Heterogeneous Oxidation of Phenolic Compounds with Photosensitizing Catalysts Incorporated into Chitosan

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Abstract: The increasing amount of hazardous micropollutants in the aqueous environment has recently become a concern, especially because they are not usually included in environmental monitoring programs. There is also limited knowledge regarding their behavior in the environment and their toxicity. This paper presents results regarding the heterogeneous photosensitized oxidation of 10 phenolic compounds under visible light. All of the selected compounds are classified as pollutants of emerging concern. For the first time, the application of photosensitizing catalysts incorporated into a chitosan carrier was investigated from several points of view, namely, structure characterization, singlet oxygen generation potential, photodegradation ability, biodegradability, and toxicity assessment. It was found that compounds of different origins were degraded with high effectiveness. Photoactive chitosan was stable and could be reused for at least 12 cycles without losing its photocatalytic activity. The Hammett constants for all of the degraded compounds were determined. Improved biodegradability after the treatment was achieved for almost all compounds, apart from 4-hydroxybenzoic acid, and only slightly for 2-phenylphenol. The acute toxicity was assessed using bioluminescent Vibrio fischeri bacteria, indicating lower toxicity than the parent compounds.

Keywords: immobilized photosensitizer; micropollutant; singlet oxygen; time-resolved spectroscopy; Hammett constant; photodegradation

1. Introduction

Green chemistry is a strategy used to reduce environmental hazards through the use of safer solvents and raw materials from renewable sources. This approach minimizes the use of hazardous substances with efficient energy applications, promoting green solar energy. Thus, green chemistry is part of the framework for sustainable development [1,2].

Photosensitized oxidation is an indirect photochemical reaction. As a result of interactions with other molecules that absorb photons (photosensitizer/catalyst), photosensitization can occur. Visible light absorption produces an excited singlet state of the photosensitizer. Then, via intersystem crossing at a higher energy level, the excited triplet state of the photosensitizer is generated. In an oxygen-saturated environment, the excited photosensitizer transfers energy to molecular oxygen (3O2) with singlet oxygen (1O2) production, or transfers an electron to 3O2, thereby forming the superoxide radical anion (O2·−) [3]. The singlet oxygen is a short-lived, excited form of oxygen, but due to relatively high oxidation potential (2.42 V) in comparison to ozone (2.07 V) or even the hydroxyl radical (2.80 V), it may act as an effective oxidizing agent for the removal of micropollutants from wastewater [4].
Photosensitized oxidation is undoubtedly one of the most important photochemical methods, since it does not generate any additional pollutants [5]. Moreover, the advantage of photosensitized oxidation is the photooxidation of hard-degradable compounds by singlet oxygen generated in the presence of air and solar radiation [5–7], therefore, photosensitized oxidation is classified as a green chemistry process. Moreover, because the heterogeneous process is considered to leave no residue, no sludge is produced from the process. Furthermore, if the immobilized photosensitizer remains unchanged throughout the process, the photocatalyst can easily be reused.

Two general mechanisms of photosensitized oxidation for such reactions are well-known as Type I, also called the radical-involving mechanism, and Type II, the singlet oxygen mechanism [8,9]. Our previous research showed that singlet oxygen could be used for water purification. For this kind of area, the immobilization of photosensitizers onto various materials (heterogeneous system) is a desirable approach due to the easy separation from the reaction mixture and the preparation for reuse. Moreover, in homogenous systems (where the photosensitizer is dissolved in a water solution), the photosensitizer undergoes photobleaching, resulting in a rapid decrease in yield. Immobilized photosensitizers exhibit the advantages of good activity, stability against photobleaching, and repeated use [10]. Immobilization of a photosensitizer is associated with a decrease in the quantum yield of singlet oxygen [8,9]. This may be due to limitations in oxygen diffusion into and from the carrier material [9]. These disadvantages can be overcome by reusing immobilized photosensitizers in the water treatment, which has many environmental and economic benefits. Therefore, the green chemistry approach is an original and innovative way to remove impurities from the water environment.

The growing problem of the presence of micropollutants in the environment has demonstrated a need to find new, effective methods regarding removal or degradation [11–13]. Currently, there are no legal regulations in relation to contaminants of emerging concern [14,15]. However, the new Urban Waste Water Directive and the Water Framework Directive are underway, and it is likely that new guidelines will be introduced in the near future [16].

Bearing in mind the advantages of photosensitized oxidation, this process could be an eco-friendly solution for the removal and degradation of micropollutants in the aquatic environment. Nowadays, photochemical oxidation is developing to deal with the problem of micropollutants. This group of photochemical oxidation processes mostly includes advanced oxidation processes (AOPs) [17–19]. These processes show great effectiveness even in the case of textile wastewater [19,20]. Unfortunately, their implementation is expensive due to necessary requirements, including energy-intensive equipment, non-ecological oxidants, and other disadvantages (e.g., iron precipitation).

