On the Efficacy of H$_2$O$_2$ or S$_2$O$_8^{2-}$ at Promoting the Inactivation of a Consortium of Cyanobacteria and Bacteria in Algae-Laden Water

Javier Moreno-Andrés *©, Ignacio Rivas-Zaballos, Asunción Acevedo-Merino and Enrique Nebot

Abstract: Harmful algal blooms in coastal areas can significantly impact a water source. Microorganisms such as cyanobacteria and associated pathogenic bacteria may endanger an ecosystem and human health by causing significant eco-hazards. This study assesses the efficacy of two different reagents, H$_2$O$_2$ and S$_2$O$_8^{2-}$, as (pre-)treatment options for algae-laden waters. Anabaena sp. and Vibrio alginolyticus have been selected as target microorganisms. With the objective of activating H$_2$O$_2$ or S$_2$O$_8^{2-}$, additional experiments have been performed with the presence of small amounts of iron (18 µmol/L). For the cyanobacterial case, H$_2$O$_2$-based processes demonstrate greater efficiency over that of S$_2$O$_8^{2-}$, as Anabaena sp. is particularly affected by H$_2$O$_2$, for which >90% of growth inhibition has been achieved with 0.088 mmol/L of H$_2$O$_2$ (at 72 h of exposure). The response of Anabaena sp. as a co-culture with V. alginolyticus implies the use of major H$_2$O$_2$ amounts for its inactivation (0.29 mmol/L of H$_2$O$_2$), while the effects of H$_2$O$_2$/Fe(II) suggests an improvement of ~60% compared to single H$_2$O$_2$. These H$_2$O$_2$ doses are not sufficient for preventing the regrowth of V. alginolyticus after 24 h. The effects of S$_2$O$_8^{2-}$/Fe(II) are moderate, reaching maximum inhibition growth of ~50% for Anabaena sp. at seven days of exposure. Nevertheless, doses of 3 mmol/L of S$_2$O$_8^{2-}$ can prevent the regrowth of V. alginolyticus. These findings have implications for the mitigation of HABs but also for the associated bacteria that threaten many coastal ecosystems.

Keywords: harmful algal bloom; hydrogen peroxide; persulfate; fenton; cyanobacteria; marine bacteria

1. Introduction

Harmful algal blooms (HABs) have become a global concern, especially during the last few years. The mechanisms that trigger an algal bloom episode are complex and are derived from natural processes, such as variation in temperatures that induce water stratification, to human-induced processes that may increase the number of nutrients released into waters. In this context, warm temperatures, as well as nutrient and light availability, are the basic requirements to sustain an extensive bloom [1]. When it is produced, wide impacts can be derived from such (micro)algal blooms, which could involve the eutrophication of waters or the release of associated toxins that will cause significant eco-hazards in both ecosystems and for human health [2–4].

Together with these blooms, the bacteria Vibrio spp. has also been reported, suggesting that the HABs may enhance the bacterial growth of these pathogenic species. This demonstrates a positive relationship between the abundance of Vibrio spp. and harmful phytoplankton, including both cyanobacterial or dinoflagellates bloom-related species [5,6]. Vibrio spp. are ubiquitously present in marine and estuarine environments, with fewer species being reported as pathogenic Vibrios for animals and plants. Approximately one dozen species have been known to cause infections in humans [7,8]. Specifically, there are
particular pathogenic Vibrios that clearly dominate human infection; they are known as the "big four": *V. cholerae, V. vulnificus, V. parahaemolyticus* and *V. alginolyticus* [7]. These species have also been reported to be associated with the HABs events [5,6], which could increase health risk and eco-hazards in marine environments. Consequently, efficient solutions that can mitigate both harmful phytoplankton and associated bacteria are encouraged.

HABs are being reported in both freshwater and marine ecosystems. One common impact that is related is that these water masses can be used as a water source for the raw influent of drinking water treatment plants (DWTPs) [9]. For instance, some DWTPs use carbon adsorption or chlorination, among other processes; however, they are still not efficient in removing these large blooms or associated toxins [2]. Additionally, chlorination involves the potential generation of by-products that are associated with the high levels of algal organic matter in these challenging waters. Another example is the use of desalination processes, where seawater reverse osmosis is the leading technology for those purposes. In this regard, one of the major operational problems is the accumulation of organic matter together with fouling complications that can be exacerbated due to the HABs’ episodes in coastal areas [10].

In this scenario, pretreatment methods have gained attention over the last years. Common biocides such as chlorine, CuSO$_4$ or KMnO$_4$ have been investigated [11]. However, some disadvantages are also reported that are related to the formation of taste and odor compounds, disinfection by-products, etc. Accordingly, the use of alternative oxidants is also encouraged. Hydrogen peroxide can be one of them since H$_2$O$_2$ naturally degrades itself in water and oxygen. In fact, it has emerged as an attractive and environmentally friendly chemical for the selective abatement of cyanobacterial blooms in freshwater lakes [9,12,13]. The application of H$_2$O$_2$ is of special interest for cyanobacteria, which are prokaryotic cells with poorly elaborated mechanisms for H$_2$O$_2$ detoxification [3], although various sensitivities have been found among different cyanobacterial species [13]. Recently, assessing H$_2$O$_2$ oxidative stress on marine microalgae species has been reported [14–16]. However, specific studies focusing on marine cyanobacteria are limited.

On the other hand, persulfate salts have recently received widespread attention for use in water treatment but have been less studied for inactivating harmful phytoplankton [17]. One of the primary advantages of S$_2$O$_8^{2-}$ for their application in seawater is the degradation products, as increasing levels of sulfates would be inconsequential compared with the background levels in seawater [17,18].

These oxidants are also widely applied in what is known as Advanced Oxidation Processes (AOPs), for which strong radicals may be generated and can accelerate inactivation practices. A clear example is the combination of either H$_2$O$_2$ or S$_2$O$_8^{2-}$ with transition metals, such as iron, where it can efficiently react with the oxidants involving the highly reactive hydroxyl or sulfate radicals according to Equations (1) and (2) [19,20].

