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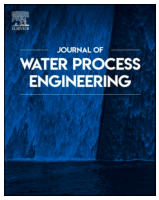

UV-LEDs combined with persulfate salts as a method to inactivate microalgae in ballast water

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ABSTRACT

The Ballast Water Management Convention (BWMC) establishes limits for viable organisms in discharged ballast water. UV-based ballast water management systems (BMWS) are among the most common, especially those with low pressure (LP) and medium pressure (MP) mercury lamps. An interesting alternative to mercury lamps could be UV LEDs that have been developing over recent years. UVA, UVB, and UVC LEDs have been tested as a method to inactivate microalgae in ballast water. For this study, the diatom *Phaeodactylum tricornutum* was selected as a target organism. Comparing the D_2 (dose required to achieve two log reductions) for *P. tricornutum* from different UV treatments, it was observed that UVC LEDs were 74.2 % more efficient than UVB LEDs and, compared with previous studies, 48.1 % more efficient than UVC LP mercury lamps. If a five day dark post-treatment was combined with the UV irradiation to avoid photoreactivation, UVC LEDs were 90 % more efficient than UVB LEDs and, compared with previous studies, 36.8 % more efficient than UVC LP mercury lamps. No damage with or without photoreactivation was caused by UVA irradiation with doses up to $4{\cdot}10^4$ mJ cm^{-2}. The combination of peroxymonosulfate (PMS) with UVA, UVB and UVC LEDs did not significantly increase the inactivation, and the combination of the peroxydisulfate (PDS) with UVC LEDs slightly decreases the inactivation compared with UVC irradiation alone. In conclusion, UVC LEDs were the most efficient for inactivating *P. tricornutum*, and the combination of PMS and PDS with UV LEDs did not notably improve it.

1. Introduction

The spread of organisms between different ecosystems occurs in various ways. One of the most important vectors is ballast water which negatively impacts economically, environmentally, and socially with invasive species. In addition, the number of invasions by non-indigenous species has been increasing over the preceding years [1–6].

Since ballast water is an important pathway for the dispersal of species in the marine environment [1,7,8], the International Maritime Organization (IMO) adopted the International Convention for the Control and Management of Ballast Water and Sediments from Ships (BWMC) [9] in 2004. It entered into force in 2017 and is currently ratified by 88 countries representing approximately 91.20 % of the world merchant fleet [10].

The Regulation D2 Ballast Water Performance Standard of the BWMC establishes limits on the allowable maximum concentration of viable organisms in discharged ballast water (Table 1). Beginning in the year 2024, ships will be required to have a ballast water management system (BWMS) in place for achieving this D2 standard. Ballast water treatment can be applied during the procedures of ballasting or the de-ballasting, in both procedures, or during the journey.

Most BWMSs consist of a mechanical filtration or separation followed by a disinfection treatment (chemical, physical, or both) with ultraviolet radiation as the most current common option [11–13].

The code for approval of ballast water management systems (BMWS Code) defines viable organisms as "organisms that have the ability to successfully generate new individuals in order to reproduce the species" [14]. Since the BWMC refers to viable organisms and UV radiation (most commonly approved BWMS) primarily affects the viability of the organisms, the evaluation of the treatment efficacy requires using

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Table 1
IMO standards for ballast water discharge [9].

Organisms size	Concentration
Greater or equal to 50 μm	Less than 10 viable organisms per m^3
Between 10 and 50 μm	Less than 10 viable organisms per mL

Indicator microbes	Concentration
Vibrio cholerae	Less than 1 colony forming units per 100 mL
Escherichia coli	Less than 250 colony forming units per 100 mL
Intestinal enterococci	Less than 100 colony forming units per 100 mL

techniques, such as growth phase modelling, that are focused on determining the organisms that are able to reproduce [15] [16,17].

