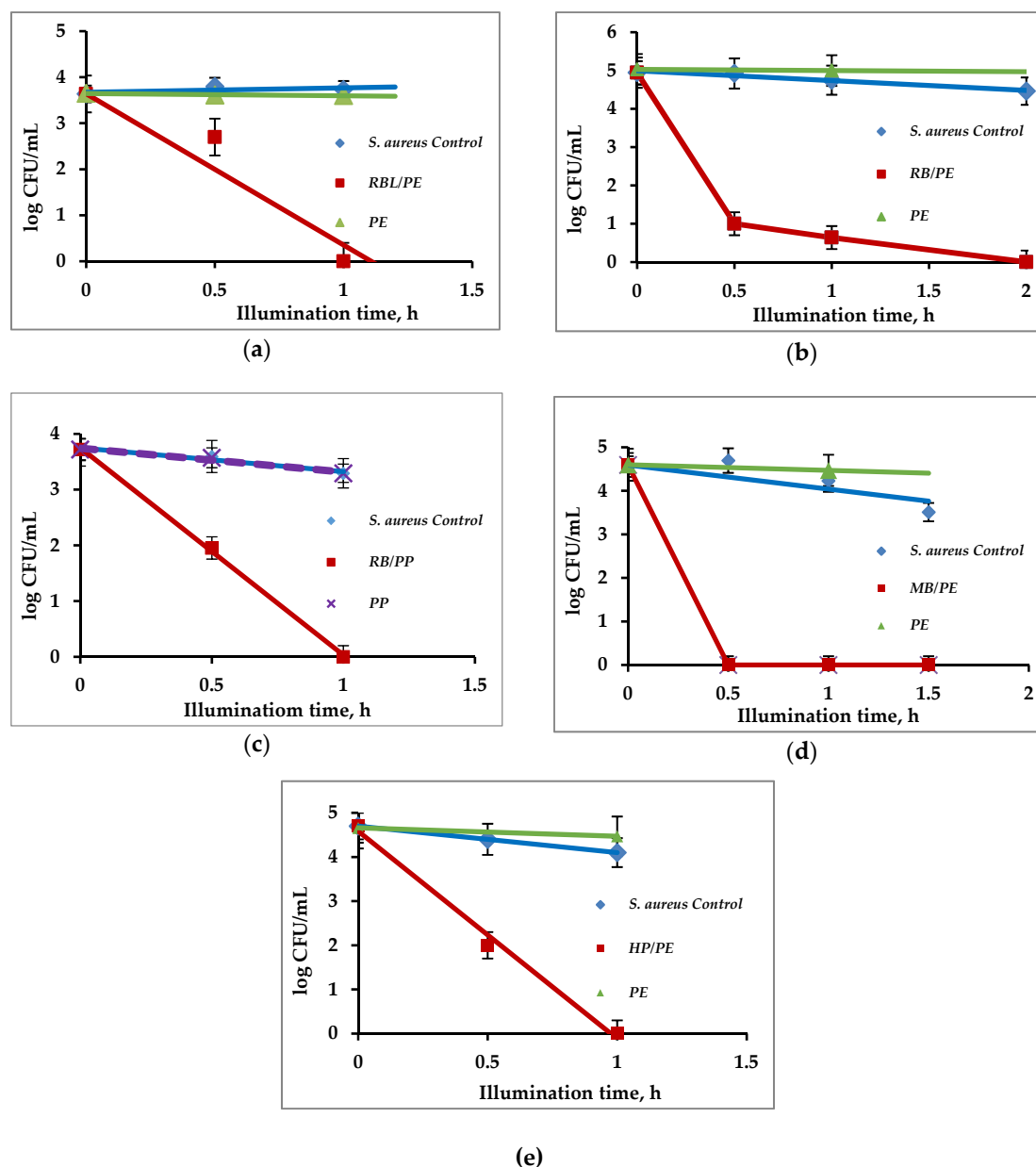


Figure 3. Effect of PSs immobilized onto polymers on the concentration of *S. aureus* under illumination with 1.25 mW/cm² white light. (a) 1% RBL/PE at a 4 g/L loading; (b) 1% RB/PE at a 20 g/L loading; (c) 1% RB/PP at a 320 g/L loading; (d) 1% MB/PE at a 220 g/L loading; (e) 1% HP/PE at a 10 g/L loading. Control experiments—*S. aureus* in the absence of any polymer and in the presence of PE or PP.

