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Comparative Study of Rhodamine B Treatment: Assessing of Efficiency Processes and Ecotoxicity of By-Products

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Abstract: In this work, a comparative study between two processes was performed—biodegradation and photocatalysis, as an advanced oxidation process—to discover which one is more efficient to degrade Rhodamine B, a synthetic dye widely used in the textile and food industries. The advantage of this study is that it correlates treatment efficiency with the ecotoxicity of the by-products resulting from the treatments. Since the COVID-19 pandemic, it has been difficult to use activated sludge because of the risk factor of COVID-19 infection. Therefore, biodegradation tests were conducted with the yeast *Saccharomyces cerevisiae* in this study. For the photocatalysis assays, TiO_2 doped with 5 per cent Cerium was used as a catalyst under UV light irradiation. *S. cerevisiae* cannot reduce RhB by biodegradation. However, a 13 per cent biosorption was observed with an uptake capacity of 4.2 mg g^{-1} dry matter of *S. cerevisiae* cultivated in the presence of 5 mg L^{-1} of RhB after 150 min. At a 5 mg L^{-1} of RhB concentration, the 6 h photocatalysis treatment led to 55% color removal and 8.6% COT reduction. The biodegradability of the photocatalyzed solution increased since the BOD_5/COD ratio raised from 0.10 to 0.42. In the presence of glucose as a source of carbon, yeast can grow on the by-products generated by photocatalysis. The phytotoxicity of RhB in solution was measured using the germination index (GI) of watercress seeds. The GI decreases by 75% for an RhB solution of 100 mg L^{-1} compared to the control sample. The by-products of the photocatalytic treatment, using crustaceans *Daphnia magna* and conducted with solutions of Rhodamine B, induced a decrease of 24% in the GI. Lethality test. After 3 or 6 h of treatment, no increase in immobilization or mortality of *D. magna* was observed compared to the negative control.

Keywords: Rhodamine B degradation; *Saccharomyces cerevisiae*; photocatalysis; TiO_2-Ce; eco-toxicity assessment

Citation: Chadelaud, T.; Zeghioud, H.; Reynoso de la Garza, A.; Fuerte, O.; Benítez-Rico, A.; Revel, M.; Chávez-Miyauchi, T.E.; Djelal, H. Comparative Study of Rhodamine B Treatment: Assessing of Efficiency Processes and Ecotoxicity of By-Products. *Processes* **2023**, *11*, 2671. https://doi.org/10.3390/pr11092671

Academic Editors: Raluca Maria Hlihor, Isabela Maria Simion and Mihaela Rosca

Received: 26 June 2023
Revised: 30 August 2023
Accepted: 31 August 2023
Published: 6 September 2023

1. Introduction

Today, many industries use synthetic dyes. However, their presence in the water discharged by these industries represents a danger to the environment, particularly to aquatic life, as well as to human health [1]. Rhodamine B (RhB), a dye commonly used in the textile, leather, and food industries [2], induces numerous ecological problems because its presence in water prevents light penetration, leading to a reduction in photosynthesis in aquatic plants [3]. Furthermore, the carcinogenicity and mutagenicity of this dye have been demonstrated [4,5]. Rhodamine B can therefore accumulate in the human or animal digestive system through diet, causing various digestive diseases such as stomach polyps and intestinal tumors and liver dysfunction [6]. The dye studied proves to be highly carcinogenic and neurotoxic for living beings [7].

Biological treatments of synthetic dyes have been studied, but these processes do not degrade dyes completely [8]. However, [9] *Saccharomyces cerevisiae* ATCC 9763 showed a high potential to degrade azo bond and to decolorize methyl red within 12 h. Jia and colleagues [10] carried out the degradation of monosodium glutamate by synergetic treatment using *S. cerevisiae* and *Coriolus versicolor* and obtained a 60% color removal efficiency. Jadhav and Govindwar observed a 85% decrease in malachite green concentration in the aerobic condition by *S. cerevisiae* [11].

Ikram et al. conducted a study on the biodegradation of the Azo dye Basic Orange 2 by a bacterium, *Escherichia coli*, and obtained a 90% removal efficiency [12]. *Trichoderma tomentosum* fungi, isolated from degraded wood wafers, had the ability to degrade Acid Red 3 R by 94.9% within 72 h [13]. The time needed to remove the dye A010 by combined adsorption on Fe_3O_4 magnetite nanoparticles and biodegradation by *Pseudomonas putida* was reduced to 5 h, knowing that it takes 11 h for biodegradation by a free microorganism [14].

Although the biological treatment of dyes is considered as a sustainable and ecofriendly process because far less reagent is used, there are many studies on treatment by adsorption and advanced oxidation processes (AOPs) for the degradation of dyes. Xiao et al. showed a 98% RhB removal in solution on activated carbon from white sugar [15]. AOPs as ozonation [16], photocatalysis [17–20], sono-photocatalysis [3], and ultrasound-assisted TiO_2 photocatalysis [21] presented hopeful results. These methods, although effective, involve the use or production of compounds whose environmental impact is not well known. Indeed, advanced oxidation reactions generally lead to the formation of by-products whose toxicity, which is not always known, may be greater than that of the original pollutant [2,22]. In addition, these processes require large amounts of electricity as the catalysts need to be photo-stimulated, mostly by UV radiation, whose high cost is a big disadvantage over biological methods [16,23]. A study on the decolorization of Rhodamine B dye using novel V_2O_5-rGO photocatalyst under solar irradiation was carried out and obtained a degradation efficiency of more than 90% in 50 min. The methods of coupling advanced oxidation and biodegradation processes using pure strain [24] or activated sludge with dyes have already shown promising results [18].