Considering the importance of water-treatment methods, which should remove a large amount of pollutants in a cost-efficient manner, the present study focused on a process involving visible light and a reusable insoluble photoactive chitosan carrier. The application of visible-driven photoactivation of Al(III) Phthalocyanine immobilized into chitosan beans was examined for the removal of well-known hazardous aquatic contaminants, including phenol (pH-OH), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), methyl paraben (MeP), benzyl paraben (BeP), 4-hydroxybenzoic acid (pHBA), 3,4-dihydroxybenzoic acid (3,4dOHBA), 2-phenylphenol (2-PP), and 3-phenylphenol (3-PP). To the best of our knowledge, this is the first time that such an extended investigation focused on heterogeneous photosensitized oxidation. The main objective was to study the influence of various substituents in the aromatic ring on the rate of degradation. The detailed evaluation included: (1) A time-resolved spectroscopy application to determine the lifetime of \( ^1\text{O}_2 \), as well as singlet oxygen quenching constants using a homologous series of chlorosubstituted phenols; (2) the characterization of catalysts using SEM, XRD, FTIR, and XPS techniques; (3) the photodegradation via singlet oxygen of 10 pollutants and quantification using the Hammett equation; (4) mineralization; and (5) biodegradability and toxicity assessments.
2. Results and Discussion

Based on our previous research, the Al(III) phthalocyanine chloride tetrasulfonic acid (AlPcS₄) has been selected as one of the most photostable photosensitizers [21]. UV–Vis absorption spectra of pure chitosan, as well as the chitosan after photosensitizer immobilization, has been performed (Figure S1) to prove the photoactivity in the visible range. As expected after the incorporation of AlPcS₄ into chitosan beans, the ability of visible light absorption was improved. Moreover, as can be seen in Figure S1, the spectrum after the reaction was almost the same as before the reaction, proving high photostability of the photoactive carrier.

The first stage of the study was to investigate the ability of the photosensitizer ability to generate singlet oxygen via an energy transfer mechanism. For this purpose, the influence of the solvent and the photosensitizer concentration on the lifetime of \( ^1\text{O}_2 \) were examined. At the same time, the rate constants of \( ^1\text{O}_2 \) quenching using a homologous series of chlorosubstituted phenols were determined and compared with the Hammett constants. Moreover, the chitosan carrier was characterized by Fourier transformation infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and X-ray diffraction (XRD) to check if changes in the structure and composition of the photosensitizer carrier occurred after the immobilization process or the photodegradation process.

Finally, the applicability of the photosensitized oxidation process in the degradation of various groups of phenolic compounds was conducted. The study focused on three stages of experiments: (1) The photodegradation experiments, (2) biodegradability, and (3) toxicity assessments.

2.1. Lifetime of Singlet Oxygen

As mentioned above, the main advantage of the photosensitized oxidation process is that the \( ^1\text{O}_2 \) is generated from the air under solar radiation. The effectiveness of this process depends on the lifetime of \( ^1\text{O}_2 \), the type of solvent, and the quenching of \( ^1\text{O}_2 \) by the carrier, photosensitizer, or chemical compound.

The lifetime of \( ^1\text{O}_2 \) has an important impact on the degradation of chemical compounds, therefore, we decided to check the lifetime of \( ^1\text{O}_2 \) in three solvents, namely, water, phosphate buffer, and acetone. \( ^1\text{O}_2 \) generation by photosensitizers (AlPc and AlPcS₄; physicochemical properties are displayed in Table S1) was checked using \( ^1\text{O}_2 \) measurements seen with infrared emission (phosphorescence) in the range of 1170–1400 nm (with the maximum at 1268 nm \( ^1\text{O}_2 \rightarrow ^3\text{O}_2 \); Figure S2).

The experimental results showed (Figure 1) that the longest lifetime of \( ^1\text{O}_2 \) was observed in the system where acetone was used as the solvent. The \( ^1\text{O}_2 \) lifetime in acetone was about 52.92 ± 4.04 µs, which was about 10 times longer than in water (4.4 ± 1.0 µs) or 0.05 M phosphate buffer solution (4.9± 0.9 µs). The difference in \( ^1\text{O}_2 \) lifetime in the solvents was caused by the different \( ^1\text{O}_2 \) rate interactions with solvent molecules [22,23].

The optimal AlPcS₄ concentration was also an essential parameter in the photosensitized oxidation process. The influence of AlPcS₄ concentration on \( ^1\text{O}_2 \) lifetime was performed for nine different concentrations ranging from \( 5 \times 10^{-5} \) M to \( 1 \times 10^{-5} \) M. The stock solution (\( 2.07 \times 10^{-5} \) M) was used to prepare the rest of the concentrations using appropriate dilutions. These results are presented in Figure S3.

In the system where acetone was used as the solvent, for AlPc concentrations below \( 4 \times 10^{-7} \) M, the \( ^1\text{O}_2 \) phosphorescence was not strong enough, making the results below this concentration unreliable due to huge measurement error. In the aqueous solutions (MQ water and phosphate buffer) this influence was not as significant (\( ^1\text{O}_2 \) lifetime was shorter, but within the error limits (Figure S3)). Therefore, although it was reasonable to determine the \( ^1\text{O}_2 \) quenching rate constants in acetone, photodegradation was easily performed without any interference on \( ^1\text{O}_2 \) in aqueous solutions.
Quenching of Singlet Oxygen

To clarify the mechanism of the photosensitized oxidation of the investigated compounds, the total singlet oxygen quenching rate constants (physical + chemical) using various chemical compounds were determined in acetone using time-resolved spectroscopy based on the Stern–Volmer relationship. Using the definition of the lifetime of the excited state (as measured by the decay time/lifetime of fluorescence) in the presence and absence of a quencher, the Stern–Volmer relationship was presented as follows:

\[
\frac{\tau^0_s}{\tau_s} = 1 + k_q \cdot \tau^0_s \cdot C_q,
\]

where values \( \tau^0_s \) and \( \tau_s \) are the fluorescence lifetimes (excited singlet state) in the absence and presence of the quencher, respectively, \( k_q \) is the decay rate constant, and \( C_q \) is the molar concentration of the quencher.

