

Original Research

**DEGRADATION OF PONCEAU S DYE BY ADVANCED OXIDATION PROCESSES:
COMPARATIVE ANALYSIS AND TOXICITY OF TRANSFORMATION
PRODUCTS**

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Abstract

*This study investigates the degradation of Ponceau S (PS), a synthetic azo dye, using advanced oxidation processes (AOPs), including UV irradiation, UV/Fe(III), and UV/Fe(III)/H₂O₂ systems. The aim was to compare the decolorization efficiency and kinetics of these processes, and to assess the toxicity of the generated products on the extremophilic microalga *Chlamydomonas acidophila* PM01. UV irradiation alone showed no significant effect on PS degradation. In contrast, systems involving Fe(III) ions led to notable decolorization. This was further enhanced in the presence of H₂O₂ due to increased generation of hydroxyl radicals. The highest degradation efficiency was observed in the UVA/Fe(III)/H₂O₂ and UVB/Fe(III)/H₂O₂ systems. Toxicity assessment revealed that untreated PS did not significantly affect microalgal growth or viability. However, degradation products formed during AOPs treatment induced a decrease in cell viability, implying the emergence of reactive and potentially harmful intermediates. These findings highlight the importance of evaluating both degradation efficiency and ecotoxicological impact when applying AOPs for dye removal from wastewater.*

Keywords: photodegradation, Ponceau S, AOPs, toxicity, *Chlamydomonas acidophila* PM01.

INTRODUCTION

Synthetic azo dyes are the most widely used classes of colorants in industries such as textiles, printing, cosmetics, food processing, and pharmaceuticals [1]. However, their presence in wastewater represents a significant environmental concern due to their high

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stability, resistance to biodegradation, and potential toxicity [2]. Also, the presence of aromatic rings and azo ($-\text{N}=\text{N}-$) bonds in their structures, makes their removal from aquatic environments by conventional wastewater treatment methods challenging [3,4]. Ponceau S (PS) is azo dye commonly used in dyeing industry of textile, leather and paper and as a staining reagent for proteins in biochemical analyses [5–7].

Advanced oxidation processes (AOPs) offer promising opportunities for degradation or even mineralization of synthetic dyes. Among AOPs, those involving hydrogen peroxide such as $\text{H}_2\text{O}_2/\text{UV}$, the Fenton process and the photo-Fenton process generate highly reactive and non-selective hydroxyl radicals $\cdot\text{OH}$. Hydroxyl radicals are also produced by photolysis of Fe(III) hydroxo complexes without addition of H_2O_2 [8,9].

The aim of this study is (a) to compare the decolorization rates of PS dye induced by UV irradiation, UV induced photolysis of Fe(III) hydroxo complexes, and UV induced photolysis of Fe(III) hydroxo complexes in the presence of H_2O_2 , and (b) to evaluate toxicity of PS and its degradation products on extremophilic microalga *Chlamydomonas acidophila* PM01.

MATERIALS AND METHODS

Reagents

The PS was purchased from Serva GmbH. Hydrogen peroxide (H_2O_2 , 30%) was obtained from Sigma-Aldrich, while $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ was supplied by Centroprom. All experiments were carried out using bidistilled, deionized ultrapure water (18 M Ω). Stock solutions of PS (1 mM), FeCl_3 (1 mM), and H_2O_2 (10 mM) were prepared in deionized water. The pH of the solutions was adjusted to pH 5 using NaOH or HCl.

UV-Vis spectroscopy

The UV-visible (UV-Vis) spectra of samples were recorded from 200 to 800 nm using UV-Vis spectrophotometer Shimadzu UV-i1900 with a spectrometric quartz cell (1 cm path length). All samples were freshly prepared, and measurements were performed immediately after preparation. Scan time was 50 s.