The by-products of the Basic Orange 2 degradation by *E. coli* are primarily aromatic amines [12], and N-deethylation is generated by RhB photocatalytic degradation [25]. Because the by-products of dyes degradation can potentially be carcinogenic [22,26], it is important to evaluate the toxicity of the obtained compounds in addition to continuing to improve the treatment of the dyes.

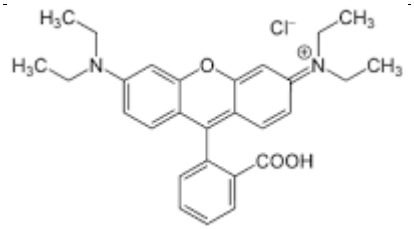
The framework of our study is the degradation of RhB according to two used strategies. The first one is based on the biodegradation by the yeast *S. cerevisiae*, which is ubiquitous in the environment, cheap, and does not present a pollution risk, such as activated sludge. It is a sustainable process. The second process tested was the TiO_2 doped with Cerium (5%)-based photocatalysis. Phytotoxicity and aquatic toxicity were studied to assess the effect of RhB and its degraded by-products with watercress seeds, yeast *S. cerevisiae*, and the crustacean *Daphnia seed* on the environment. Often enough, studies focusing on the removal of recalcitrant compounds only consider the efficiency of purification without considering the quality of the formed by-products. The uniqueness of our study lies in examining both the effectiveness of two treatment processes and the ecotoxicity of the by-products.

2. Materials and Methods

2.1. Rhodamine B

The target pollutant studied is Rhodamine B (RhB), a hydrophilic red azoic synthetic dye (Table 1). RhB was obtained from Thermo Scientific (98%), and all solutions were prepared with Ultra-Pure Water Elix Advantage 5.

Table 1. Characteristics of Rhodamine B.

Physico-Chemical Properties	Rhodamine B
Molecular structure	
Molecular formula	$C_{28}H_{31}ClN_2O_3$
Molecular weight (g mol^{-1})	479.0
Solubility (g L^{-1}) at 25 °C	8–10
λ_{max} (nm)	554

2.2. Microorganisms and Inoculum

The *Saccharomyces cerevisiae* CIP 95 strain used throughout the study was obtained from the Institut Pasteur (Paris, France) and kept refrigerated in Sabouraud agar tubes. For the preparation of inoculum, the yeasts were suspended in a sterile 150 mM KCl solution and then transferred to a preculture in a liquid medium of 2% (*w*/*v*) D-Glucose, 2% (*w*/*v*) peptone, and 1% (*w*/*v*) yeast extract and incubated at 28 °C, 130 rpm for 15 h. All reagents come from Prolabo (Paris, France).

2.3. Medium for Degradation and Biosorption Assays

Biosorption and biodegradation tests were carried out in the following mineral medium (mg L^{-1}): 1040 KH_2PO_4; 120 $MgSO_4$,$7H_2O$; 15 $CaCl_2$,$6H_2O$; 10 $FeSO_4$,$7H_2O$; 3 $ZnSO_4 7H_2O$; 7.9×10^{-5} $CuSO_4$,$5H_2O$; 1.5 H_3BO_3; 2×10^{-3} KI; 5×10^{-3} Na_2Mo_4,$2H_2O$; 3.2 $MnSO_4$,H_2O; 5.6×10^{-3} $CoCl_2$,$6H_2O$; 10 EDTA, to which NH_4Cl (10 mM) was added. All reagents came from Prolabo (Paris, France). The media could be supplemented with RhB (5 mg L^{-1}) and/or D-Glucose (10 g L^{-1}) before sterilization. All media were sterilized by autoclaving at 120 °C for 20 min after adjusting the pH to 7 ± 0.2.

2.4. Biosorption Assays

The prepared inoculum, as detailed in Section 2.2, was centrifuged (5000 rpm, 4 °C, 5 min), and the pellet was resuspended in KCl (150 mM). This was repeated twice before the suspension was resuspended in 25 mL of KCl (150 mM). In total, 5 mL of this suspension was placed in three erlenmeyers containing 100 mL of mineral medium (Section 2.3), to which RhB (5 mg L^{-1}) was added. The erlenmeyers were incubated at 28 °C (New Brunswick Scientific innova 40, Edison, NJ, USA), 130 rpm, and the RhB concentration was monitored for 180 min. This experiment was carried out with active and inactive cells. The inactivation of microorganism cells was conducted in an autoclave at 120 °C for 20 min before being suspended in the mineral medium. Each experiment was carried out in triplicate.

The sorption of the RhB was calculated using the following Equation (1):

$$\text{Sorption}(\%) = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where A_0 and A correspond to the initial and final absorbance of the RhB at 554 nm, respectively.

2.5. Biodegradation Assays

The mineral medium (described in Section 2.3) to which either RhB (5 mg L^{-1}), glucose (10 g L^{-1}), or RhB and glucose were added before sterilization was inoculated as described before (described in Section 2.4). The culture was incubated at 28 °C, 130 rpm agitation for

5 days, during which time cell growth and the RhB concentration were monitored. Each experiment was carried out in triplicate.