The picture for Gram-negative *E. coli* was different from that obtained for *S. aureus*. Figure 4 shows the rates of *E. coli* inactivation by immobilized PSs. It can be seen that in all cases, the inactivation time was much higher than for *S. aureus*. It took 3 h for RBL/PE to eradicate *E. coli* cells (Figure 4a), 6 h for RB/PE (Figure 4b), 4 h for MB/PE (Figure 4c), whereas HP/PE did not kill the cells even after 20 h (Figure 4d). In the latter case the curve did not differ from control of PE—*p*-value was 0.96.

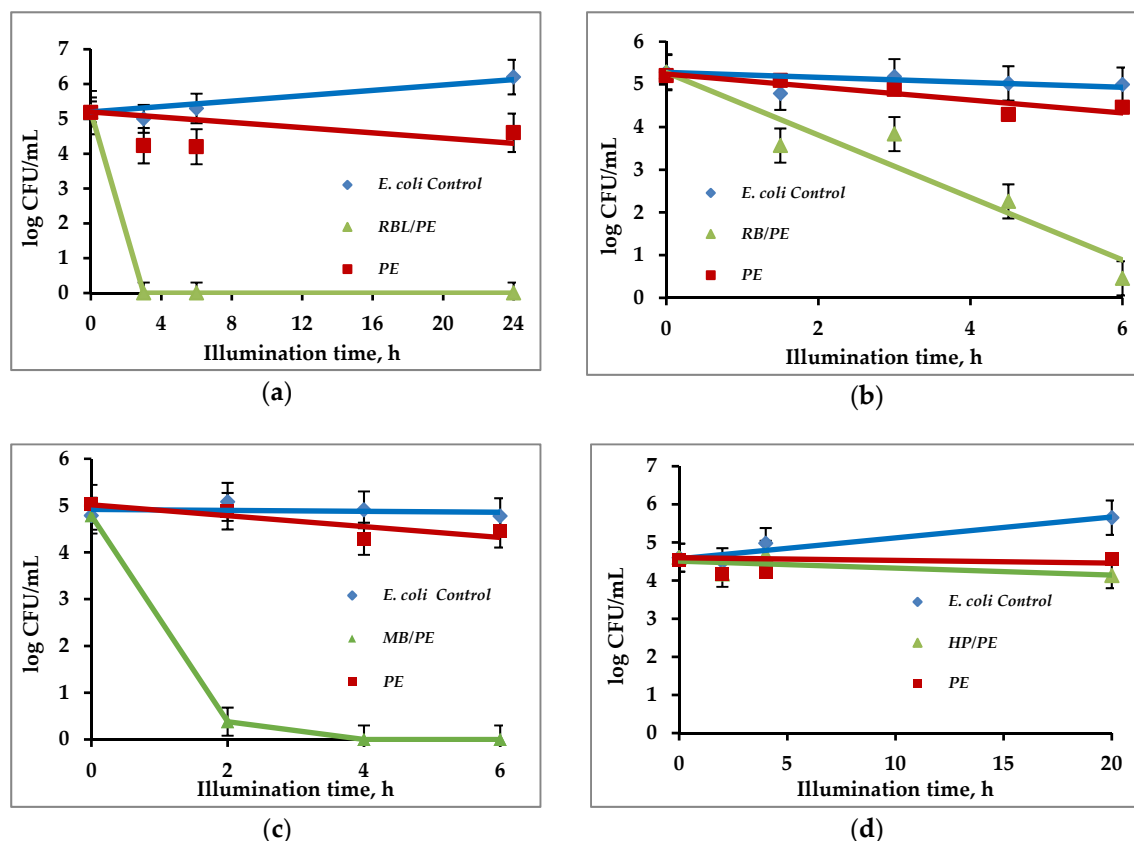


Figure 4. Effect of PSs immobilized onto polymers on the concentration of *E. coli* under illumination with 1.25 mW/cm^2 white light. (a) 1% RBL/PE at a 320 g/L loading; (b) 1% RB/PE at a 320 g/L loading; (c) 1% MB/PE at a 220 g/L loading; (d) 1% HP/PE at a 400 g/L loading. Control experiments—*E. coli* in the absence of any polymer and in the presence of PE.