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}
\]

\[
\text{Fe}^{2+} + \text{S}_2\text{O}_8^{2-} \rightarrow \text{Fe}^{3+} + 2\text{SO}_4\cdot^-
\]

The use of these oxidants in the abatement of both cyanobacteria and related toxins together with bacteria has shown promising results in freshwaters [2,21–23]; however, the seawater scenario is less studied [17]. These studies make use of moderately high iron concentrations [17,21,23], which is important to consider because of the ecological risk of the residual metal after treatment. Additionally, the behavior of both cyanobacteria and bacteria inactivation in the consortium is limited [13]. Accordingly, the main goal of this study is to assess the efficacy of two different oxidants, H$_2$O$_2$ or S$_2$O$_8^{2-}$, for the treatment of algae-laden waters.
2. Materials and Methods

2.1. Target Microorganisms

As target microorganisms, the marine cyanobacterium *Anabaena* sp. (Strain: CCMM 01/0101, provided by the Marine Microalgal Culture Collection of the Institute of Marine Sciences of Andalusia, ICMAN-CSIC; Supplementary Materials Figure S1) has been selected as representative of cyanobacterial fraction based upon their occurrence in source water supplies, the availability of a monoalgal culture, and the ability to produce odorous or toxic metabolites [24,25]. In parallel, the marine pathogen *Vibrio alginolyticus* (CECT521T; ATCC 17749) has been selected as the associated bacterial fraction [5,6,26].

*Anabaena* sp. were cultured in ground saltwater from the University Campus of Puerto Real at the University of Cadiz (pH = 7.65; salinity = 35.8) and enriched with Guillard f/2 medium (Guillard and Ryther, 1962). For experiments with Fe(II), the same f/2 medium was used but without trace metals and the EDTA complexing agent in order to avoid interferences with experimentation by adding iron as a possible catalyst (See Section 2.2). Experimentation was carried out in conditions that were able to simulate the algal bloom in a water source, i.e., with an initial cellular density of approx. $10^5$–$10^6$ cells·mL$^{-1}$. All cultures were maintained in a culture chamber at 20 °C illuminated by two LED lamps (Phillips LED tube, 18 W, 1600 lm, cool daylight) that provide photosynthetically active radiation of 130 $\mu$einsteins m$^{-2}$ s$^{-1}$, with a 14:10 light:dark cycle.

The monitoring of the *Anabaena* sp. cultures was followed by means of absorbance at 680 nm (Jenway 7315) and fluorescence (Microplate Fluorescence Reader “Tecan infinite F200”; excitation wavelength: 370 nm; emission wavelength: 670). Both measurements were correlated with cell concentration measured by microscopy (Leica, DM 750). Accordingly, the fluorescence or absorbance measurements and their corresponding values of cell concentration measured with Neubauer plates were subjected to a linear regression analysis. The intercept was not significant ($p = 0.2380$ and $p = 0.8186$ for absorbance and fluorescence, respectively), thus the cell density was linearly correlated with absorbance and fluorescence measurements with a slope of $2 \times 10^{-7}$ ($R^2 = 0.7579$) and $6 \times 10^{-4}$ ($R^2 = 0.8591$), respectively.

Alternatively, *V. alginolyticus* was cultured in marine broth (Panreac) and inoculated in either ground saltwater or cyanobacterial cultures to obtain a concentration of approx. $10^5$ CFU·mL$^{-1}$. The reactivation of cryovials was performed as detailed elsewhere [27,28]. Bacterial survival after treatment was assessed with standard plate counts with the TCBS Agar (VWR Chemicals) [27].

2.2. Experimental Approach

Several experiments were performed with $H_2O_2$ (Scharlab 30% w/v) and peroxydisulfate salt (PDS), $S_2O_8^{2-}$ (AppliChem 98%), which were applied in the range of 0.015–0.29 mmol/L and 0.05–5 mmol/L for $H_2O_2$ and $S_2O_8^{2-}$, respectively. In this first step, the authors aim to determine the effect of both oxidants on *Anabaena* sp. on its own. Thus, growth inhibition tests were performed up to seven days in order to determine the effective values for 50% inhibition after 72 h but also the regrowth capability at longer times, which the authors consider of interest based on the aim of the present work.

Once the effect on *Anabaena* sp. caused by the addition of a single oxidant was determined, the possible enhancement of growth inhibition by the addition of low amounts (18 $\mu$mol/L) of Fe(II) (from FeSO$_4$ (99%, Scharlab)) was studied in order to promote the generation of hydroxyl (•OH) or sulfate radicals ($SO_4^{2-}$) from $H_2O_2$ and $S_2O_8^{2-}$, respectively (Equations (1) and (2)). In that case, selected concentrations of $H_2O_2$ and $S_2O_8^{2-}$ based on the first experimental results were used, and similar growth inhibition tests were performed with the objective of observing if the presence of iron can increase the growth inhibition of cyanobacteria.

Finally, the efficiency of both oxidants together with the presence/absence of iron was assessed in co-cultures of *Anabaena* sp. and *Vibrio alginolyticus* as a consortium of cyanobacterial and marine bacteria. This was carried out to determine if the presence of bacteria
can alter the results obtained from single cultures of cyanobacteria. The summarized experimental design is depicted in Table 1.

Table 1. Experimental approach for assessing H$_2$O$_2$- or S$_2$O$_8$$^{2-}$-based processes on *Anabaena* sp. and *V. alginolyticus* in a consortium.

| Target Organism | Treatment | [H$_2$O$_2$] (mmol/L) | [S$_2$O$_8$$^{2-}$] (mmol/L) |
|-----------------|-----------|----------------------|-----------------------------|
| *Anabaena* sp.  | Single oxidant | 0.015–0.29 | 0.05–5 |
|                 | + Fe(II) [Fe(II)] = 18 µmol/L | 0.059, 0.118$^1$ | 1, 3, 5$^1$ |
| *Anabaena* sp.  | Single oxidant | 0.088, 0.29$^2$ | 3$^2$ |
| + *V. alginolyticus* | + Fe(II) [Fe(II)] = 18 µmol/L | 0.088, 0.29$^2$ | 3$^2$ |
| *V. alginolyticus* | Single Oxidant | 0.29 | 3 |
|                 | + Fe (II) [Fe(II)] = 18 µmol/L | 0.29 | 3 |

$^1$ Reagent concentrations selected based on the growth inhibition percentages obtained in Section 3.1; $^2$ Reagent concentrations selected based on the growth inhibition percentages obtained in Section 3.2.