2.6. Bio-Acclimation Assays

The *S. cerevisiae* bio-acclimation tests were performed by subculturing the strain from a Sabouraud agar tube into Petri dishes containing a mineral medium (MM) (described in 1.3), to which agar (18 g L^{-1}), NH_4Cl (10 mM), RhB (5 mg L^{-1}), and different glucose (Glc) concentrations (mg L^{-1}: 1; 0.75; 0.5; 0.25, 0) were added. The cells were successively transferred from the dishes with the highest concentration of glucose to those with the lowest concentration after 48 h of incubation at 28 °C. The same tests were performed with the mineral medium prepared directly in the 3 h treated RhB solutions to acclimatize *S. cerevisiae* to the treatment effluents (Figure 1). Each experiment was carried out in triplicate.

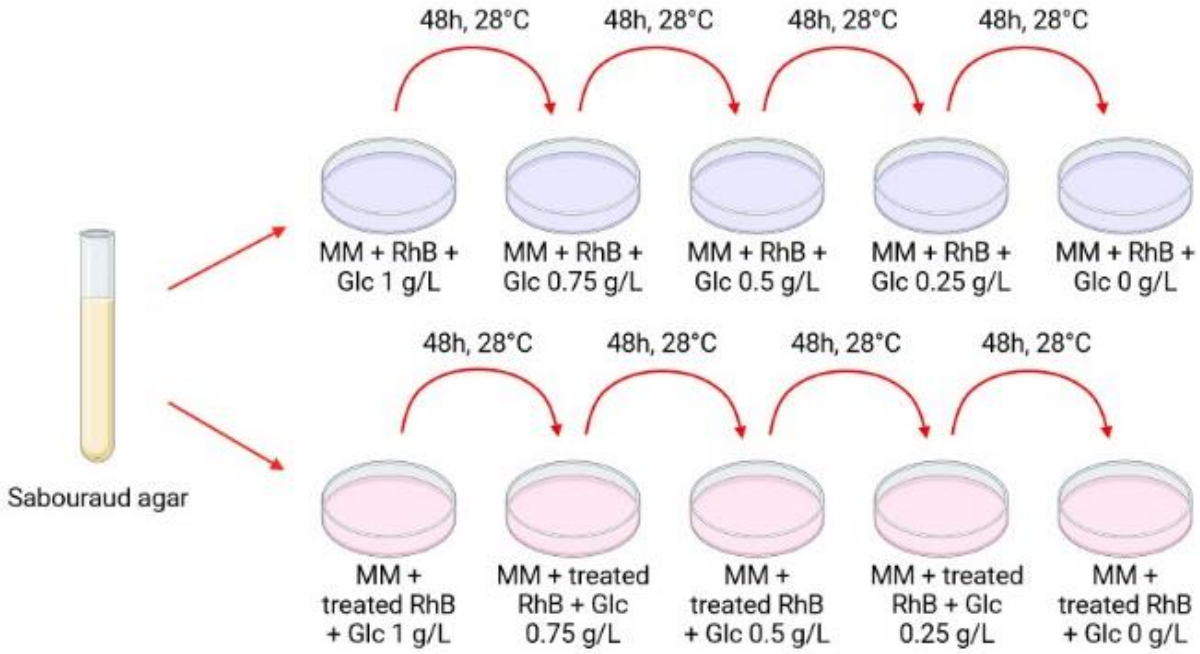


Figure 1. Experimental protocol for the acclimatization of *S. cerevisiae*.

2.7. Photocatalysis Assays

Photocatalysis tests on Rhodamine B solutions were carried out using Cerium (5%)-doped TiO_2 photocatalyst under 254 nm UV-C radiation (OSRAM Puritec 11 W UV-C lamp) in a batch reactor with a capacity of 500 mL (Figure 2). The preparation and the characterization of nanosized Cerium-doped Titania used have been well presented and discussed by Reynoso et al. [27]. The 40 milligrams of photocatalyst powder were dispersed in a RhB solution at ambient temperature and under continuous magnetic stirring at 400 rpm. Before starting photocatalysis, the RhB solution with catalyst was kept in the dark at the beginning of each photocatalytic assay for 30 min to establish an adsorption–desorption equilibrium. RhB solutions (5 mg L^{-1}) were treated by photocatalysis for 3 or 6 h. Each experiment was carried out in triplicate.