The graphically-determined singlet oxygen quenching constants for phenol and its chlorinated derivatives in acetone are shown in Figure 2. The determined quenching rates constant of \( ^1{\text{O}_2} \) are presented in Table 1.

The obtained rate constants of \( ^1{\text{O}_2} \) quenching were compared with data found in the literature. 2,4,6-TCP and 2,4-DCP were oxidized by \( ^1{\text{O}_2} \) at similar rates due to almost the same approximated \( k_q \) values. These values showed that the pH-OH reaction with \( ^1{\text{O}_2} \) was the slowest of the four compounds. The determined constants for the predominantly undissociated forms of compounds were slightly higher than those obtained by Tratnyek and Hoigne [24], but they were of the same order of magnitude.
The quenching constants changed depending on the concentration range, as shown in Figure 2. The quenching constant determined for 2,4-DCP concentration values below 2·10⁻⁵ M (insert) indicated a greater ability to quench singlet oxygen than concentrations above 2·10⁻⁵ M.

Figure 2. Determined values of the quenching rate constant of \( ^1 \text{O}_2 \) for phenol and chlorophenols in acetone solution.

Table 1. Determined rate constants of singlet oxygen quenching by pH-OH, 2-CP, 2,4-DCP, and 2,4,6-TCP in acetone solution.

| Compounds  | Rate Constant of Singlet Oxygen Quenching | References [24] |
|------------|-------------------------------------------|-----------------|
| pH-OH      | \((8.17 \pm 0.91) \cdotp 10^6 \text{ M}^{-1}\text{s}^{-1}\) | \((2.6 \pm 4.0) \cdotp 10^6 \text{ M}^{-1}\text{s}^{-1}\) |
| 2-CP       | \((1.11 \pm 0.08) \cdotp 10^7 \text{ M}^{-1}\text{s}^{-1}\) | \((9.2 \pm 9.4) \cdotp 10^6 \text{ M}^{-1}\text{s}^{-1}\) |
| 2,4-DCP    | \((2.98 \pm 0.22) \cdotp 10^7 \text{ M}^{-1}\text{s}^{-1}\) | \((5.1 \pm 4.7) \cdotp 10^6 \text{ M}^{-1}\text{s}^{-1}\) |
| 2,4,6-TCP  | \((2.83 \pm 0.20) \cdotp 10^7 \text{ M}^{-1}\text{s}^{-1}\) | \((1.7 \pm 0.7) \cdotp 10^7 \text{ M}^{-1}\text{s}^{-1}\) |

2.2. The Chitosan CARRIER ANALYSIS

XRD analysis showed that the chitosan carrier was a stable substance of an amorphous structure. Neither the immobilization of dye (phthalocyanine) onto the surface of chitosan, nor the reaction of dye with chitosan changed the structure of the chitosan carrier, which was confirmed XRD diffractograms (Figure S4).

XPS analysis, which allowed for the identification of elements in the sample and analysis of their binding, showed that immobilization of dye on the chitosan surface did not significantly affect the composition of the samples (Table 2).

The greatest difference was observed for the sodium atom, which made up 2.8% of pure chitosan compared to chitosan after immobilization of the dye and after the reaction. This may be due to the fact that it was not possible to elute the sodium hydroxide from the chitosan samples. However, the increase in the nitrogen and oxygen contents in the sample with the dye most probably resulted from the fact that the dye was present in the analyzed sample (chitosan + dye).
Table 2. Percentages of elements of analyzed carriers.

| Elements | Chitosan Pure | Chitosan + Dye | Chitosan + Dye after the Reaction |
|----------|---------------|----------------|----------------------------------|
| %        | %             | %              | %                                |
| C        | 61.5          | 61.4           | 42.9                             |
| O        | 29.4          | 30.7           | 43.2                             |
| N        | 4.9           | 6.6            | 3.8                              |
| Na       | 2.8           | 0.3            | 10.0                             |
| Si       | 1.5           | 1.1            | not detectable                   |

The percentage of elements in the sample after the reaction showed significant changes in its composition; an increase in the amount of oxygen and sodium in the chitosan after oxidation was observed. This was probably due to adsorption of the decomposed chemical compound or products of its decomposition. Due to the low concentration of the phosphoric buffer, its absorption could not be determined. It should be noted that the influence of some undetectable variables on the structure may have occurred, but could not be measured.

The surface studies performed using SEM for pre- and post-reaction samples (Figure S5A–C) showed no major changes. After the reaction, the adsorbed molecules on the surface of the carrier were observed. However, the amount of adsorbed substances was so low that it did not affect the process.