For *Anabaena* sp., a period of 6–9 days has been defined to determine the treatment effect. In the case of the bacterial assays (*V. alginolyticus*), the exposure time has been fixed for 72 h, according to the specific bacterial growth rates. All experiments were conducted in duplicate at least. Each chemical was added in a single dosage to reach the desired concentration in a total volume of 50 mL of culture. An aliquot of each culture was extracted daily to determine cell density (by means of absorbance or fluorescence measurement), together with oxidant decay and dissolved iron in selected cases. Dissolved iron, H$_2$O$_2$ and S$_2$O$_8$$^{2-}$ were monitored spectrophotometrically according to the methods explained in Spuhler et al. 2010 [29], Eisenberg 1943, DIN 38 409 H15 [30]; and Liang et al. 2008 [31]. In all cases, the maximum volume to be extracted from each treated sample was limited to half of the original volume in order to avoid possible effects due to the loss of volume.

2.3. Data Treatment

In the case of cyanobacterial cells (*Anabaena* sp.), the effects from the different treatments were assessed by growth monitoring after the treatment. The growth inhibition (%) was calculated by the variation of cell density before and after treatment on each sampling day, as explained elsewhere [32]. For determining effective concentrations (EC50%) of specific reagents (H$_2$O$_2$ and S$_2$O$_8$$^{2-}$), the growth inhibition (%) versus effective concentrations were fitted according to the model proposed by Hampel et al., 2001 [33]. Thus, the EC$_{50}$% ± the standard error was obtained as a significant coefficient ($p < 0.001$) in the model.

In the case of bacteria (*V. alginolyticus*), the effects from the different treatments were assessed by analyzing inactivation profiles with a logarithmic reduction in the survival microorganisms (Log (N/N$_0$)) versus time. The detection limit was determined as 10 CFU·mL$^{-1}$, which corresponds to a 4.53–5.03 Log Removal Values (LRV).

3. Results and Discussion

3.1. Effects of H$_2$O$_2$ or S$_2$O$_8$$^{2-}$ on *Anabaena* sp.

Firstly, in order to determine the damage that both oxidants can cause in *Anabaena* sp., H$_2$O$_2$ and S$_2$O$_8$$^{2-}$ were applied in a wide range of doses to determine an effective concentration for both reagents. Although absorbance and fluorescence were valid for measuring cell concentration, the use of the fluorescence was selected due to its better correlation and higher sensitivity, which was essential in some cases when the concentration of the cultures is rather low. Additionally, the associated errors between replicates were reduced by means of fluorescence measurements (Supplementary Materials Figure S2).
In the case of H₂O₂ (Figure 1), low amounts were required to reach an effective concentration, EC50%, at 72 h = 0.0712 mmol/L ± 0.007 (R² = 0.9845). In fact, concentrations equal to or higher than 0.088 mmol/L caused an inhibition percentage >90% (at 72 h) with respect to the control samples. This effect is somewhat modified throughout time since the EC50% on day 6 slightly increased up to 0.088 mmol/L ± 0.534 (R² = 0.9628), suggesting that the damage cannot be reparable during this time frame at concentrations ≥0.088 mmol/L (2.99 mg H₂O₂/L).

On the other hand, S₂O₈²⁻ (Figure 2) needs higher concentrations to cause perceptible cell damage since inhibition percentages of 8.71–28.40% were reached at 72h with oxidant doses of 0.5–5 mmol/L of S₂O₈²⁻. The estimated EC50% values at 72 h were obtained as 6.80 mmol/L ± 1.33 (R² = 0.6678). In this case, those values were reduced up to 5.40 mmol/L ± 1.04 (R² = 0.6878) on day 7, which suggests that the S₂O₈²⁻ react slowly with cells. Thus, the effects associated with S₂O₈²⁻ became perceptible at longer times. Nonetheless, the inhibition percentage is notably less if compared with the H₂O₂.
The consumption of oxidants was monitored during experimentation. In this context, differences were also evidenced according to the type of oxidant. When H$_2$O$_2$ was applied, rapid and total consumption (<24 h) was recorded for initial concentrations up to 0.18 mmol/L, whereas at least 72 h was necessary for total H$_2$O$_2$ consumption in the range 0.23–0.029 mmol/L. In respect to the PDS, longer consumption periods were detected. PDS consumption was quantified in the range of 25–55% after 7 days of exposure. These slow PDS consumption percentages can be related to the higher concentrations that were used but also to the higher stability of this salt. These results suggest the major efficiency of H$_2$O$_2$ over that of S$_2$O$_8^{2-}$ when it is applied as a single oxidant, especially in cyanobacterial species.

Generally, hydrogen peroxide has shown significant sensitivity to cyanobacterial species with very low concentrations (2 mg H$_2$O$_2$/L; 0.059 mmol/L) [12,34]. These results corroborate the authors’ experiments with *Anabaena* sp., for which the EC50% values obtained (0.0712 mmol/L ± 0.007) agree with those reported in the literature with other cyanobacterial species, such as *Microcystis aeruginosa* [35,36]. These matches are interesting in the way *Anabaena* sp. differs from *M. aeruginosa* in cell morphology (filaments formed in *Anabaena* cultures) and also the seawater matrix used in this study.

The application of H$_2$O$_2$ for inactivating cyanobacteria in freshwater ecosystems was successfully applied in real blooms [3,12]. Thus, the specific sensitivity to H$_2$O$_2$ for the cyanobacterial species over that of other eukaryotic organisms was reported, showing that cyanobacterial species are more sensitive than other species of green algae or diatoms that show greater resistance to hydrogen peroxide [12,13,15,16,34]. The higher sensitivity of cyanobacteria to H$_2$O$_2$ can be related to the lack of major antioxidant enzymes, such as catalases or ascorbate peroxidase, which permits the degradation of substantial quantities of intracellular H$_2$O$_2$ [36,37]. The lack of these enzymes can be attributed to the fact that cyanobacteria do not have to deal with similar levels of intracellular H$_2$O$_2$ as do other eukaryotic microorganisms; therefore, cyanobacteria have less elaborate H$_2$O$_2$ detoxification routes [3,36,38].

On the other hand, the use of PDS salt (S$_2$O$_8^{2-}$) results in low growth inhibition for *Anabaena* sp. even at higher concentrations of 5 mmol/L. The results in this study agree with previous studies in which the biocide efficacy of PDS was tested against natural groundwater microalgae [39] or green alga *Dunalieila tertiolecta* [17]. Results from these studies reported low biocidal activity of PDS. The results obtained in the present study suggest a minimum effect of PDS against cyanobacteria. Although some studies suggest possible intracellular damage provoked by the penetration of sulfate (that is in excess in the extracellular environment from S$_2$O$_8^{2-}$) through the sulfate permeases (membrane-protein transporters) [40,41], it appears to be minimal. Indeed, the low consumption rates obtained suggest a slow reaction rate of PDS in seawater, supporting that PDS presents high stability in seawater [17].