Irradiation

Samples were prepared in deionized water pH 5 (sample volume 1 mL), placed in a quartz cuvette (1 cm $\times$ 1 cm $\times$ 5 cm), and exposed to UV radiation up to 10 minutes using a lamp equipped with a filter for visible light (Carl Roth GmbH, Karlsruhe, Germany, H469.1), and UV tubes (Sankyo Denki, Tokyo, Japan). Measurements were carried out immediately after sample preparation (for non-irradiated systems), or 30 s after the end of UV irradiation. The UV A and UV B lamps emitted maximum radiation at wavelengths of 312 nm and 352 nm, respectively. The irradiation took place in a temperature-controlled chamber, and the irradiance was set at 17 J m $^{-2}$ s $^{-1}$ for UV A and 7.7 J m $^{-2}$ s $^{-1}$ for UV B.

Algae culture and growth analysis

C. acidophila strain PM01 was isolated from acid mine drainage from Parys Mountain, Anglesey North Wales, UK. Microalgae were grown under photoautotrophic conditions in an optimal, nutrient sufficient modified MAM medium, with a composition prepared according to Olaveson and Stokes (1989) [10]. The initial pH of the medium was set to ~ 3 . Microalgal

culture replicates were added to 50 mL of MAM medium in 100 mL Erlenmeyer flasks at the initial density of 5×10^5 cells/mL. Microalgae were grown at 22 °C on orbital shakers (120 rpm) in a growth cabinet with a continuous photon flux of $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ (MST TL-D Reflex 36W840 1 SLV/25 tubes, Philips, Amsterdam, The Netherlands). The culture's growth was monitored on the three independent samples by optical density at 750 nm (OD_{750}) and 680 nm (OD_{680}), and the viability of the cell cultures, until the reaching of late stationary phase of growth (19 days). Cultures were treated using degradational products of PS in final concentrations 50 μM PS, 20 μM Fe^{3+} and 100 μM H_2O_2 . After 24 h of treatment, both treated cultures and corresponding controls (untreated) were assessed for the biomass concentration (OD_{750}), an indirect indicator of chlorophyll a content (OD_{680}) [11]. Absorbance at 750 nm was used to correct for turbidity, and the $\text{OD}_{680}/\text{OD}_{750}$ ratio was taken as a relative chlorophyll a per unit biomass [12]. OD was estimated using a UV-Vis spectrophotometer (Jenway Genova Plus, Staffordshire, UK). For measurements, samples were diluted to maintain the OD values below 1. The effects of PS degradation products on cell viability were tested using the Evans Blue stain, commonly used measure of cell viability [13]. Non-viable cells have compromised or ruptured membranes with increased permeability, which enables the uptake and accumulation of Evans Blue dye. Cell viability is expressed as the percentage of Evans Blue negative cells. At least 100 cells were analyzed per sample.

RESULTS AND DISCUSSION

The effect of UV irradiation on the degradation of PS azo dye in aqueous solution at pH 5 was investigated using UV–Vis spectroscopy (Figure 1).

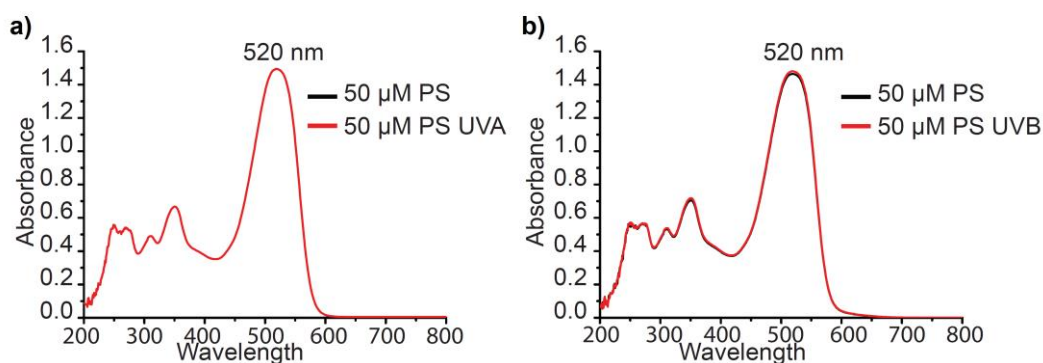


Figure 1 Effect of UV irradiation on 50 μM PS in aqueous solution at pH 5 under: a) UVA irradiation; b) UVB irradiation