It should be mentioned that in all control experiments in the case of Gram-positive and Gram-negative bacteria polymer beads not containing PS exhibited no antibacterial activity (Figures 3 and 4). Immobilized PS were also inactive against bacteria in the control dark experiments.

It was interesting to examine how loading of immobilized PSs and bacterial concentration affected the cell eradication rate. For this purpose, a series of experiments was performed in which immobilized PSs were added either at various loadings to suspensions of *S. aureus* at a constant concentration (Figure 5), or at constant loadings to *S. aureus* at various concentrations (Figure 6). RBL/PE at a loading of 4 g/L totally eradicated *S. aureus* added at a concentration of 3×10^5 CFU/mL after 1 h of illumination, but cell inhibition after 0.5 h was not complete. At loadings of 10 g/L or more, the cells were killed already after 0.5 h (Figure 5a). After 1 h of illumination, RB/PE at loadings of 20 g/L or less caused partial eradication of *S. aureus* when the cell concentration was 5×10^4 CFU/mL, whereas all bacteria were killed at loadings of 40 g/L or more (Figure 5b). MB/PE destroyed all *S. aureus* cells added at a concentration of 2×10^4 CFU/mL after 1 h of illumination at a loading of 80 g/L.

However, the immobilized MB showed only limited activity at lower loadings (Figure 5c). HP/PE at loadings of 10 g/L or more eradicated *S. aureus* added at a concentration of 10^6 CFU/mL after 1 h of illumination. However, after 0.5 h it caused total bacterial eradication only at loadings of 40 g/L or more (Figure 5d).

Bacterial concentration was also a very significant factor. After 1 h of illumination, RB/PE at a loading of 10 g/L eradicated all *S. aureus* cells at a concentration of 10^4 CFU/mL, but cell inactivation was not complete at concentrations of 10^5 and 10^6 CFU/mL, where the cell concentration decreased by only 3.8 and 3.5 log₁₀, respectively (Figure 6).