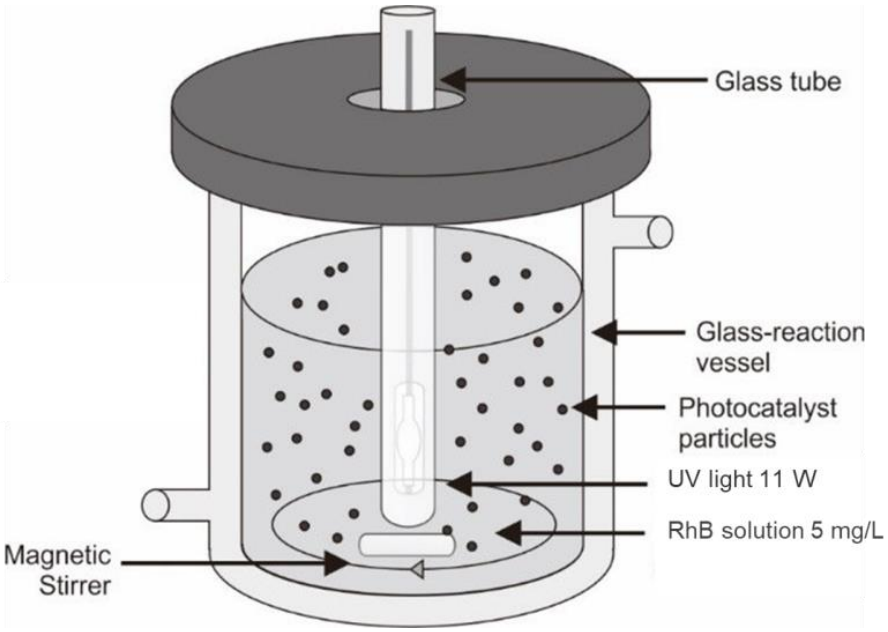


Figure 2. Photocatalytic reactor.

The decolorization efficiency E of the solutions was calculated using the following Equation (2):

$$E = \left(1 - \frac{C}{C_0}\right) \times 100 \quad (2)$$

where C_0 and C are the initial and final concentrations of RhB, respectively.

2.8. Toxicity Assays

2.8.1. Impact of Rhodamine B, Catalyst, and Treated Solutions on the Growth of *S. cerevisiae*

The impact of RhB, catalyst TiO_2-Ce 5%, and photocatalysis-treated RhB solutions (Section 2.6) on the growth of *S. cerevisiae* was assessed. Petri dishes were poured with Sabouraud's agar, to which different concentrations of RhB (1; 5; 10; 25; 50; 100 mg L^{-1}) or catalyst (1.5 g L^{-1}) were added before sterilization. Sabouraud's agar was also prepared directly in the photocatalysis-treated RhB solutions to test their impact on *S. cerevisiae*. The Petri dishes previously prepared and sterilized were inoculated by yeast and incubated at 25°C for 48 h.

2.8.2. Impact of Rhodamine B, Catalyst, and Treated Solutions on the Germination of Watercress Seeds

The phytotoxicity of RhB, the TiO_2-Ce 5% catalyst, and RhB solutions after photocatalysis treatment was assessed by studying their impact on the germination of watercress seeds (*Lepidium sativum*, Alénois Commun de Fourche & Compagnie). The tests were conducted as described by Zeghioud et al. [18]. The germination index (GI) was determined from the calculation of the average number of seeds that sprouted, and the average number of root shoots based on the following equation:

$$GI(\%) = \left(\frac{\text{Seed germination}(\%) * \text{average of root lenght of the sample}}{\text{Seed germination}(\%) * \text{average of root length of the control}}\right) * 100 \quad (3)$$

The value obtained for a solution allows us to classify it according to three categories: high phytotoxicity (GI% < 50%), moderate phytotoxicity (50% < GI% < 80%), or no phytotoxicity (GI% > 80%).

2.8.3. Impact of Rhodamine B and Treated Solutions on the Immobilization of the Crustacean *Daphnia magna*

The invertebrate *D. magna* was used to evaluate the ecotoxicity of Rhodamine B (subject to no treatment), Rhodamine B after 3 h of treatment, and Rhodamine B after 6 h of treatment. Organisms were cultured in the laboratory according to the OCDE procedure using a reconstituted M4 medium mainly containing g L^{-1}: $NaHCO_3$ (64.8); $CaCl_2$ $2H_2O$ (293.8); $MgSO_4$ $7H_2O$ (123.3); and KCl (5.8) with a pH between 7 and 8, hardness between 140 and 250 mg L^{-1} of $CaCO_3$, and constant oxygenation [28]. *D. magna* were placed in aquariums and kept in an experimental chamber with a temperature of 20 ± 2 °C and an artificial photoperiod of 16 h/8 h (light:dark). Organisms were fed daily, 5 days a week with freshwater algae *Chlorella vulgaris* (8×10^6 cells m L^{-1}), and the medium was renewed every week.

All assays were conducted in a chamber under the same abiotic conditions as the culture, and test conditions as well as analysis were carried out in accordance with the international OECD Guidelines for the Immobilization and survival test of *Daphnia magna*—48 h from OECD, 2004. For each replicate (4 in total), 5 organisms aged under 24 h were placed in 10 mL of M4 medium (control) and initial Rhodamine B (diluted in M4 medium) with the following dilutions: 0; 0.1; 1; 10; 50; and 100 g L^{-1}, and for Rhodamine B after 3 or 6 h of treatment: 0; 10; 15; 25; 40; and 50% diluted in a M4 medium. The number of dead organisms was assessed using a binocular microscope after 48 h of exposure [29]. The acute toxicity to *D. magna* was defined as the average concentration that induces immobilization (EC50) and mortality (LC50) in 50% of the test organisms using Probits analysis on excel.