The UV–Vis spectrum of PS exhibits four distinct absorption maxima in the 200–400 nm range, along with a prominent absorption band in the visible region at 520 nm, which can be attributed to $n \rightarrow \pi^*$ transitions of the $\text{N}=\text{N}$ chromophore [14]. After exposure of PS to UVA and UVB irradiation (Figure 1a,b), no changes in the absorption maxima or band positions were observed. These results indicate that UVA and UVB irradiation do not promote degradation of PS via photo induced reactions under the applied experimental conditions.

On the other hand, the effects $\cdot\text{OH}$ radicals produced by UV induced photolysis of Fe(III) hydroxo complexes on the degradation of PS (50 μM) are shown in Figures 2a,b). A noticeable decrease in absorbance at 520 nm is observed upon UV irradiation of PS in the presence Fe^{3+} ions. Under UVA irradiation, the absorbance decreases progressively, as confirmed by the time-dependent plot (Figure 2a), where A_{520} drops significantly over 10 minutes. Similar results are observed under UVB irradiation (Figure 2b). Time-dependent measurements further demonstrate that the degradation process follows a gradual decline in absorbance intensity, indicating a continuous transformation or degradation of the absorbing species. Additionally, the reduction in absorbance at 520 nm is more pronounced in the UV irradiated system containing Fe^{3+} ions and H_2O_2 , compared to Fe^{3+} alone (Figure 2c,d).