FTIR analyses of the pure chitosan carrier, the carrier with the immobilized dye, and the carrier with the dye after the process of photosensitized oxidation were performed. The obtained results (Figure 3) showed that there were no significant changes in the obtained spectra. Comparing the IR spectra of the chitosan sample before and after the immobilization of the dye, an increase in the adsorption signal associated with the O-H and N-H groups (range 3600–3100 cm\(^{-1}\)) was observed after immobilization of the photosensitizer.

![Figure 3. FTIR spectra for the chitosan before and after photosensitizer immobilization.](image-url)
2.3. Photodegradation

In order to investigate the photodegradation of the phenolic compounds, photolysis and photosensitized oxidation were applied.

2.3.1. Photolysis

The possibility of decomposition of the target compounds due to photolysis was investigated. The results of degradation by photolysis are given in Figure 4. The lamp used in the research had an emission spectrum that was very close to the spectrum of sunlight. The main difference in the spectra of the lamp and the sun was the lack of a UV radiation range in the lamp spectrum.

![Figure 4. Relative reduction of compound concentration in the solution after 180 min of photolysis. Absorption spectra of compounds and lamp emission spectrum (insert).](image)

After 180 min of photolysis, none of the compounds showed susceptibility to degradation with simulated solar light. The efficiency of photodegradation was below 5% of the initial concentration for all of the compounds. Such a small decay in the phenolic compounds during photolysis was caused by the fact that the absorption spectra of the compounds did not overlap with the spectrum emitted by the lamp. Based on these results, as well as results found by other researchers [25], it could be concluded that the photodegradation of phenolic compounds in the natural environment occurs very slowly. The results of the tests determining the decrease in the concentrations of chemical compounds during reactions conducted in the light, air, and pure chitosan system (without incorporated photosensitizer) are presented in Figure S6.

2.3.2. Photosensitized Oxidation

Due to the negligible degradation of the selected compounds by photolysis, the next step of the investigation was to examine the effect of the photosensitized oxidation. The process of photosensitized oxidation was repeated three times for each compound. The results presented in Figure 5 are the average values obtained from the three experiments.
Figure 5. The relative concentration decreases of the phenolic compounds due to photosensitized oxidation in the aqueous solutions depending on the reaction time. (A) phenol and chlorophenols; (B) pHBA and its derivatives; (C) phenol and phenylphenols

The extent of the degradation of the investigated chlorophenolic compounds appeared in the order 2-CP > 2,4-DCP ≥ 2,4,6-TCP > pH-OH, which was similar to results obtained by Vinu et al. [26], who oxidized chlorophenols with other types of photosensitizers and TiO$_2$. Among the compounds undergoing the oxidation process, most were decomposed by more than 50%. As seen in Figure 5A, phenol was the slowest to be decomposed by $\text{^1O}_2$.

It was concluded that chlorine substituents generally accelerated the photodecomposition of chlorophenols. However, an increase in the number of chlorine atoms in the compounds (2,4-DCP and 2,4,6-TCP) did not cause the expected acceleration of the decomposition rate. According to the quenching constant determined in the acetone solution, 2,4,6-TCP and 2,4-DCP should have been oxidized by $\text{^1O}_2$ the fastest. This trend was observed in the aqueous solution of 2,4-DCP and 2,4,6-TCP
compounds in the undissociated form [27]. However, in the buffered solution, these compounds were predominantly in dissociated form, which was probably the reason that this correlation was not observed.

Compounds belonging to the group of pHBA derivatives (Figure 5B) were all degraded to about 75%. It should be noted that the fastest degradation was observed for 3,4dOHBA. This unusual behavior could be explained by the oxidative ortho-cleavage mechanism of the aromatic ring [26,28].

As previously mentioned, the pH of the solution possibly affected the degree of oxidation of the compounds. Most of the examined compounds in the buffer solution occurred in a dissociated form, which promoted the reaction with the singlet oxygen [10]. However, when we compared the pKa values of pHOH (pKα = 9.99), 2-PP (pKα = 10.01), and 3-PP (pKα = 9.5) (Figure 5C), none of the compounds were predominantly in the dissociated form. Degrees of dissociation α were, respectively, αpH-OH = 9.3%, α2-PP = 8.9%, and α3-PP = 24%. The fastest photodegradation occurred in the case of 2-PP (about 77%), despite the fact that in the solution at pH = 9, more than 90% of the compound occurred in the non-dissociated form. Based on this observation, it was concluded that, apart from the predominant form of the compound (dissociated or undissociated), the rate of degradation of a chemical compound was also influenced by the position of the substituent.

Comparing compounds possessing two aromatic rings in their structure (BeP, 2-PP and 3-PP), despite BeP being predominantly in the dissociated form, it decomposed 5% and 10% more than 2-PP and 3-PP, respectively.

Bearing in mind that immobilization of a photosensitizer allows for its recovery and reuse, multi-cycle reuse was investigated. After each use, the chitosan carrier with the immobilized photosensitizer was collected by filtration, rinsed several times with distilled water, and dried under ambient conditions. The recovered photoactive chitosan carrier was added into a fresh reaction solution and the next cycle was carried out under the same conditions. Solutions of the same concentration were oxygenated by air and exposed to radiation. The stability and recyclability of photosensitizing catalysts incorporated into chitosan were important features for practical applications. As seen in Figure 6, the photoactive chitosan exhibited superior photocatalytic activity after twelve photodegradation cycles, which clearly and significantly demonstrated the stability of the photocatalyst. The reaction rate of 2,4DCP degradation (as an example of a phenolic compound) slightly decreased during the 5th and 12th cycles, which may have been due to accumulation of intermediates on active surface sites of the photocatalyst. However, this decrease fell within the limits of measurement error. The obtained results proved that photoactive chitosan could be considered a reusable green catalyst that photo-degrades phenolic compounds in aqueous environments.