### 3.2. Inactivation by the Presence of Fe (II)

As both H$_2$O$_2$ and S$_2$O$_8^{2-}$ can be activated by the presence of transition metals, it seems interesting to know if the presence of small amounts of iron can be enough to increase the growth inhibition on *Anabaena* sp. For that purpose, selected concentrations of 0.059, 0.118 mmol/L of H$_2$O$_2$ and 2.3,4 mmol/L of S$_2$O$_8^{2-}$ were combined by the addition of 18 µmol/L of Fe(II). Control tests with the single addition of iron were performed, and similar growth curves were observed as those without Fe(II), assuring that the addition of this metal in tested concentrations does not inhibit the growth of *Anabaena* sp. This accords with studies that are more specific, which suggests that the dominance of *Anabaena azotica* was between 18 and 36 µmol Fe/L rather than at other Fe concentrations [42].

The inhibition growth (%) obtained for specific days 2, 3, and 7 are shown in Figure 3. In the case of H$_2$O$_2$, two different scenarios were observed. When concentrations of H$_2$O$_2$ were rather low (0.059 mmol/L), little effect in *Anabaena* sp. is observed (approx. 11% of growth inhibition on days 2 and 3), which becomes minimal on day 7 (3.17%))).
a recovery of these cells. In this case, \([\text{H}_2\text{O}_2] = 0.059 \text{ mmol/L}\), the combination with Fe(II) results in extra damage by obtaining an inhibition percentage of approximately 30% in respect to the control samples, which was maintained during the seven days of experimentation. Nonetheless, although higher growth inhibition was observed with the presence of iron in respect to single \(\text{H}_2\text{O}_2\), this is still low for a possible abatement of the \textit{Anabaena} blooms. Higher concentrations of \(\text{H}_2\text{O}_2\) (0.118 mmol/L) imply a notably greater effect on the inhibition of growth, especially from day 3 onwards (> 80%, Figures 1 and 3). These higher growth inhibition percentages deter from properly quantifying possible extra damage caused by the presence of iron, for which similar growth inhibition percentages were obtained (Figure 3).

![Graphs showing growth inhibition percentages](image)

**Figure 3.** Specific growth inhibition (%) obtained at days 2, 3 and 7 for \textit{Anabaena} sp. exposed to \(\text{H}_2\text{O}_2\) or \(\text{S}_2\text{O}_8^{2-}\) in the presence or absence of Fe(II).

When PDS (\(\text{S}_2\text{O}_8^{2-}\)) is assessed as a source of radicals (Equation (2)), a low-moderate effect of \(\text{S}_2\text{O}_8^{2-}\) itself in \textit{Anabaena} sp. was observed, which did not exceed 36% after seven days of experimentation (Figure 3). The addition of Fe(II) notably increased the effect of single PDS, for which the growth inhibition was enhanced by a factor of ~3.5 with 3 and 4 mmol/L of PDS on day 2. However, this improvement was decreasing with longer exposure time, where the increase in growth inhibition was by a factor of ~1.20 on day 7 for 3 and 4 mmol/L of PDS + Fe(II).

The addition of Fe(II) was spiking (in a single dosage) to the target cultures; hence, the presence of Fe(II) in the extracellular environment might lead to reactive radicals according to Equations (1) and (2), which can be responsible for cell damage in the bulk. However, the saline matrix, together with the basic pH of the \textit{Anabaena} cultures, was probably responsible for the rapid oxidation of Fe(III) into Fe(II), decreasing the reaction rate among Fe(III) and \(\text{H}_2\text{O}_2\) or \(\text{S}_2\text{O}_8^{2-}\) [19,43]. In fact, in the case of PDS, the reaction with Fe(III) is unknown [20].

Additionally, it would also involve the precipitation of iron hydroxides. In this regard, occasional measurements of dissolved iron were performed, which decreased up to 0.33–0.42 mg Fe/L within the first 24 h. It might entail heterogeneous Fenton-like reactions in the bulk to some extent and especially in the case of \(\text{H}_2\text{O}_2\) [22]. On the other hand, an extracellular iron reduction from Fe(III) to Fe(II) could also occur facilitated by a specific outer membrane transporter on the cell surface or siderophore or other DOM-mediated mechanisms in the cultures [44–46], which could maintain the residual levels of dissolved Fe detected. These possible pathways were perhaps responsible for the enhanced growth inhibition at longer exposure times, specially for \(\text{S}_2\text{O}_8^{2-}\) (Figure 3).
Intracellular mechanisms might have also been responsible for the growth inhibition of *Anabaena* sp. after the iron addition in the presence of H$_2$O$_2$ or S$_2$O$_8^{2−}$. The existence of these reagents, together with Fe in bulk, might have been transported to the intracellular domain [22,43]. The presence of additional H$_2$O$_2$ at the intracellular level can be fatal for cyanobacteria due to the lack of scavenging enzymes, as discussed in the previous Section 3.1. Thus, similar growth inhibition percentages were obtained with the presence/absence of iron at an H$_2$O$_2$ concentration of 0.118 mmol/L. However, S$_2$O$_8^{2−}$, together with extracellular added iron, could have interacted with membrane transporters, favoring diffusion through the cell wall membrane. These hypothetic intracellular S$_2$O$_8^{2−}$, together with the presence of extra iron, could have promoted an enhanced intracellular PDS/Fe(II) process, causing the enhancement observed in Figure 3 [22,40].

Although some improvements for the use of H$_2$O$_2$ or S$_2$O$_8^{2−}$ in combination with low amounts of Fe(II) were detected, there was still a wide range to obtain higher growth inhibition in *Anabaena* sp. (especially for the PDS case), such as the addition of other activation factors (e.g., UV-radiation) or by increasing the iron concentration [32].

### 3.3. Behavior of *Anabaena* sp. Inactivation in Consortium with *V. alginolyticus*

As a next step, mixture experiments were performed with *Anabaena* sp. and *V. alginolyticus*, a marine pathogenic bacterium that has been associated with HABs.