UV radiation has a number of advantages such as the effectiveness on damaging DNA (preventing the reproduction of the organisms) or other cellular structures (resulting in cell death in certain cases), the safety of its use, or the minimum production of toxic disinfection by-products [18–20]. One disadvantage of UV radiation as a treatment is the lack of residual effect that allows the regrowth of organisms once they are exposed to favourable environmental conditions [21,22]. Regrowth can be due to organisms that were not affected by the treatment as well as those that recovered their viability through DNA repair processes, such as photoreactivation and dark-repair with the former being more effective [23,24]. Nonetheless, it has been demonstrated that photo-repair processes can be limited by the combination of UVC radiation and the absence of light during storage in the ballast tanks [25].

Ultraviolet light can be divided into three wavelength regions: UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm) [26]. The action mode of UV radiation can differ according to the UV emissions range. As the DNA maximum absorption peak is at 260 nm [27], the UVC presents the higher inactivation because it is highly absorbed by the DNA but also presents much more photoreactivation that could considerably decrease the effectiveness of the treatment. The UVB is partially absorbed by the DNA and causes less damage than the UVC and also presents less photoreactivation. The UVA is not absorbed by the DNA, but it is capable of damaging cell membranes and other cellular components by oxidative damage [28–31].

The combination of UV radiation with persulfate salts, usually applied in the form of peroxymonosulfate (PMS) or peroxydisulfate (PDS), could increase the inactivation effect with the formation of radicals, principally sulfate radicals ($SO_4^{\bullet-}$) with high reactivity [32–34]. It can result in an Advanced Oxidation Processes (AOP) that can also advantageously prolong the effect of the treatment, allow disinfection beyond the time of exposure to UV radiation, and avoiding possible growth during the storage in the ballast tanks thanks to the residual oxidants that are formed. Another advantage of this disinfection enhancement is the possibility of reducing the necessary UV dose required to reach the D2 standard and consequently lower energy consumption. To date, most of the studies on the combination of UV with PMS and PDS in the search for AOPs with LEDs as a source of UV radiation have been with bacteria and not with microalgae.

During the preceding years, the development of UV light emitting diode (UV LED) technologies have made UV LEDs a promising source to replace UV mercury lamps for water disinfection processes. UV LEDs are safer because of the absence of mercury, have a higher energy efficiency, longer lifetime, faster start-time (no need for a warm-up time), lower heat generation (easier control of the temperature), and high design flexibility because of the small size of the units [35–37].

The objective of the present paper is to evaluate the inactivation effectiveness of the three different UV emission wavelengths (UVA, UVB, UVC) provided by UV LEDs, the photoreactivation associated with them, and the improvement of the inactivation by their combination with persulfate salts (PMS and PDS). The diatom *Phaeodactylum tricornutum* was used as a standard organism for the experiments, and growth modelling was the technique employed to determine the inactivation of each treatment.

2. Material and methods

2.1. Organisms, culture medium, and pre-treatment procedure

The target organism used in this work was the diatom *Phaeodactylum tricornutum* oval morphotype (CCMM 07/0402) that was provided by the Marine Microalgal Culture Collection of the Institute of Marine Sciences of Andalucía. The selection of *P. tricornutum* was based on a number of general favourable characteristics for experimentation such as its worldwide distribution [38] and its use as a standard organism for water quality bioassays [39]. Further, several other organism's features made it specifically interesting for this work. On one hand, its high growth rate [40] was advantageous for growth modelling. On the other hand, its relatively high sensitivity to UV radiation allows analysing the inactivation overcoming the problem of the low output power of actual UVC LEDs (few milliwatts) and the consequential relatively low doses associated with it [37,41].