2.9. Analytical Methods

Cell growth of *S. cerevisiae* was monitored at 600 nm wavelength, the concentration of RhB was measured at 554 nm wavelength, and the maximum adsorption capacity was measured using a UV-vis spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan). Prior to the lecture, samples were centrifuged (5000 rpm, 4 °C, 5 min). Dry matter (DM) determination was performed by centrifuging 25 mL (V) of the KCl suspension (5000 rpm, 4 °C, 5 min) in a pre-weighed tube (m_0). The supernatant was then discarded, and the tube was placed in an oven at 105 °C for 24 h. A second weighing (m_1) was then carried out, and the dry matter concentration was measured using the following formula:

$$MS = (m_1 - m_0)/V \quad (4)$$

Chemical Oxygen Demand (COD) measurements were made using the Merck Spectroquant COD Cell Test kit (4.0–40.0 mg L^{-1}). The analysis of Biological Oxygen Demand (BOD_5) was carried out as described by Zeghioud et al. [18]. The inoculum used for the determination of BOD_5 was made as described by Cherif et al. [22]. The total organic carbon (TOC) was measured using a TOC-L Total Organic Analyzer, Shimadzu, Japan.

3. Results and Discussion

3.1. Biodegradation Tests on Rhodamine

3.1.1. Saccharomyces cerevisiae Growth in Presence of Rhodamine B

Before the biodegradation tests of RhB by *S. cerevisiae,* the growth of the yeast was carried out in a solid Sabouraud medium (15 g L^{-1} agar), to which RhB was added before sterilization. After 48 h of incubation at 25 °C, at all RhB concentrations, *S. cerevisiae* was able to grow (Figure 3). So, RhB did not prevent the yeast from growing, meaning it can be used in biodegradation tests.

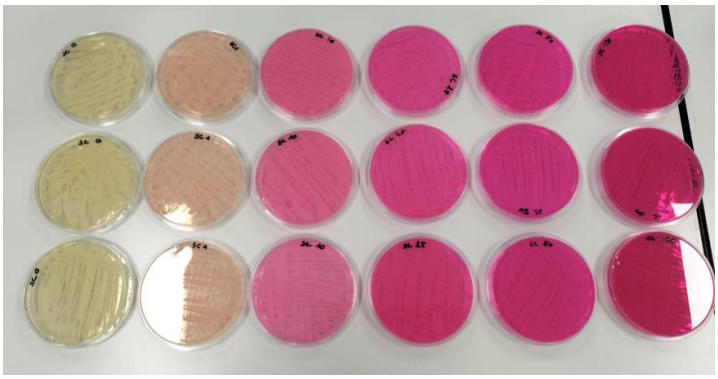


Figure 3. *Saccharomyces cerevisiae* growth inoculated on Sabouraud's agar plates containing RhB at 0, 5, 10, 25, 50, and 100 mg L^{-1} after 48 h of incubation.

3.1.2. Biosorption

The aim of the biosorption test was to verify if the elimination of the target compound was due to biodegradation or sorption on the cell's walls. In this study, the tests were carried out over 150 min to assess the sorbtion capacity of RhB on living and dead *S. cerevisiae* cells (Figure 4). A decrease in the RhB concentration of 13.0% and 14.7%, respectively, for living and dead cells was observed after 30 min of testing. In the present study, the results showed that the uptake capacities of *S. cerevisiae* were 4.2 mg g^{-1} DW and 3.2 mg g^{-1} DW, respectively, for living cells and dead cells for 150 min of culture. Beyond that, no decrease was observed. The decrease does not derive from an active phenomenon of the cells and could be explained by a phenomenon of the sorption of RhB on the surface asperities of *S. cerevisiae*. Ternes et al. [30] suggest that sorption is possible because the cells are charged negatively and there is an electrostatic interaction with positively charged groups of the chemical. The concentration of RhB did not increase in the culture medium during the test, suggesting that there was no release of the molecule previously adsorbed onto the cells.

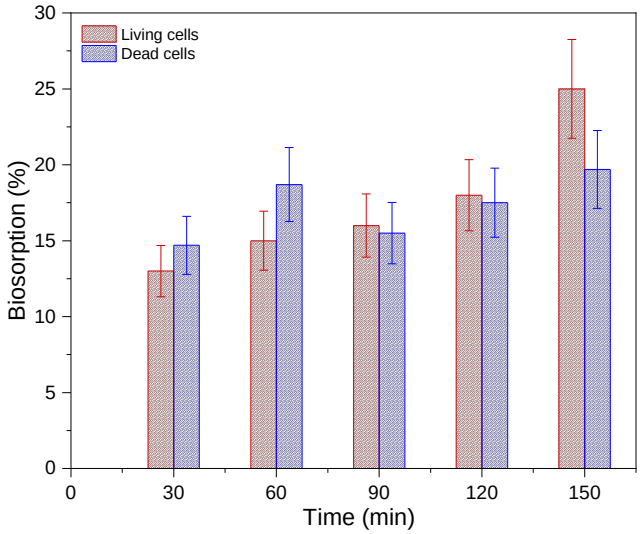


Figure 4. Assessment of the biosorption of Rhodamine B on living and dead cells and dead (Error < 5%).