![Figure 6](image-url)  
Figure 6. Recyclability of photosensitizing catalysts incorporated into chitosan for the photodegradation of 2,4DCP after 12 cycles.
2.4. The Hammett Constant

Hammett and Taft’s works focusing on the influence of substituents on the rate of chemical reactions initiated a new look into chemical structure description. The basic Hammett Equation (2) relates the dissociation constant of a substituted molecule characterized by the $\sigma_i$ (substituent constant, i.e., the constant assigned to a specific substituent) $K_i$, and the reference constant of the unsubstituted parent compound $K_0$.

\[
\lg\left(\frac{K_i}{K_0}\right) = \rho \sum \sigma_i, \quad (2)
\]

\[
pK_i - pK_0 = \rho \sum \sigma_i, \quad (3)
\]

\[
\Delta pK_a = \rho \sum \sigma_i, \quad (4)
\]

where $\rho$ is the Hammett constant.

This allows a quantitative description of the molecule by showing the influence of the substituents. For aromatic rings with more than one substituent, the additive nature of the $\sigma$ coefficients can be assumed (with some caution) [29,30]. The substituent constants found in the literature are presented in Table 3 [31,32]. Due to the possibility of the mesomeric phenomenon occurring at the ortho (-o) position substituents, the designated substituent constants for some substituents could not be found.

| Substituent | -o | -m | -p |
|-------------|----|----|----|
| –OH         | −0.28 | 0.12 | −0.37 |
| –Cl         | 0.68 | 0.37 | 0.23 |
| –C₆H₅       | 0.06 | 0.37 | −0.01 |
| –COOH       | 0.37 | 0.45 |
| –COOMe      | 0.37 | 0.45 |
| –COOPh      | 0.37 | 0.44 |

The plot of the Hammett equation $\Delta pK_a = f(\sum \sigma)$ for the phenolic compounds is presented in Figure 7.

![Figure 7. Hammett plot for phenolic compounds.](image)

A good correlation was found between $\Delta pK_a$ and substituent $\sigma$ values (the correlation coefficient was $R^2 = 0.941$). The highest reaction rate for 2,4,6-trichlorophenol and the lowest rate for phenol were observed. The slope of the correlation line provided a Hammett constant of $\rho = 2.4$, and the
positive sign of the slope indicated that oxidation was accelerated for more substituted compounds than phenol.

The correlation of the Hammett plot for the chlorinated compound presented in the insert (Figure 7) was even better ($R^2 = 0.998$) than for all of the investigated compounds, but the Hammett constant was slightly different.

2.5. Biodegradability

The photosensitized oxidation process with the application of the photoactive chitosan allowed for the degradation of the chemical compounds. The mineralization degree of the target compounds was carried out by TOC measurements before and after the treatment. The results of the relative decreases in TOC and COD for the target compound are presented in Figure 8. The TOC decrease indicated partial mineralization of the tested compounds. The best reduction in TOC (20%) was obtained for pHBA. A very similar TOC reduction was obtained for 3,4-dOHBA, a compound that differed from pHBA by the absence of one hydroxyl group. The reduction of TOC at the level of approximately 15% was also obtained for 2-PP and 3-PP. The loss of compounds in the tested solutions and the decrease in the TOC value indicated that the oxidation time was not long enough for further products to be released. As expected, the COD decrease was higher than relative reduction of TOC.

![Figure 8](image_url)

**Figure 8.** Comparison of the relative decreases in COD and TOC.

A very slight TOC decrease was observed in the chlorinated phenols 2-CP, 2,4-DCP, and 2,4,6-TCP. The compounds, despite their total disappearance in solutions, showed only a 5%–10% decrease in TOC value, indicating that one of the resulting oxidation products was less susceptible to further effects of singlet oxygen than the parent compounds.

To assess the biodegradability of the solutions before and after treatment, the changes in the degree of oxidation were monitored. More oxidized reaction products were not a direct indicator of improved biodegradability. Likewise, the average oxidation state (AOS) parameter provided indirect information regarding biodegradability; however, because it correlated very well with the $\text{BOD}_5/\text{COD}$ index, it was not necessary to determine $\text{BOD}_5$ [33].
The AOS and COD/TOC are parameters used in wastewater biodegradability assessment [34]. The AOS parameter takes values between +4 for CO$_2$, the most oxidized state of carbon, and −4 for CH$_4$, the most reduced state of carbon, and is expressed by the following formula (5):

$$\text{AOS} = 4 \cdot (\text{TOC} - \text{COD}) / \text{TOC},$$  \hspace{1cm} (5)

where TOC and COD are expressed in mM of C and O$_2$, respectively [33].

The COD/TOC factor is also an indicator of the degree of carbon compound oxidization. In the case of the COD/TOC parameter, lower values correspond to higher oxidation states. The calculated AOS and COD/TOC values are shown in Figures 9 and 10, respectively.