According to the previous results described in Sections 3.1 and 3.2, 0.088 mmol/L of H$_2$O$_2$ and 3 mmol/L of PDS were selected as the oxidant concentration, together with 18 µmol/L of Fe(II). Control experiments for assuring the regular growth of both *Anabaena* sp. and *V. alginolyticus* in co-cultures were performed, showing no growth inhibition for neither *Anabaena* sp. nor *V. alginolyticus*.

Regarding the use of PDS (Figure 4), similar trends were observed for *Anabaena* sp. when PDS was tested in monocultures. Some differences in growth inhibition were detected within the first days of exposure among PDS or PDS/Fe(II); however, similar growth inhibition percentages were observed on day 7 (44.29–47.73%). This suggests that similar damage was caused by these reagents in the presence of bacteria.

![Figure 4](image_url)

*Figure 4.* Growth curves of *Anabaena* sp. in co-culture with *V. alginolyticus* for single S$_2$O$_8^{2−}$ or S$_2$O$_8^{2−}$ + Fe(II) treatments. Inset: Specific growth inhibition (%) obtained on day 3 or 7 for *Anabaena* sp.

Experiments with H$_2$O$_2$ are depicted in Figure 5. Initially, a concentration of 0.088 mmol/L of H$_2$O$_2$ was used, for which growth inhibition was expected to some extent (see Section 3.1). However, with the presence of *V. alginolyticus*, the inhibition growth obtained for *Anabaena* sp.
was quantified rather low, i.e., 23.91% and 30.57% (on Day 3) for both H$_2$O$_2$ and H$_2$O$_2$/Fe(II) treatments, respectively. The obtained results differ from those obtained by single Anabaena sp. cultures (Figure 1) as Anabaena sp. (in co-culture) is able to grow similar to control samples on day 7 (Figure 5). Accordingly, the authors decided to increase the concentration of H$_2$O$_2$ up to 0.29 mmol/L of H$_2$O$_2$, for which complete inhibition was observed in monocultures. Interestingly, the growth inhibition now decreased down to 60% on day 7 with the presence of V. alginolyticus (compared to the 99.01% obtained in monocultures, Figure 1). In addition, the differences were now very clear among H$_2$O$_2$ and H$_2$O$_2$/Fe(II) treatments where the inhibition percentage of Anabaena sp. reached 94% on day 7 with 0.29 mmol/L of H$_2$O$_2$ + Fe(II).

![Figure 5](image_url)

**Figure 5.** Growth curves of Anabaena sp. in co-culture with V. alginolyticus for single H$_2$O$_2$ or H$_2$O$_2$ + Fe(II) treatments. Inset: Specific growth inhibition (%) obtained at day 3 or 7 for Anabaena sp. exposed to H$_2$O$_2$ or H$_2$O$_2$ + Fe(II) treatments.

Related to V. alginolyticus, no differences were detected by PDS or PDS/Fe(II) (Figure 6), although a delayed inactivation was observed for V. alginolyticus with the presence of Anabaena sp. It is important to note that PDS was not entirely consumed during experimentation, i.e., on day 7; 79.3% and 92% of the initial PDS amounts (3 mmol/L) were consumed by PDS and PDS/Fe(II), respectively. This remaining oxidant could be one of the reasons why V. alginolyticus was not able to regrow after treatment. With respect to the effect of hydrogen peroxide on V. alginolyticus survival (Figure 6), a 1.31–1.48 LRV or 2.44–2.72 LRV was obtained (within 24 h) in co-cultures or monocultures, respectively. However, after 24 h, bacteria regrowth was observed in both cases, which was probably caused by the bacteria that survived. This observed phenomenon could be due to the fact that H$_2$O$_2$ was entirely consumed within the first 24 h of the experiment. It could have permitted the surviving bacteria to grow, which is contrary to what happens with PDS, for which the high concentrations involved a residual oxidant that might have avoided the regrowth of bacteria. Nonetheless, treated samples did not reach control samples, suggesting the growth rate slows down, especially when V. alginolyticus was in a co-culture with Anabaena sp. (Figure 6). It might imply that the remaining bacteria are somehow damaged by the addition of H$_2$O$_2$ (+Fe(II)) or by cyanobacterial-derived organic matter that was probably released during the treatment, which can affect the growth of V. alginolyticus [26].
The results obtained in co-cultures of *Anabaena* sp. and *V. alginolyticus* indicate the protection of cyanobacteria (extremely sensitive to H$_2$O$_2$) by the presence of bacteria, such as an increment of H$_2$O$_2$ to reach a growth inhibition of 50% was needed from 0.071 mmol/L (2.41 mg H$_2$O$_2$/L) up to 0.29 mmol/L (10 mg H$_2$O$_2$/L) in monocultures and co-cultures, respectively. It suggests that *Anabaena* sp. can survive at much higher H$_2$O$_2$ concentrations in a co-culture with marine bacteria. This effect was also recently reported by Weenink et al. 2021 [47], who demonstrated that green algae (*Chlorella sorokiniana*) could protect cyanobacteria (*Microcystis aeruginosa*) against oxidative stress originated by H$_2$O$_2$. Similar experiments, but with different approaches, were performed by Pulgarin et al. 2020 [22], in which the photo-Fenton process was tested against fecal bacteria (*E. coli*) in microalgal cultures (*Chlorella vulgaris*), suggesting, in this case, some protective effect for *E. coli* in *C. vulgaris* cultures. Thus, the presence of bacteria or green algae, with much developed cellular defenses against Reactive Oxygen Species, would degrade H$_2$O$_2$ more efficiently and could protect cyanobacteria against oxidative stress.

In addition, the effect of the Fenton process has also been evidenced in co-cultures where an increase of 58.4% in growth inhibition (on day 7) was observed for H$_2$O$_2$/Fe(II) compared to single H$_2$O$_2$. As previously stated (see Section 3.2), the rapid oxidation of Fe(II) into Fe(III) involves the iron precipitation in co-cultures; thus, the concentration of dissolved iron decreased within 24 h. Consequently, the Fenton reaction in an extracellular environment is expected to be rather slow. However, it is known that internal Fenton reactions can also occur [48]. Taking into account that marine cyanobacteria have iron-rich photosynthetic machinery with an extensive range of iron stress responses (due to the iron limitation in marine environments) [43], intracellular processes might become important. These could be responsible for observed growth inhibition with the presence of additional iron in the bulk. In combination with H$_2$O$_2$ and the lack of specific enzymes that could degrade it, the internal Fenton processes would be favored for the cyanobacterial case.