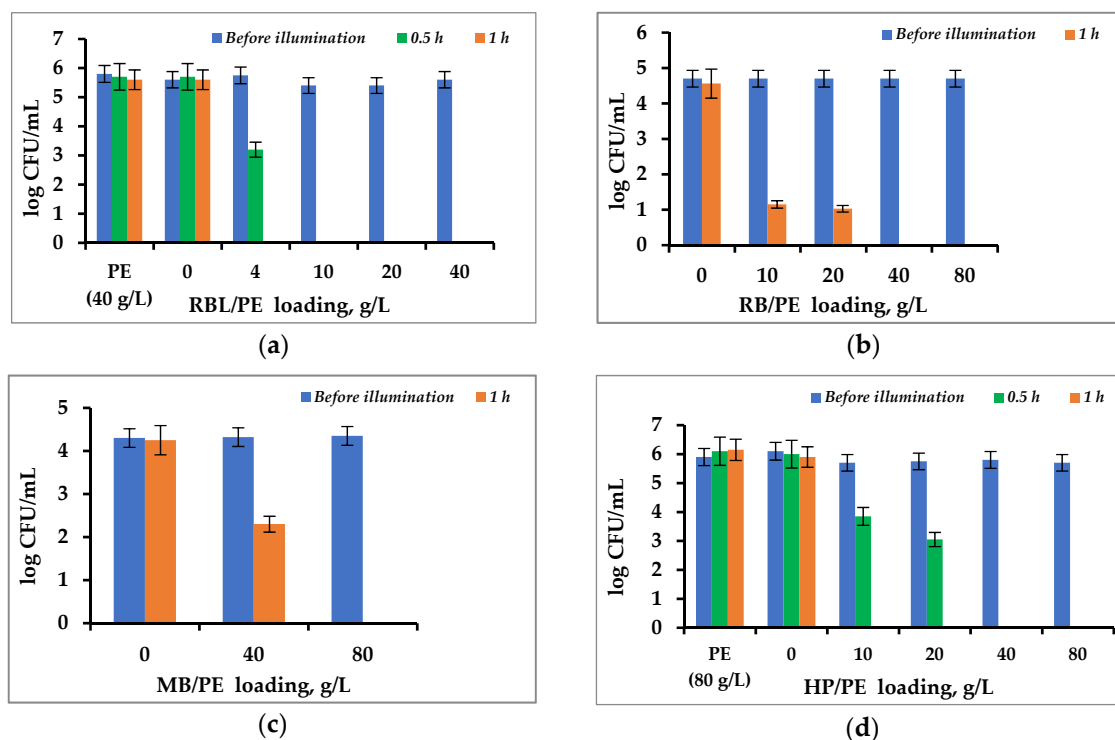


Figure 5. Effect of PS/PE loadings as an amount of PS/PE beads per volume on the eradication of *S. aureus* under illumination with 1.25 mW/cm² white light. (a) 1% RBL/PE at an initial cell concentration of 10⁶ CFU/mL; (b) 1% RB/PE at an initial cell concentration of 10⁵ CFU/mL; (c) 1% MB/PE at an initial cell concentration of 10⁴ CFU/mL; and (d) 1% HP/PE at an initial cell concentration of 10⁶ CFU/mL.

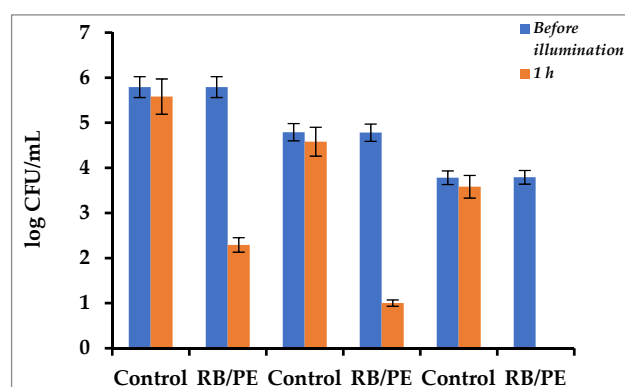


Figure 6. Effect of the initial *S. aureus* cell concentration on the antibacterial activity of 1% RB/PE at a loading of 10 g/L under illumination with 1.25 mW/cm² white light.

3.4. Continuous Eradication of Waterborne Bacteria by Immobilized PS

A photoreactor based on RB/PE beads packed in a vertical column was built in order to examine whether immobilized PSs can be applied for continuous disinfection of water. The photoreactor was permanently illuminated by luminescent lamps. Suspensions of *S. aureus* or *E. coli* were fed to the inlet of the column and sampling was performed for testing of bacterial concentration at the column's inlet and outlet. Additional columns packed with PE beads were connected in parallel to the columns with RB/PE so that the bacterial suspension at the inlets of both columns was fed from the same source, in order to prove that the decrease in bacterial concentration was due to the immobilized RB and not to the hypothetical retention of bacterial cells by simple filtering (Figure 7).

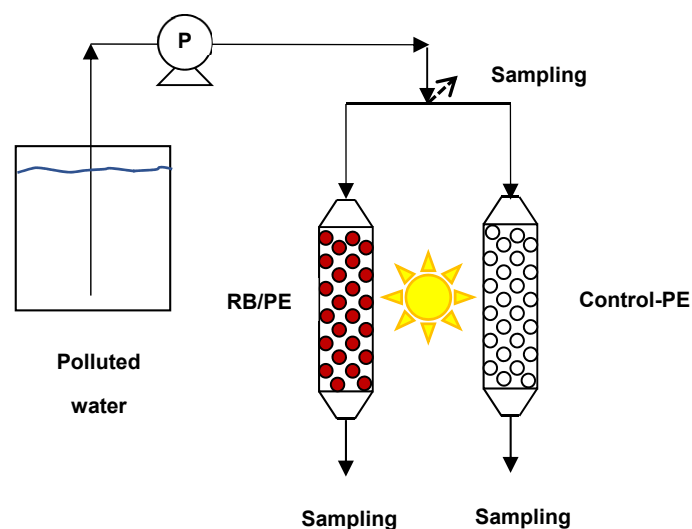


Figure 7. A scheme of continuous treatment of water polluted with pathogenic bacteria by immobilized RB (left column) and PE control beads (right column).