3.1.3. Biodegradation Study

During the tests, the absorbance at 600 nm was measured every 24 h to verify the effect of Rhodamine B on the growth of *S. cerevisiae* (Figure 5). The glucose was added as a co-substrate to enhance the growth of the yeast and biodegradation capability of *S. cerevisiae*. A maximum growth was reached between 48 and 72 h for both cultures in the presence of glucose, followed by a stable plateau during the rest of the tests. There

was no impact of Rhodamine B on the growth of *S. cerevisiae*. However, the cells culture on RhB shows no growth, and *S. cerevisiae* are unable to use the target compound as a carbon substrate so they cannot remove it. In the presence of glucose, the RhB was removed by 10% (Figure 6). However, this reduction corresponds to the part of the biosorption of RhB and not to biodegradation. The use of Bacillus subtilis 'RA29' for the decolorization of Rhodamine B (RhB) resulted only in a 6.69% degradation, whereas, under the same conditions, the Congo red, Amido black, and Acid orange dyes were eliminated to the extents of 98.23%, 8.32%, and 6.69%, respectively [31]. These results do not match those of the work on the degradation of methyl red by *S. cerevisiae* ATCC 9763. Indeed, the authors observed a total decolorization after 12 h of treatment [9]. The studies using *S. cerevisiae* MTCC 463 for the removal of triphenylmethane dyes concluded that yeast decolorize malachite green thanks to biotransformation, while methyl violet, crystal violet, and cotton blue decolorize via biosorption [11]. Ikram et al. observed that 90% of the biological degradation of azo dye basic Orange 2 by *Escherichia coli* happens in the presence of glucose, NaCl, and sodium benzoate as redox mediators [12]. The authors put forward a strategy for the biodegradation of rhodamine B by isolating a microorganism from an industrial effluent containing this dye. The isolated bacterium, *Brevundimonas diminuta*, enabled a removal of 90–95% under optimal conditions [32].

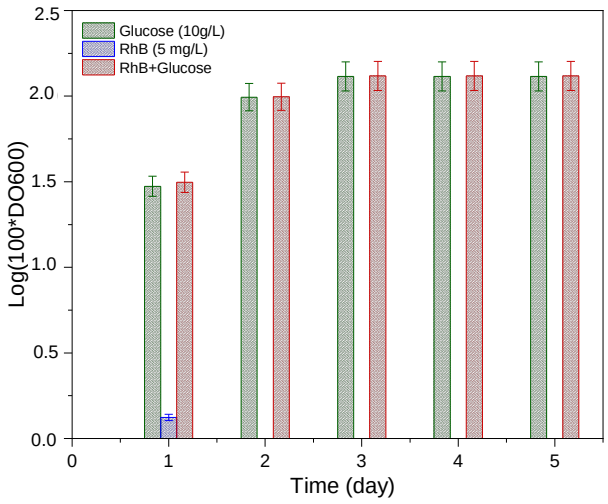


Figure 5. Growth monitoring of *S. cerevisiae* in mineral medium in the presence or absence of glucose and/or RhB (Error < 5%).

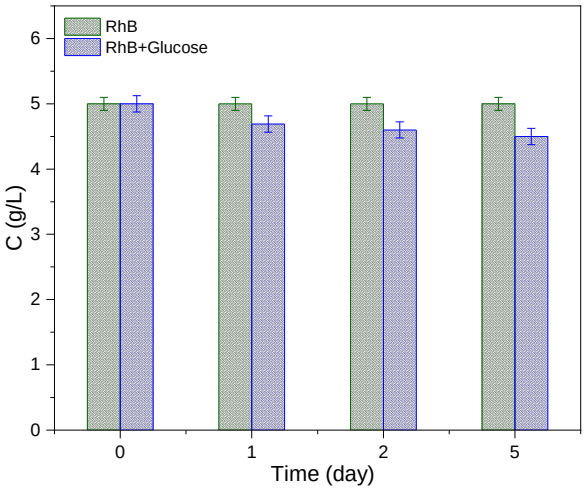


Figure 6. Assessment of concentration of RhB (C) in the presence of *S. cerevisiae* in mineral medium with and without glucose (Error < 5%).

3.1.4. Photocatalytic Study

Due to the difficulty of removing RhB by *S. cerevisiae*, the photocatalysis, as an advanced oxidation process (AOP), was tested for RhB elimination with TiO_2-Ce 5% photocatalyst. The assessment of the degradation of the target compound was carried out after 3 and 6 h of treatment. In total, 42 and 54% of RhB elimination were observed after 3 and 6 h of photocatalysis, respectively (Table 2). It seems that 8% supplementary photodegradation efficiency was obtained by doubling the treatment time. While the COD removal remains significant after 6 h of treatment, the mineralization yield increased by 43%.

Table 2. Results of Rhodamine B, COD, TOC removal efficiency, and BOD_5/COD ratio after 3 and 6 h of photocatalytic treatment (Error < 5%).

	RhB (%)	TOC (%)	COD (%)	BOD_5/COD
3 h	42.5	4.9	0.0	0.20
6 h	54.0	8.6	55.2	0.42

In the scientific literature, many catalysts have been tested for the degradation of RhB using a photocatalysis process. It should be noted that the photocatalytic treatment of RhB by ZnO nanoparticles as a catalyst was reported to degrade the dye by 95% within 70 min under UV irradiation [33]. With the catalyst chitosan-SnO_2 nanocomposites, the dye studied was removed by 51% within 100 min [34]. The use of the catalyst CS/SNO_2 leads to the removal of RhB by 95% within 60 min [20]. So, the catalyst used, and its dose plays an important part on the degradation rate. An important point to be highlighted is the increase in the biodegradability of degradation molecules (based on an increase in the ratio of BOD_5/COD). Then, it would be possible to combine the photocatalysis treatment with the biological one. Previously, some studies showed the possibility of combining two treatment processes for intensifying the degradation of dyes. For example, in their study, Sharma et al. combined the photocatalysis treatment used for the degradation of the Reactive Black 5 and Reactive Yellow 15 with the biological treatment based on the culture of *Pseudomonas fluorescens* [24]. The degradation of the azo dye methyl red was enhanced by the combination of sequential photocatalysis and biological treatments [35]. Table 3 shows data about the Rhodamine B photocatalytic performance compiling various experimental setups.