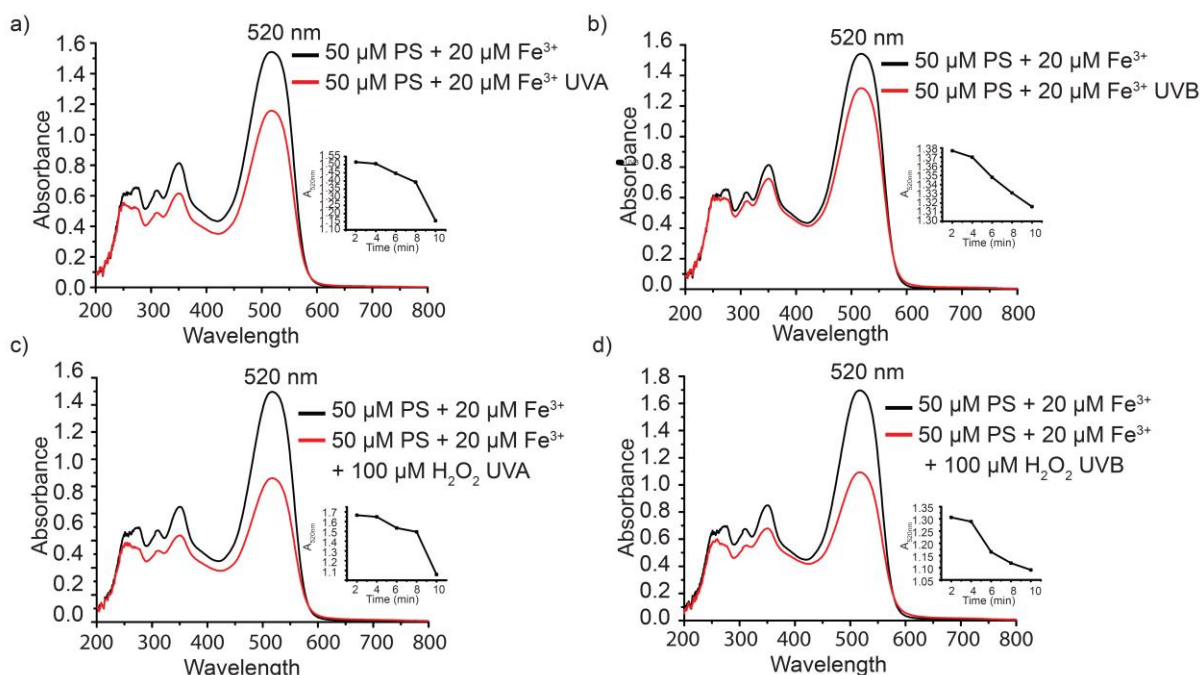


Figure 2 Effect of UV irradiation on a 50 μM PS aqueous solution in the presence of 20 μM Fe^{3+} at pH 5 under: a) UVA irradiation; b) UVB irradiation; as well as in the presence of 20 μM Fe^{3+} and 100 μM H_2O_2 under: c) UVA irradiation; d) UVB irradiation

The efficiency of the UV/ Fe^{3+} and UV/ Fe^{3+} / H_2O_2 systems, as well as the reaction kinetics for these processes are presented at Figure 3. Under UVA irradiation (Figure 3a), the decolorization efficiency gradually increase with time for both systems. However, the UVA/ Fe^{3+} / H_2O_2 system consistently showed higher removal efficiency than UVA/ Fe^{3+} alone. After 10 minutes, the decolorization reached approximately 35% in the presence of H_2O_2 , compared to about 24% without it. The rate constant for UVA/ Fe^{3+} / H_2O_2 ($k = 0.01983 \text{ min}^{-1}$) is higher than that of UVA/ Fe^{3+} ($k = 0.01469 \text{ min}^{-1}$), confirming the positive role of H_2O_2 in decolorization process. This improvement can be attributed to the photo-Fenton-like reaction, where H_2O_2 reacts with Fe^{2+} to produce highly reactive $\cdot\text{OH}$ radicals. Under UVB irradiation (Figure 3b), the decolorization efficiency was evidently higher for the UVB/ Fe^{3+} / H_2O_2 system compared to the UVB/ Fe^{3+} system. The UVB/ Fe^{3+} / H_2O_2 system achieved approximately 35% removal within 10 minutes, whereas the UVB/ Fe^{3+} system

reached only about 12%. This enhanced performance under UVB can be attributed to its higher photon energy, which facilitates more efficient photolysis of H_2O_2 and faster regeneration of Fe^{2+} , thereby accelerating hydroxyl radical production. The kinetic constants further support this observation: the rate constant for the UVB/ $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ system ($k = 0.02506 \text{ min}^{-1}$) is significantly higher than that for the UVB/ Fe^{3+} system ($k = 0.00598 \text{ min}^{-1}$), indicating a substantially faster reaction rate when both UVB and H_2O_2 are present.