![Figure 9. Average oxidation states for samples before and after treatment.](image)

Despite the decreases in TOC and COD after the reaction, the results showed that not all of the degraded compounds were mineralized, or their biodegradability was not improved. The best biodegradability was achieved in the case of phenol and chlorophenols (the AOS value increased by more than 1). The AOS values of BeP, MeP, and 3-PP increased by 0.213, 0.514, and 0.732, respectively, meaning that the process improved the biodegradability of these photooxidation products. The AOS value also proved that the products of the oxidation process were more likely to be degradable than the parent compounds. Unfortunately, photosensitized oxidation did not change the biodegradability of some of 2-PP, which is very interesting because of its very similar structural formula to 3-PP. Moreover, the photodegradation of 2-PP in the reaction solution was better, possibly indicating that some products of the degradation of 2-PP were more resistant to photooxidation than the products of 3-PP degradation. Also, the AOS value for 3,4dOHBA after photosensitized oxidation improved (increased) very slightly, although its depletion in solution was quite fast. The reason of such poor AOS improvement was possibly the same reason as for 2-PP, i.e., that one of the products of 3,4dOHBA oxidation was hard to oxidize using singlet oxygen, or that the oxidant was more specific for some by-products in the reaction solution. In the case of pHBA, the AOS value decreased so it was probably that the by-products created during the process were more difficult to degrade than the parent compound.
The obtained results of COD/TOC, for most of the tested solutions, were satisfactory and confirmed the increase in the degree of carbon oxidation during the treatment. Although the greatest decrease in the COD/TOC ratio was obtained for 2,4-DCP (more than 1), the values obtained for pH-OH, 2-CP and 2,4,6-TCP were also satisfactory (decreased by more than 0.6). The pHBA solution was similar to the AOS parameters, whereby the COD/TOC ratio indicated a reduction in the degree of carbon compounds oxidation. The most probable explanation for this would be that during purification, more complex compounds were formed than the parent compounds. In the case of 2-PP, the COD/TOC results, which were similar to the AOS values, showed that there was no change in the degree of carbon compounds oxidation during treatment of 2-PP, meaning that there was a really small improvement in biodegradability. The treatment appeared to improve biodegradability in all cases, except that of pHBA.

2.6. Toxicity

The obtained EC$_{50}$ results showed that BeP was the most toxic compound among all of the examined compounds, and its determined EC$_{50}$ value was 0.0339 mg·dm$^{-3}$. Due to the fact that BeP is used as a preservative, such high, acute toxicity was not particularly strange, and the value obtained fell within the range of values obtained by other researchers (Table 4). High toxicity (low value of EC$_{50}$) was also characterized by 2-PP and 3-PP, with respective values of 1.932 and 0.4932 mg·dm$^{-3}$. The value obtained for 2-PP was also in the range determined in earlier studies, while the EC$_{50}$ value for 3-PP was not found in earlier publications. In this case, the position of the substituting phenolic group into phenol significantly affected the toxicity of the compounds. Both compounds (2-PP and 3-PP) showed higher toxicity than other commonly-used preservatives, e.g., MeP. In the case of phenol and its chlorinated derivatives, the toxicity of the compounds increased with an increasing number of chlorine atoms.
Table 4. The EC$_{50}$ values after 15 min and toxic units for the compounds tested before and after the photosensitized oxidation process.

| Compounds | EC$_{50}$ mg dm$^{-3}$ | Time 0 min | Time 120 min | References |
|-----------|-----------------------|------------|--------------|------------|
| pH-OH     | 20.22 (17.41–23.47)   | 404.4      | 261.7        | [35,36]    |
|           | 18.50                 | 0.25       | 0.38         |            |
| 2-CP      | 4.875 (3.704–6.032)   | 303.0      | 206.1        | [36,37]    |
|           | 4.875                 | 0.33       | Non toxic    | -          |
| 2,4-DCP   | 3.152 (1.349–7.366)   | 97.5       | 7.14         | [36]       |
|           | 1.932                 | 1.03       | 0.49         |            |
| 2,4,6-TCP | 1.509 (2.474)         | 34.91      | 45.85        | [38]       |
|           | 0.4932 (0.4544–0.5353)| 9.81       | 32.62        |            |
| 2-PP      | 1.09 (1.509–2.474)    | 34.91      | 21.8         |            |
|           | 1.09                  | 2.86       | 2.54         |            |
| 3-PP      | 0.4932 (0.4544–0.5353)| 9.81       | 32.62        |            |
| pHBA      | 3.717 (3.717–9.091)   | 127.3      | 108.58       | [39]       |
|           | 17.08 (9.109–32.02)   | 0.79       | 0.92         |            |
| 3,4dOHBA  | 1.509–2.474          | 34.91      | 21.8         | [38]       |
|           | 1.509                 | 2.86       | 2.54         |            |
| MeP       | 4.41 (1.062–16.23)    | 91.91      | 125.02       | [40–43]    |
|           | 1.09                  | 1.09       | 0.80         |            |
| BeP       | 0.033 (0.0313–0.0367) | 3.6        | 28.19        | [40,41]    |