4. Conclusions

In this study, the efficacy of two different oxidants, H$_2$O$_2$ and S$_2$O$_8^{2-}$ (PDS), was assessed against *Anabaena* sp. as bloom-forming and noxious cyanobacteria. A summary of the key findings is reported in Table 2.

The effects of both oxidants differed when they were assessed in monocultures of *Anabaena* sp. since H$_2$O$_2$ shows greater efficiency over that of PDS. *Anabaena* sp. was very sensitive to H$_2$O$_2$ (EC$_{50}$% at 72 h = 0.0712 mmol/L), while PDS showed a moderate effect on growth inhibition (EC$_{50}$% at 72 h = 6.80 mmol/L). With respect to H$_2$O$_2$, the addition of Fe(II) at 18 µmol/L did not increase the growth inhibition of *Anabaena* sp. due to the
substantial sensitivity of the H\textsubscript{2}O\textsubscript{2} itself. On the other hand, the growth inhibition (on day 3) was increased by a factor of ~3.5 with 3 and 4 mmol/L of PDS. This enhancement disappears at longer exposure times, reaching inhibition percentages never higher than 50%.

Table 2. Summary table with key findings related to the use of H\textsubscript{2}O\textsubscript{2} or S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−} for inactivate Anabaena sp. and V. alginolyticus in (co)-cultures.

| Target Organism | Treatment | Key Findings |
|-----------------|-----------|--------------|
| Anabaena sp.    | H\textsubscript{2}O\textsubscript{2} | High growth inhibition (EC\textsubscript{50} 72h = 0.0712 mmol H\textsubscript{2}O\textsubscript{2}/L ± 0.007). The strong effects of H\textsubscript{2}O\textsubscript{2} itself inhibit properly quantifying possible extra damage caused by the presence of Fe(II). |
| Anabaena sp. (+V. alginolyticus) | S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−} | Low–moderate growth inhibition ((EC\textsubscript{50} 72 h = 6.80 mmol S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−}/L ± 1.33)). The addition of Fe(II) notably increases growth inhibition in the first 48–72 h. However, this improvement decreased with longer exposure times, e.g., on day 7. |
| Anabaena sp. (+V. alginolyticus) | H\textsubscript{2}O\textsubscript{2} | The presence of bacteria implies increasing the H\textsubscript{2}O\textsubscript{2} concentration up to 0.29 mmol H\textsubscript{2}O\textsubscript{2}/L to obtain a growth inhibition (at 72 h) of 55% ± 5.73, indicating that the presence of bacteria can protect cyanobacteria from H\textsubscript{2}O\textsubscript{2} exposure. The effect of H\textsubscript{2}O\textsubscript{2} + Fe(II) was evidenced in co-cultures, increasing the growth inhibition by 58.4% compared to single H\textsubscript{2}O\textsubscript{2}. |
| V. alginolyticus (+Anabaena sp.) | S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−} | Similar growth inhibition percentages were observed when S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−} was tested in monocultures (44.29–47.73% at day 7). This suggests that similar damage was caused by S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−} in the presence of bacteria. |

By co-culturing Anabaena sp. and V. alginolyticus, an incremental addition of H\textsubscript{2}O\textsubscript{2} (from 0.071 mmol/L to 0.29 mmol/L) was required to achieve growth inhibition percentages higher than 50%, indicating that the presence of bacteria can protect cyanobacteria from H\textsubscript{2}O\textsubscript{2} exposure. In addition, the effect of H\textsubscript{2}O\textsubscript{2}+Fe(II) was evidenced in co-cultures, increasing the growth inhibition by 58.4% compared to single H\textsubscript{2}O\textsubscript{2}. However, these H\textsubscript{2}O\textsubscript{2} concentrations were not enough to prevent the regrowth of V. alginolyticus after 24 h.