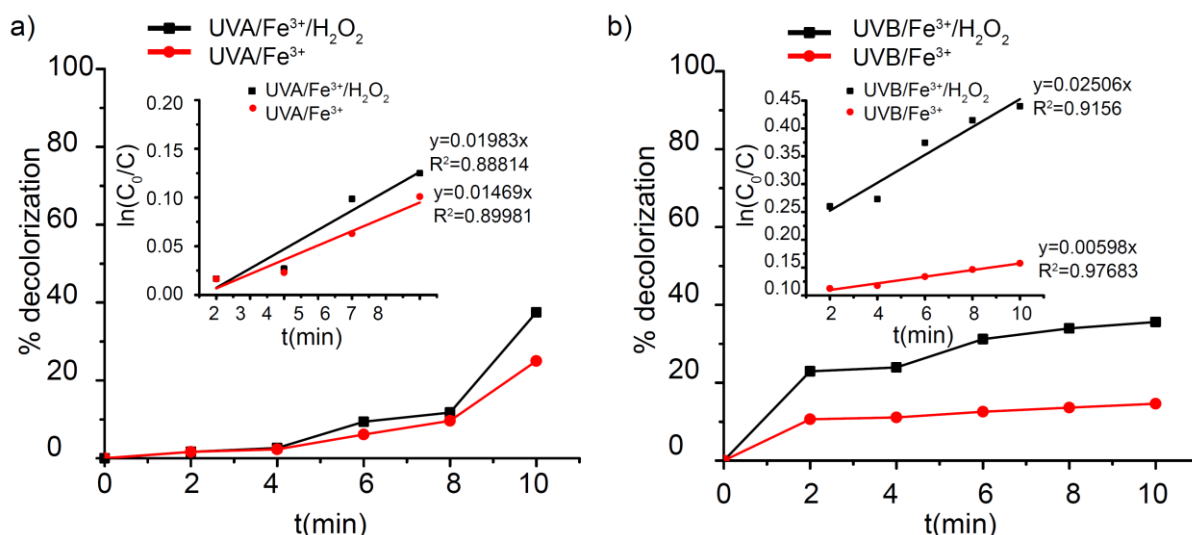


Figure 3 Comparison of PS decolorization by UV/ Fe^{3+} and UV/ $\text{Fe}^{3+}/\text{H}_2\text{O}_2$ systems under: a) UVA; b) UVB irradiation. Experimental conditions: $[\text{PS}] = 0.05 \text{ mM}$, $[\text{H}_2\text{O}_2] = 1 \text{ mM}$, $[\text{Fe}^{3+}] = 0.02 \text{ mM}$, pH 5. Inset: Kinetics of the decolorization processes

Azo dyes and their degradation products have been widely reported to exert toxic effects on aquatic organisms, including microalgae, with impacts on growth and photosynthetic pigments as part of stress responses [15]. Regardless of the wavelength, OD values followed a similar trend (Figure 4a). Treatments with H_2O_2 led to an increase in OD, likely reflecting an oxidative stress response (Figure 4a). Low-dose exposure to H_2O_2 can trigger stress acclimation responses in microalgae, which may temporarily enhance metabolic activity without causing immediate damage [16]. An increase in OD values was also observed in the case of PS exposed to UV irradiation (Figure 4a). Since we established that no significant photodegradation occurs under UV irradiation alone (Figure 1), this suggests that the effects are attributable to the parent azo dye [17]. On the other hand, treatment using pure PS exhibited similar OD values to the control (Figure 4a). Although, to the best of our knowledge, no data are available on the effects of PS on microalgae, other azo dyes, including typical monoazo compounds, have been reported to exert toxic effects on microalgae [18,19]. Therefore, rather than outright cytotoxicity, PS exposure may instead elicit transient metabolic responses that can include stress-induced changes in photosynthetic activity. Interestingly, the A_{680}/A_{750} ratios, which reflect chlorophyll content relative to biomass, were very similar across all treatments. This ratio is commonly used as an indicator of photosynthetic activity and the physiological state of microalgal cells [12] suggesting that

photosynthetic function was largely maintained in all treated groups. However, peaks were observed in the Fe treatment and in all combinations with UVA (Figure 4a).