The results of this experiment are presented in Figure 8. For *S. aureus*, the flow of the bacterial suspension through the columns was chosen to ensure a bacterial retention time of 1 h, since after this period the bacteria were eradicated at loadings of 40 g/L or more, and the loading of RB/PE in the column was 350 g/L. Figure 8a shows that there were no live bacteria at the outlet from the column after 1 h, whereas the bacterial concentration at the outlet from the control column was the same as at the inlet. For *E. coli*, the retention time was increased to 4 h in order to provide enough time for cell inactivation. Figure 8b shows that the bacteria at the outlet from the column were eradicated already after 5 h, and this status continued during all five days of the experiment. In contradistinction, the bacterial concentration at the outlet from the control column remained unchanged.

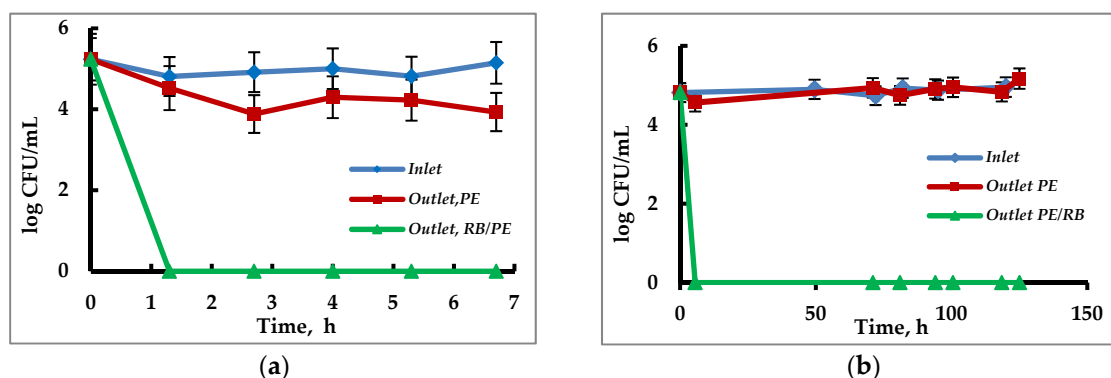


Figure 8. Results of continuous disinfection of water polluted with (a) *S. aureus* and (b) *E. coli* by 1% RB/PE under illumination with 1.25 mW/cm² white light.

3.5. Photostability of Immobilized PSs

The photostability of immobilized PSs was studied by following the bacterial concentration in two pools with tap water in which daily suspensions of Gram-positive *S. epidermidis* were added. In the first pool, strips of immobilized RB/PP were added to a loading of 4.7 g/L. The strips floated on the surface of the pool. The second pool served as a control. The water in the pools was stirred at a low rate by magnetic stirrer. Both pools were illuminated from the top by a luminescent lamp. Bacterial concentration was tested daily in both pools before the addition of a fresh portion of bacteria. It was found that the bacterial concentration in the control pool remained unchanged during the entire experiment, whereas the water in the pool with immobilized RB contained no bacterial cells during

the first 11 days, after which the bacterial concentration began to increase (Figure 9), indicating that RB ceased to disinfect the water, probably due to photobleaching.

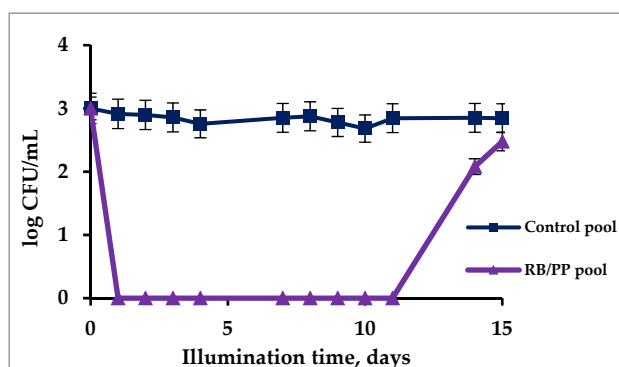


Figure 9. Testing the photostability of RB immobilized in PP under illumination with 1.25 mW/cm² white light by its activity against waterborne *S. epidermidis* added daily to an experimental pool with 4.7 g/L of 1% RB/PP and to a control pool not containing any immobilized RB.