Table 3. Comparison of photocatalysis performance of Rhodamine B.

Catalyst	Removal Performance (%)	Reference
ZnO nanoparticles	95	[33]
Chitosa-SnO_2	51	[34]
CS/SNO_2	95	[20]
TiO_2-Ce	54	This study

The elaboration of the degradation pathway is usually difficult. Nevertheless, Ajiboye et al. have proposed one. The elaboration, about the degradation pathway of RhB by reduced graphene-oxide-based photocatalyst, revealed that the degradation of the RhB commenced by the removal of ethyl-attachments on the nitrogen heteroatom. This ethyl removal was followed by the cleavage of the chromophore and opening the rings. Complete mineralization of the intermediates formed into nitrogen-containing nitrogen ions, water, and carbon dioxide [36].

3.1.5. Effect of Acclimation

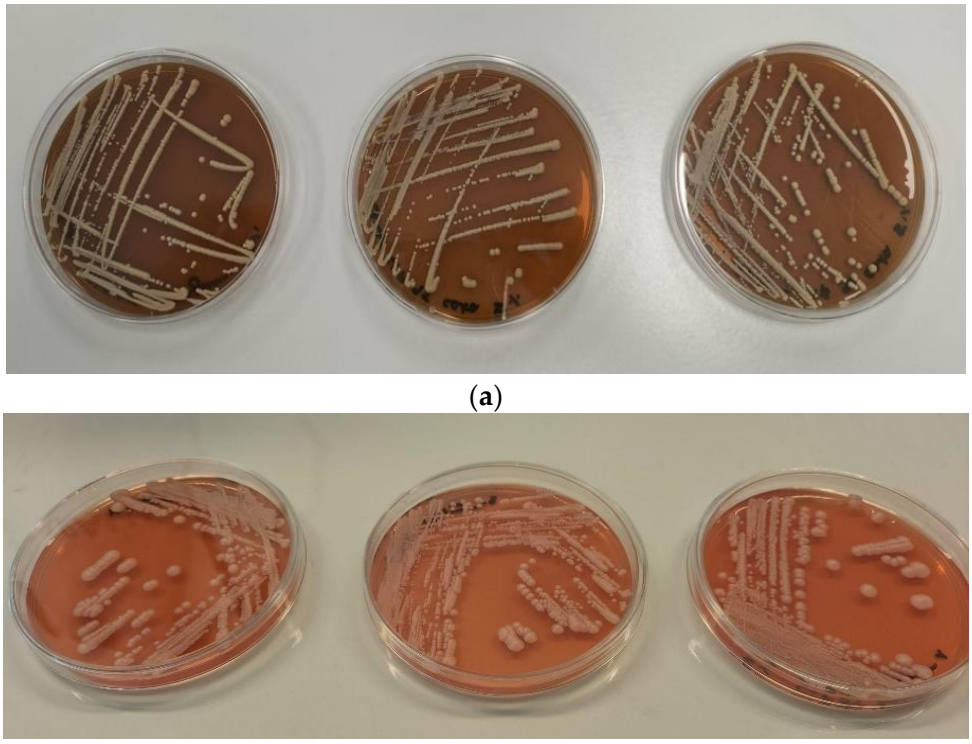
The bio-acclimation test of *S. cerevisiae* with Rhodamine B (5 mg L^{-1}) as a carbon substrate or a solution of Rhodamine B (5 mg L^{-1}) treated by photocatalysis for 6 h showed no growth in the first plates (RhB or RhB treated for 6 h + glucose at 1 g L^{-1}) after 120 h.

There was no subculturing in the Petri dishes with a lower concentration of glucose as the cells did not appear to be able to grow under these culture conditions. The yeast does not seem to be able to acclimate to RhB and use it as a carbon source, which explains the lack of biodegradation.

3.2. Assessment of the Toxicity of RhB, the Catalyst, and Photocalysis By-Products

3.2.1. Effect on *Saccharomyces cerevisiae* Growth

The catalyst TiO_2 doped with Cerium at 5% did not affect the growth of the yeast (Figure 7a). The same observation was conducted with the presence of the by-products from the photocatalysis process in the culture medium (Figure 7b). Therefore, in the presence of glucose contained in the Sabouraud medium, the growth of *S. cerevisiae* was possible despite the presence of photocatalysis by-products.



(**a**)

(**b**)

Figure 7. *Saccharomyces cerevisiae* inoculated on Sabouraud's agar plates containing the catalyst (**a**) and with the addition of the treated solution (**b**) after 48 h of incubation (Error < 5%).