A marked reduction in viability was observed in *C. acidophila* following 24 h treatment with H_2O_2 and Fenton reagents, and was likewise evident in samples treated with PS in combination with Fe^{3+} or H_2O_2 (Figure 4b). Hydrogen peroxide and Fenton-type reactions are well known to induce cytotoxicity through the generation of highly reactive hydroxyl radicals, leading to oxidative damage, lipid peroxidation, and cell death [20]. High impact on cell viability was observed following PS photodegradation under UVB irradiation (Figure 4b), where no changes in absorption maxima or band positions were detected (Figure 1b). This effect may be attributed to light screening and photophysical interference in the presence of the azo dye rather than to the degradation products themselves, as strongly absorbing dyes can attenuate incident radiation through absorption and inner filter effects [21]. Further, the trend in viability largely mirrored that observed during the decolorization process (Figures 3 and 4). Thus, PS in combination with Fe^{3+} and H_2O_2 and UVA induced a decrease in viability of more than 10% compared to the corresponding control (Figure 4b). A similar trend, although to a lesser extent, was also observed for treatments using photolysis products of PS under both types of irradiations (Figure 4b). However, this did not reflect the relationship observed in decolorization efficiency (Figure 3). Specifically, photolysis under UVA irradiation was approximately twice as effective, whereas the percentage of viable cells remained nearly the same for both treatments (Figure 4b). These results suggest that, under the applied AOPs for PS degradation, cytotoxic effects may arise due to the formation of reactive and potentially toxic transformation products [22].

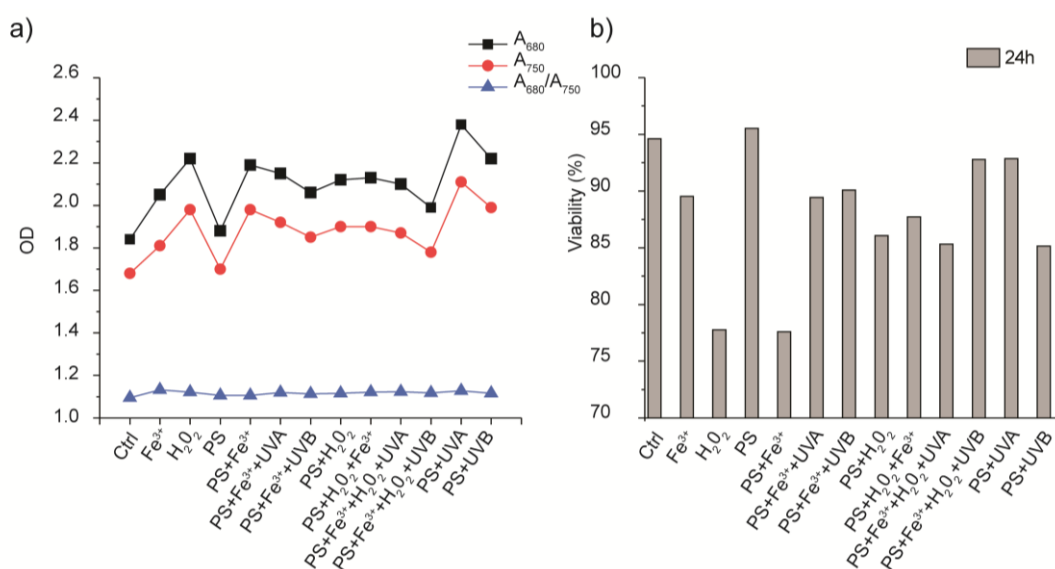


Figure 4 a) Growth (optical density at 750 nm and 680 nm); and b) cell culture viability (Evans Blue staining) determined 24h after the exposure to PS and its degradation products

CONCLUSION

The results of this study demonstrate that UV irradiation alone is not effective for the degradation of PS, confirming its photostability under the applied conditions. In contrast, the

inclusion of Fe(III) ions significantly enhances the decolorization process, while the addition of H₂O₂ further improves efficiency due to intensified hydroxyl radical production via photo-Fenton-like reactions. Among the tested systems, the UVA/Fe(III)/H₂O₂ and UVB/Fe(III)/H₂O₂ processes showed the highest degradation rate and overall efficiency.

Toxicity assessment revealed that the parent dye did not significantly affect the growth and viability of *Chlamydomonas acidophila* PM01. However, degradation products formed during AOPs treatment exhibited appreciable effects on cell viability, indicating the generation of reactive and potentially harmful intermediates. These findings emphasize that, in addition to removal efficiency, the formation and impact of transformation products must be carefully considered when applying AOPs for wastewater treatment.

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