4. Discussion

All immobilized PSs examined in the present work were active against waterborne Gram-positive bacteria under moderate illumination, and their relative efficiency was as follows: RBL/PE > HP/PE > RB/PE > MB/PE (Figures 3 and 5). Only three of the tested PSs were efficient against Gram-negative bacteria. In this case their relative antibacterial activity was: MB/PE > RBL/PE > RB/PE >> HP/PE (Figure 4). Since Gram-negative bacteria in general, and coliform bacteria in particular, are a very essential part of the microbial population in wastewater, only those PSs which are active against them can be recommended for wastewater disinfection.

Varying the loadings of immobilized PSs enables presetting conditions for the necessary extent of bacterial eradication (Figure 5). This finding, and the fact that the bacterial eradication rate depends on the initial bacterial concentration, indicate that direct contact between the bacteria and the surfaces of the immobilized PSs is necessary. In order to provide such contact, mass transfer can be improved by stirring floating beads of immobilized PSs in bacterial suspension in the case of batch processes, or by transferring polluted water via a column packed with PS/polymer beads, i.e., performing disinfection in a continuous mode. Reasonable time periods of water disinfection enable using immobilized PSs not only in batch schemes but also in continuous processes. Application of the latter mode is limited only by the photostability period of the immobilized PSs, which is estimated at approximately 10 days (Figure 9), i.e., cartridges with PS/polymer beads can be used for water disinfection over the course of several days without replacement and with no need for introducing any additional external cytotoxic agents into the treated water. In any case, water disinfection by PACT requires good light penetration into the treated system. This method is, therefore, applicable only for wastewater after secondary treatment of wastes at the water treatment plants, which decreases the water turbidity.

The results of continuous eradication of both Gram-positive and Gram-negative bacteria (Figure 8) show good applicability of the method for water disinfection. These experiments confirm, once again, that prolonged killing of bacteria is due to immobilized and not to leaked PSs, since only thoroughly pre-washed PS/polymer beads were used. The method for PS immobilization by the co-extrusion of PSs and polymers, suggested in the present work, does not require any additional chemicals, and can thus be considered as a reagent-less and totally “green” method. PSs are well-retained in the polymeric matrix, and minor PS leakage to an aqueous phase cannot interfere with applying immobilized PSs for water disinfection, since the leaked PS can also contribute to the overall antimicrobial activity. However, it does not lead to a significant increase in the total organic compounds load in the wastewater, since the concentration of leaked PSs does not exceed the ppm level even in the absence of preliminary washing of PS/polymer beads. The proposed PS immobilization method can compete with immobilization

of PSs by adsorption [20,21], which is also a reagent-less method of immobilization, except for the possibility of PS leakage and adsorption of other components from the treated wastewater.

Further development of the method may include using sunlight as the illumination source, as proposed previously by García-Fresnadillo [7,26,27]. Since bacterial eradication by immobilized PSs occurs effectively even at a very moderate light fluence rate (1.25 mW/cm^2), exposure to much more intense sunlight is expected to significantly increase the efficiency of water disinfection. The problem of a dark period can be solved by accumulation of solar energy during the day and conversion of the accumulated energy into light energy at night.

5. Conclusions

Immobilization of PSs onto a solid phase can be performed by a simple, cheap and “green” method of co-extrusion of PSs and polymers. The obtained strips and beads of PSs incorporated into polymers exhibit high antibacterial activity and can be used for water disinfection in batch and continuous regimes.

6. Patent

M. Nisnevitch, F. Nakonechny and A. Valkov, Antimicrobial Composition Made of a Thermoplastic Polymer and a Photosensitizer, US 14/995,414.

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