3.2.2. Phytotoxicity Study

The results obtained shows an absence of phytotoxicity (GI% > 80) in Rhodamine B solutions up to 5 mg L^{-1}. Above these concentrations, RhB is moderately toxic (80 > GI% > 50) for concentrations of 10 mg L^{-1} and 25 mg L^{-1} and then highly toxic for concentrations of 50 mg L^{-1} and 100 mg L^{-1} (Figure 8). The TiO_2 catalyst doped with 5% Cerium shows a moderate toxicity (GI% = 64.2). The germination index obtained for the treated solutions is lower than that obtained for the untreated solution (5 mg L^{-1}), and the phytotoxicity of the solution after 3 h of treatment is found to be higher than that of the solution treated for 6 h (GI% of 59.8 and 65.2, respectively). This higher toxicity could be due to the production of toxic subspecies of Rhodamine B during the advanced oxidation treatment. Moreover, a longer treatment would enable the phytotoxicity of the treatment effluents to be limited by also oxidizing the toxic by-products formed during the first hours of treatment.

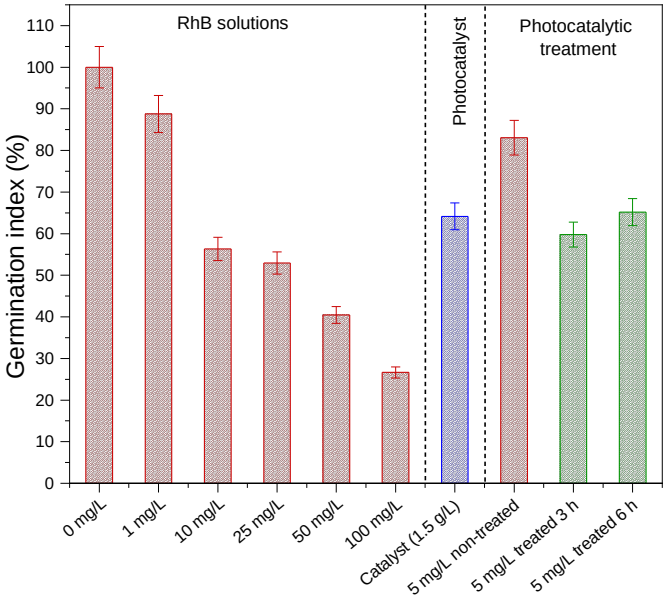


Figure 8. The phytotoxicity of the RhB solutions at different concentrations and the photocatalyst at 1.5 g L^{-1} and the solution after photocatalytic treatment at 3 and 6 h (Error < 5%).

Work on the removal of the dye Reactive Green 12 by photocatalysis TiO_2-impregnated polyester as a supported catalyst under UV light has shown the opposite results [18]. However, [22] observed that the catalyst ZnO calcined at 250 °C increases the phytotoxicity of the Reactive Bleu 19 dye. As for the azo dye methyl red, the phytotoxicity on *Sorghum vulgare* and *Phaseolus mango* decreases seriously after a photocatalysis treatment coupled with a biological process [33].

Daphnia magna Test

Toxicity tests showed an increase in immobilization and mortality in *D. magna* exposed to Rhodamine B without treatment with an EC50 and LC50 of 18.56 and 38.96 mg/L, respectively. The value of LC50 is in the range of the data from the study of [37] with LC50 of 25 mg L^{-1} of Rhodamine B. Immobilization and mortality tests conducted with solutions of Rhodamine B after 3 or 6 h of treatment did not show any increase in the immobilization or mortality of *D. magna* compared to the negative control. This resulted in a correlation with the study based on the photocatalysis process of RhB, with BiOI-modified $MgCr_2O_4$ nanosphere as a catalyst, showing the by-products to have no harmful effect on Daphnia [38].

4. Conclusions

In this work, the degradation of Rhodamine B by two processes was conducted. First, the yeast *Saccharomyces cerevisiae* can eliminate RhB by 13%. However, this quantity corresponds to the adsorption of the target compound on the yeast and not to the biodegradation process. The degradation of RhB using a photocatalysis process with TiO_2 doped with 5% Cerium was observed under 254 nm UV radiations. The mineralization reached 8.6% after 6 h of treatment. The study on the eco-toxicity of the photocatalysis by-products showed that *S. cerevisiae* cannot grow on a culture medium with by-products as a sole carbon source. However, with a supplemented assimilable carbon source such as glucose in the by-product solutions, growth is possible. This means that the catalyst and the by-products did not have impacts on the yeast growth. A decrease of 24% in the germination index (GI) was observed when watercress seeds were cultivated on photocatalytic treatment by-products. Nevertheless, no effect of the by-products was observed on the mobility of *Daphnia magna*. Additional tests to evaluate the ecotoxicity of the treatment by-products would be valuable. This would allow us to make informed judgments about the effectiveness of the treatment

processes and the quality of the discharged effluents. By assessing the potential environmental impact of the by-products, we can gain a more comprehensive understanding of the overall efficiency and sustainability of the treatment methods used.

Author Contributions: Conceptualization, H.D. and H.Z.; methodology, H.D. for the biological part, A.B.-R. and T.E.C.-M. for catalyst part; H.Z. for photocatalysis part, M.R. for the *Daphnia magna* test; manipulation, T.C., A.R.d.l.G., and O.F.; writing—original draft preparation, H.D., T.C., M.R., and H.Z.; writing—review and editing, H.D.; supervision, H.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Marie-Anne Hairan from Unilasalle-EME Rennes-France for the correction of the English language.

Conflicts of Interest: The authors declare no conflict of interest.

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