The results that were obtained demonstrated that oxidants such as H\textsubscript{2}O\textsubscript{2} or PDS salts (S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−}) can be applied to reduce both cyanobacteria and associated pathogenic bacteria in marine waters. Although some improvements about the use of H\textsubscript{2}O\textsubscript{2} or S\textsubscript{2}O\textsubscript{8}\textsuperscript{2−} in combination with Fe(II) have been detected, there is still a wide range to promote higher growth inhibition in Anabaena sp., especially for PDS, for which moderate effects have been obtained.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/microorganisms10040735/s1, Figure S1. Anabaena sp. (Strain: CCMM 01/0101, ICAMN-CSIC). Figure S2. Growth curves of Anabaena sp. after adding different concentrations of H\textsubscript{2}O\textsubscript{2}. A. Cell density obtained by means of fluorescence measurements. B. Cell density obtained by means of absorbance (λ = 680 nm). C. Growth inhibition rate for Anabaena sp. at 72 h exposed to H\textsubscript{2}O\textsubscript{2} by means of fluorescence of absorbance measurements.
Author Contributions: Conceptualization, J.M.-A.; Data curation, I.R.-Z.; Formal analysis, J.M.-A. and I.R.-Z.; Funding acquisition, J.M.-A. and E.N.; Investigation, J.M.-A. and I.R.-Z.; Methodology, J.M.-A. and I.R.-Z.; Project administration, J.M.-A. and E.N.; Resources, J.M.-A. and E.N.; Supervision, A.A.-M. and E.N.; Validation, E.N.; Writing—original draft, J.M.-A.; Writing—review and editing, I.R.-Z. and E.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union under the 2014–2020 ERDF Operational Programme and by the Department of Economy, Knowledge and Business at the University of the Regional Government of Andalusia. Project reference: FEDER-UCA18–108023. J.M.-A. acknowledges Grant JIC2020–042741-I funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References
1. Anderson, D.M.; Cembella, A.D.; Hallegraeff, G.M. Progress in Understanding Harmful Algal Blooms: Paradigm Shifts and New Technologies for Research, Monitoring, and Management. *Ann. Rev. Mar. Sci.* 2012, 4, 143–176. [CrossRef] [PubMed]
2. Munoz, M.; Ciré, S.; de Pedro, Z.M.; Colina, J.A.; Velásquez-Figueroa, Y.; Carmona-Jiménez, J.; Caro-Borrero, A.; Salazar, A.; Santa Maria Fuster, M.C.; Contreras, D.; et al. Overview of toxic cyanobacteria and cyanotoxins in Ibero-American freshwaters: Challenges for risk management and opportunities for removal by advanced technologies. *Sci. Total Environ.* 2021, 761, 143197. [CrossRef] [PubMed]
3. Kibuye, F.A.; Zamyadi, A.; Wert, E.C. A critical review on operation and performance of source water control strategies for cyanobacterial blooms: Part I—chemical control methods. *Harmful Algae* 2020, 109, 102099. [CrossRef] [PubMed]
4. Costa, P.R.; Costa, S.T.; Braga, A.C.; Rodrigues, S.M.; Vale, P. Relevance and challenges in monitoring marine biotoxins in non-bivalve vectors. *Food Control* 2017, 76, 24–33. [CrossRef]
5. Bellés-Garulera, J.; Vila, M.; Borrell, E.; Riób, P.; Franco, J.M.; Sala, M.M. Variability of planktonic and epiphytic vibrios in a coastal environment affected by Ostreopsis blooms. *Sci. Mar.* 2016, 80, 97–106. [CrossRef]
6. Greenfield, D.I.; Gooch Moore, J.; Stewart, J.R.; Hilborn, E.D.; George, B.J.; Li, Q.; Dickerson, J.; Keppler, C.K.; Sandifer, P.A. Temporal and Environmental Factors Driving Vibrio Vulnificus and V. Parahaemolyticus Populations and Their Associations With Harmful Algal Blooms in South Carolina Detention Ponds and Receiving Tidal Creeks. *GeoHealth* 2017, 1, 306–317. [CrossRef]
7. Baker-Austin, C.; Trinanes, J.; Gonzalez-Escalona, N.; Martínez-Urtaza, J. Non-Cholera Vibrios: The Microbial Barometer of Climate Change. *Trends Microbiol.* 2017, 25, 76–84. [CrossRef]
8. Grimes, D.J. The Vibrios: Scavengers, Symbionts, and Pathogens from the Sea. *Microb. Ecol.* 2020, 80, 501–506. [CrossRef]
9. Sukenik, A.; Kaplan, A. Cyanobacterial Harmful Algal Blooms in Aquatic Ecosystems: A Comprehensive Outlook on Current and Emerging Mitigation and Control Approaches. *Microorganisms* 2021, 9, 1472. [CrossRef]
10. Villacorte, L.O.; Assiyeh, S.; Tabatabai, A.; Anderson, D.M.; Amy, G.L.; Schippers, J.C.; Kennedy, M.D. Seawater reverse osmosis desalination and (harmful) algal blooms. *Desalination* 2015, 360, 61–80. [CrossRef]
11. Xu, H.; Brookes, J.; Hobson, P.; Pei, H. Impact of copper sulphate, potassium permanganate, and hydrogen peroxide on Pseudanabaena galatea cell integrity, release and degradation of 2-methylisoborneol. *Water Res.* 2019, 157, 64–73. [CrossRef]
12. Matthijs, H.C.P.; Visser, P.M.; Reeze, B.; Meese, J.; Slot, P.C.; Wijn, G.; Talens, R.; Huisman, J. Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Res.* 2012, 46, 1460–1472. [CrossRef]
13. Lusty, M.W.; Gobler, C.J. The Efficacy of Hydrogen Peroxide in Mitigating Cyanobacterial Blooms and Altering Microbial Communities across Four Lakes in NY, USA. *Toxins* 2020, 12, 428. [CrossRef]
14. Barone, M.E.; Parkes, R.; Herbert, H.; McDonnell, A.; Conlon, T.; Aranysos, A.; Fierli, D.; Fleming, G.T.A.; Touzet, N. Comparative Response of Marine Microalgae to H2O2-Induced Oxidative Stress. *Appl. Biochem. Biotechnol.* 2021, 193, 4052–4067. [CrossRef]
15. Romero-Martínez, L.; Rivas-Zaballos, I.; Moreno-Andrés, J.; Moreno-Garrido, I.; Acevedo-Merino, A.; Nebot, E. Improving the microalgae inactivating efficacy of ultraviolet ballast water treatment in combination with hydrogen peroxide or peroxymonosulfate salt. *Mar. Pollut. Bull.* 2021, 162, 111886. [CrossRef]
16. Mathijs, G.D.; Robbert, G.; Mark, A.J. Time and Concentration Dependency in the Potentially Affected Fraction of Species: The case of hydrogen peroxide treatment of ballast water. *Environ. Toxicol. Chem.* 2008, 27, 746–753.
17. Ahn, S.; Peterson, T.D.; Righter, J.; Miles, D.M.; Tratnyek, P.G. Disinfection of ballast water with iron activated persulfate. *Environ. Sci. Technol.* 2013, 47, 11717–11725. [CrossRef]
18. Moreno-Andrés, J.; Farinango, G.; Romero-Martínez, L.; Acevedo-Merino, A.; Nebot, E. Application of persulfate salts for enhancing UV disinfection in marine waters. *Water Res.* 2019, 163, 114866. [CrossRef]
19. Pignatello, J.J.; Oliveros, E.; MacKay, A. Advanced oxidation processes for organic contaminant destruction based on the fenton reaction and related chemistry. Crit. Rev. Environ. Sci. Technol. 2006, 36, 1–84. [CrossRef]

20. Rodríguez-Chueca, J.; Giannakis, S.; Marjanovic, M.; Kohantorabi, M.; Gholami, M.R.; Grandjean, D.; de Alencastro, L.F.; Pulgarín, C. Solar-assisted bacterial disinfection and removal of contaminants of emerging concern by Fe(II)-activated HSO₃⁻ vs. S₂O₅²⁻ in drinking water. Appl. Catal. B Environ. 2019, 248, 62–72. [CrossRef]

21. Song, Q.; Niu, X.; Zhang, D.; Song, X.; Li, Y.; Ma, J.; Lai, S.; Yang, Z.; Zhou, S. The behaviors of Microcystis aeruginosa and microcystins during the Fe²⁺/persulphate (PS) preoxidation-coagulation and floc storage period. Environ. Res. 2020, 186, 109549. [CrossRef]

22. Pulgarín, A.; Giannakis, S.; Pulgarín, C.; Ludwig, C.; Refardt, D. A novel proposition for a citrate-modified photo-Fenton process against bacterial contamination of microalgae cultures. Appl. Catal. B Environ. 2020, 265, 118615. [CrossRef]