A comparison of toxicity before and after the oxidation process revealed that three of the ten tested substances formed products that were more toxic than the parent compounds. Despite the fast degradation of 3,4dOHBA, the toxicity of the solution after treatment increased by 45%, showing that the products of the oxidation were more toxic. Furthermore, the small change in the AOS value after the treatment also showed that the oxidation product compounds were harder to biodegrade than 3,4dOHBA. The toxicity values obtained for phenol and its degradation products increased by about 40%, however, it should be noted that after the entire photosensitizing process, the loss of phenol was 41%. For the pHBA, the increase in the toxicity of the solution after the reaction was not more than 30%, despite 68% degradation of the parent compound after the oxidation process. However, the highest decrease in toxicity after the photooxidation process was observed in the case of BeP. Although the BeP solution still showed acute toxicity after the process, it was nearly 10 times lower than the solution before the process, despite 21% of the compound remaining after the process.

An alternative way of expressing toxicity data is as a function of the undiluted sample. This form of expression is known as the toxic unit (TU), and is defined as

$$TU = \frac{100}{EC_{50}}.$$  (6)

Using TU to express toxicity was advantageous in the regard that toxicity increased with increasing TU values. The obtained results are shown in Table 4.

According to Persoone et al. [44], the toxicity of the wastewater was divided into five groups:

I—no acute toxicity TU < 0.4;
II—slightly acute toxicity 0.4 ≤ TU < 1;
III—acute toxicity 1 ≤ TU < 10;
IV—highly acute toxicity 10 ≤ TU < 100; and
V—very highly acute toxicity 100 ≤ TU.

By assigning the results to the division proposed by Persoone et al. [44], BeP, which was the most toxic compound studied, belonged to the highly acute toxicity group, but 3-PP also belonged to the same group of toxicity. Most of the compounds used in this research showed toxicity, which was classified according to Persoone et al. [44], at the level of group III, i.e., acute toxicity. After the treatment, five compounds moved into a toxicity group. The degradation products of 2,4,6-TCP showed such low toxicity that after 120 min of photodegradation, the solution was classified as group
I—no acute toxicity. The toxicity of the BeP solution after treatment decreased almost 10 times, and the solution changed from group IV to group III. This means that after 120 min of treatment, the BeP solution degradation products were acutely toxic. The same change was observed in the case of 3-PP. The 3-PP solution after phototreatment was still toxic. The MeP and 2,4-DCP solutions after treatment were classified as group II.

3. Materials and Methods

3.1. Materials

Al(III) phthalocyanine chloride tetrasulfonic acid (a mixture of regio-isomers; AlPcS$_4$) was used as the photosensitizer and was purchased from Frontier Scientific (Logan, UT., USA). Phthalocyanine was immobilized on chitosan beads according to a procedure presented elsewhere [24]. Na$_2$HPO$_4$ and NaOH were used to prepare 0.05 mol dm$^{-3}$ buffer solutions in concentration at pH 9.

The photoactive chitosan carrier was prepared in the form of beads. Hydrogel beads were produced using phase inversion from an aqueous solution of chitosan in acetic acid. The acetic acid chitosan solution was dropped with a needle into NaOH, where coagulation was carried out. Photosensitizer immobilization of chitosan carriers was carried out by adsorption of the photosensitizer from the aqueous solution. When the photoactive chitosan carrier was put into the reactor, the reaction solution was added. The mass ratio of photosensitizer to chitosan was 1:2750.

All reaction solutions were prepared in distilled water, treated additionally in a Millipore Milli-Q Plus System (18.2 MΩ). All degraded compounds are presented in Table S2. The purity and providers of the chemical compounds that were used are described in Text S2. The concentration of all pollutants was 5 mg dm$^{-3}$.

3.2. Methods

Reactions were conducted in a glass reactor with a volume of 0.5 dm$^3$. The buffered reaction mixtures were agitated by air stream at a flow rate of 75 dm$^3$h$^{-1}$. Simulated solar light (HPS lamp, Lumatek 600 W, (Lumatek LTD, Rochester, U.K.)) was used to excite the photosensitizers (mass of photoactive chitosan beads: 27 g). The constant temperature of the reaction mixture, set at 25 °C, was maintained using the reactor equipped with a cooling jacket.

Reaction progress was monitored by concentration determination using an Agilent 1220 Infinity LC HPLC apparatus equipped with a Poroshell 120 C18 column (2.7 µm) (Agilent Technologies Inc., Hamburg, Germany). The 0.1% formic acid water solution (A) and the 0.1% formic acid methanol solution (B) were used as eluents. The columns were thermo-stated at 40 °C. The method involved gradient elution (presented in Text S3) with a flow rate of 0.7 mLmin$^{-1}$ for methyl paraben (MeP), benzyl paraben (BeP), 2-phenylphenol (2-PP), and 3-phenylphenol (3-PP) and 0.8 mLmin$^{-1}$ for phenol (pH-OH), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6- TCP), 4-hydroxybenzoic acid (pHBA), and 3,4-dihydroxybenzoic acid (3,4OHBA). The injection volume was 10 µL for parabens and phenylphenols, while the phenolic derivative were 70 µL in volume. Under these conditions, very good linearity was obtained (at least R$^2 = 0.998$) for every compound. The limits of quantification were 0.0066 mg dm$^{-3}$, 0.0058 mg dm$^{-3}$, 0.0078 mg dm$^{-3}$, 0.0166 mg dm$^{-3}$, and 0.0468 mg dm$^{-3}$ for pH-OH, 2-CP, 2,4-DCP, 2,4,6- TCP, and 3-PP respectively.