23. Zhang, X.; Ma, Y.; Tang, T.; Xiong, Y.; Dai, R. Removal of cyanobacteria and control of algal organic matter by simultaneous oxidation and coagulation—Comparing the H₂O₂/Fe(II) and H₂O₂/Fe(III) processes. Sci. Total Environ. 2020, 720, 137653. [CrossRef]

24. Wert, E.C.; Dong, M.M.; Rosario-Ortiz, F.L. Using digital flow cytometry to assess the degradation of three cyanobacteria species after oxidation processes. Water Res. 2013, 47, 3752–3761. [CrossRef]

25. Moreno-Andrés, J.; Morillo-Ponce, J.; Ibáñez-López, M.E.; Acevedo-Merino, A.; García-Morales, J.L. Disinfection enhancement of single ozonation by combination with peroxymonosulfate salt. J. Environ. Chem. Eng. 2020, 8, 104335. [CrossRef]

26. Eiler, A.; Gonzalez-Rey, C.; Allen, S.; Bertilsson, S. Growth response of Synechococcus sp. PCC 7002 Reduces Amorphous and Crystalline Iron Forms in Synthetic Seawater Medium. Environ. Sci. Pollut. Res. 2021, 126, 248. [CrossRef]

27. Moreno-Andrés, J.; Acevedo-Merino, A.; Nebot, E. Study of marine bacteria inactivation by photochemical processes: Disinfection kinetics and growth modeling after treatment. Environ. Sci. Pollut. Res. 2018, 25, 27693–27703. [CrossRef]

28. Spuhler, D.; Andres Rengifo-Herrera, J.; Pulgarín, C. The effect of Fe²⁺ on cyanobacterial photosynthesis. Front. Microbiol. 2007, 1043, 363–369. [CrossRef]

29. Liang, C.; Huang, C.F.; Mohanty, N.; Kurakalva, R.M. A rapid spectrophotometric determination of persulfate anion in ISCO. Vibrio cholerae sp. PCC 7120. Environ. Microbiol. 2012, 14, 1655–1670. [CrossRef]

30. Eisenberg, G.M. Colorimetric Determination of Hydrogen Peroxide. Ind. Eng. Chem. 1943, 15, 327–328. [CrossRef]

31. Wang, B.; Zhang, Y.; Qin, Y.; Li, H. Removal of Microcystis aeruginosa and control of algal organic matter by Fe(II)/peroxymonosulfate pre-oxidation enhanced coagulation. Environ. Sci. Total Environ. 2020, 731, 137653. [CrossRef]

32. Yuan, Y.; Jiang, M.; Zhu, X.; Yu, H.; Otte, M.L. Interactions between Fe and light strongly affect phytoplankton communities in a phytoplankton community study on the consequences for phytoplankton community and diversity. Front. Microbiol. 2015, 6, 714. [CrossRef]

33. Latifi, A.; Ruiz, M.; Zhang, C.-C. Oxidative stress in cyanobacteria. FEMS Microbiol. Rev. 2009, 33, 258–278. [CrossRef]

34. Farinelli, G.; Giagnorio, M.; Ricceri, F.; Giannakis, S.; Tiraferri, A. Evaluation of the Effectiveness, Safety, and Feasibility of 9 Potential Biocides to Disinfect Acidic Landfill Leachate from Algae and Bacteria. Water Res. 2021, 191, 116801. [CrossRef]

35. Berruti, I.; Oller, I.; Polo-López, M.I. Direct oxidation of peroxymonosulfate under natural solar radiation: Accelerating the simultaneous removal of organic contaminants and pathogens from water. Chemosphere 2021, 279, 130555. [CrossRef]

36. Xiao, R.; Liu, K.; Bai, L.; Minakata, D.; Seo, Y.; Kaya Göktas, R.; Dionysiou, D.D.; Tang, C.-J.; Wei, Z.; Spinney, R. Inactivation of pathogenic microorganisms by sulfate radicals: Present and future. Chem. Eng. J. 2019, 371, 222–232. [CrossRef]

37. Yuan, Y.; Jiang, M.; Zhu, X.; Yu, H.; Otte, M.L. Interactions between Fe and light strongly affect phytoplankton communities in a eutrophic lake. Ecol. Indic. 2021, 126, 107664. [CrossRef]

38. Hunnestad, A.V.; Vogel, A.I.M.; Armstrong, E.; Digernes, M.G.; Van Ardelan, M.; Hohmann-Marriott, M.F. From the Ocean to the Lab—Assessing Iron Limitation in Cyanobacteria: An Interface Paper. Microorganisms 2020, 8, 1899. [CrossRef]

39. Hunnestad, A.V.; Vogel, A.I.M.; Digernes, M.G.; Van Ardelan, M.; Hohmann-Marriott, M.F. Iron Speciation and Physiological Analysis Indicate that Synechococcus sp. PCC 7002 Reduces Amorphous and Crystalline Iron Forms in Synthetic Seawater Medium. J. Mar. Sci. Eng. 2020, 8, 996. [CrossRef]

40. Stevanovic, M.; Hahn, A.; Nicolsien, K.; Mirus, O.; Schleif, E. The components of the putative iron transport system in the cyanobacterium Anabaena sp. PCC 7120. Environ. Microbiol. 2012, 14, 1655–1670. [CrossRef]
46. Moreno-Andrés, J.; Vallés, I.; García-Negueroles, P.; Santos-Juanes, L.; Arques, A. Enhancement of Iron-Based Photo-Driven Processes by the Presence of Catechol Moieties. *Catalysts* 2021, 11, 372. [CrossRef]

47. Weenink, E.F.J.; Matthijs, H.C.P.; Schuurmans, J.M.; Piel, T.; van Herk, M.J.; Sigon, C.A.M.; Visser, P.M.; Huisman, J. Interspecific protection against oxidative stress: Green algae protect harmful cyanobacteria against hydrogen peroxide. *Environ. Microbiol.* 2021, 23, 2404–2419. [CrossRef]

48. Giannakis, S.; Voumard, M.; Rtimi, S.; Pulgarin, C. Bacterial disinfection by the photo-Fenton process: Extracellular oxidation or intracellular photo-catalysis? *Appl. Catal. B Environ.* 2018, 227, 285–295. [CrossRef]