An acute ecotoxicity bioassay was conducted using a Microtox® Model 500 analyzer (Modern Water, New Castle, DE, USA) using original Microtox® solutions and reagents with the marine bacteria Vibrio fischeri as a bioluminescent indicator. The “81.9% basic test” protocols, which were available with the MicrotoxOmniTM analyzer software, were used for the ecotoxicity assessment. The toxicity was expressed as an effective concentration (EC$_{50}$), meaning that the toxicant caused 50% inhibition of the luminescence, as well as toxicity units, according to calculation presented in [44]. The toxicity analysis was performed before and after the photosensitized oxidation. The samples were incubated in contact with the bacteria for 15 min.
The singlet oxygen lifetime was determined in water, phosphate buffer, and acetone solutions using the high-performance fluorescence lifetime system FluoTime 200 (PicoQuant, Berlin, Germany). The singlet oxygen quenching constants for the used compounds were also determined. Samples were excited by the laser and fluorescence was collected in the usual 90° angle orientation.

XPS analysis was carried out with a Kratos AXIS Ultra spectrometer (Kratos Analytical Ltd, Manchester, UK) with the use of a monochromatic Al Kα X-ray source of excitation energy at 1486.6 eV. The spectra were obtained using an analysis area of 300 µm × 700 µm. The power of the anode was set at 150 W and the hemispherical electron energy analyzer was operated at a pass energy of 20 eV for all high-resolution measurements. The use of a charge neutralizer during spectra collection was necessary due to the insulating character of the samples. Reproducibility was ensured by taking more than three measurements of the analyzed sample.

XRD analysis was used to identify any structural changes in the chitosan samples. The powder X-ray diffraction patterns were collected at ambient temperature using a PANalytical X’Pert Pro MPD diffractometer with Bragg–Brentano reflecting geometry (PANalytical BV, Almelo, The Netherlands). Copper Cu Kα radiation from a sealed tube was applied to these measurements.

4. Conclusions

This paper presents the results regarding the heterogeneous photosensitized oxidation of 10 phenolic compounds with visible light. This is the first time such an extensive study of the heterogeneous system has been presented. The application of photosensitive chitosan carriers was investigated from several points of view, including structure characterization, singlet oxygen generation potential, photodegradation ability, biodegradability, and toxicity assessment.

The results of XPS, XRD, SEM, and FTIR showed no major changes in the carrier structure, even after the process of photooxidation. The determined constants of $^1\text{O}_2$ quenching by the compounds in acetone were similar to those obtained by scientists for deuterium water when the compounds were in the most non-dissociated form. The order of photooxidation decay of chlorinated phenol derivatives in the buffered solution was 2-CP > 2,4-DCP ≥ 2,4,6-TCP > pH-OH. In the group of p-hydroxybenzoic acid derivatives, the 3,4-dOHBA underwent the most rapid oxidation, with a high probability of ortho-cleavage of the compound ring. The high degradation effectivity of different origin compounds was correlated with the predominant form of the compound, whether this was dissociated or non-dissociated, as well as the position of the substituent. The increasing value of the AOS parameter after photosensitive oxidation provided indirect information regarding the biodegradability improvement of the reaction solution. The TOC analysis indicated slight mineralization but, at the same time, a large reduction in toxicity was achieved for seven compounds. The acute toxicity assessment showed that most of the products formed during the oxidation were less toxic than the parent compounds. Small increases in the acute toxicity for pH-OH, pHBA, and 3,4dOHBA were observed, which were probably due to hydroquinone generation. The obtained results proved that photoactive chitosan could be considered as a reusable green catalyst that photo-degrades phenolic compounds in aqueous environments.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/9/11/891/s1. Figure S1: UV-Vis absorption spectra of photoactive chitosan beans before and after reaction, and the UV-Vis spectrum of pure chitosan. Table S1: Physicochemical properties of Al(III) phthalocyanine chloride and Al(III) Phthalocyanine chloride tetrasulfonic acid. Figure S2: 3D view of the luminescence signal spectrum of $^1\text{O}_2$ generated by AlPc in acetone. Figure S3: Change in $^1\text{O}_2$ lifetime in relation to AlPc concentration. Text S1: Chitosan carrier analysis. Figure S4: XRD diffractograms of chitosan carriers. Figure S5: Pictures of the chitosan surface before and after the reaction using SEM analysis. Figure S6: Decrease in the concentration of 2,4-DCP and BeP in solution with the carrier without an incorporated photocatalyst. Text S2: Purity and providers of used chemicals compounds. Table S2: Physical and chemical properties of used chemical compounds. Text S3: Gradient method used during chromatographic analysis.

Author Contributions: M.G. conceived the experiments; M.F. and M.G. designed the experiments; M.F. prepared the carriers, performed the experiments, and performed the chemical analyses; M.G. and L.B. performed the
carrier analyses; M.F., L.B., and M.G. analyzed the data; M.F. wrote the paper; L.B., M.G., and S.L. revised the paper; M.G. and S.L. supervised the project.

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