The culture medium was ground saltwater from the Campus of Puerto Real of the University of Cadiz (pH = 7.65 and conductivity = 48.9 mS cm^{-1}) that was sterilized in autoclave and enriched with Guillard f/2 medium [42] and 500 μg L^{-1} of silicate. The cultures were maintained in a culture chamber at 20 °C with continuous light with a photosynthetically active radiation of 36 μEinstein m^{-2} s^{-1} (QSL-2100 Radiometer, Biospherical Instruments Inc., San Diego, CA, USA). After dilution in a fresh medium, the organisms needed time to acclimatize [43,44]. In the case of *P. tricornutum*, the dilution in the fresh medium two days before the experiment was sufficient for ensuring acclimatization and the application of the treatment in the exponential growth phase.

2.2. Experimental procedure

2.2.1. UV reactor

Samples were irradiated with a collimated beam reactor (CBR) with UVA, UVB, and UVC independent LEDs (Photolab LED275-0.01/300-0.03/365-1cb; APRIA Systems S.L.; Guarnizo, Spain). Main parameters of the UV LEDs are shown in Table 2. The distance from the LEDs to the sample surface was 12.2 cm, and the irradiance reaching the surface of the target culture was measured with a radiometer (HD2102.1, Delta OHM Srl., PD, Italy) that was equipped with the corresponding UV probe according to the wavelength range (Delta OHM LP471UVA for UVA, Delta OHM LP471UVB for UVB, and Delta OHM LP471UVBC for UVC). The mean intensity (I_m) was calculated according to the protocol of Bolton & Linden [87], based on the reactor morphometry, and measured irradiance and water transmittance; the UV dose was determined as the product of the I_m and the exposure time.

2.2.2. UV irradiation and incubation

In each experimental series, aliquots of 20 mL of the target culture were placed in a Petri plate of 5.5 cm of internal diameter and subsequently irradiated with one wavelength of the UV LEDs (Fig. 1). During the UV irradiation, the cultures were continuously homogenized with a magnetic stirrer. Considering the different action mode of the three wavelengths, the exposure times of each wavelength were defined according to their specific emission: UVA (2.5–46.6 min; $2{\cdot}10^3$–$4{\cdot}10^4$ mJ

Table 2
Principal parameters of the UV LEDs.

	LED UVA	LED UVB	LED UVC
Wavelength emission (λ)	365–370 nm (λ_{max}: 365 nm)	295–305 nm (λ_{max}: 300 nm)	265–285 nm (λ_{max}: 275 nm)
Irradiance (W/m^2)	140.41 ± 1.81	1.94 ± 0.05	0.72 ± 0.01

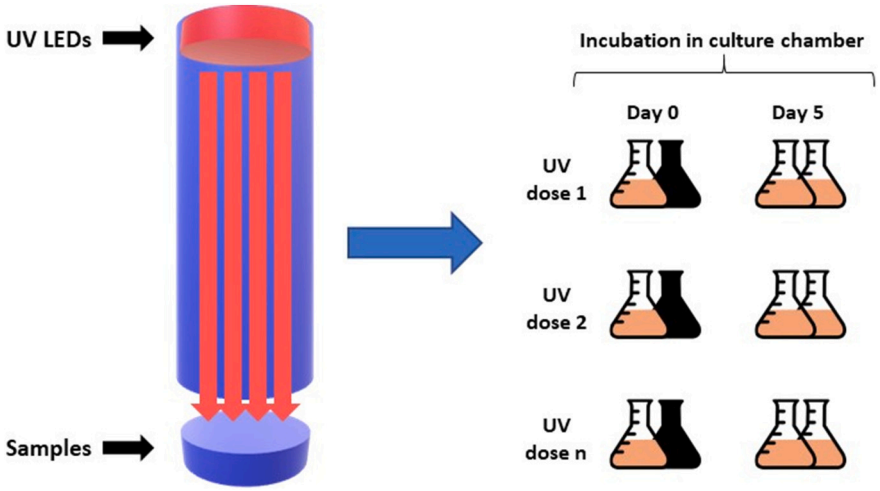

Fig. 1. Collimated beam reactor (CBR) and experimental procedure.

cm^{-2}), UVB (1.2–30.0 min; 10.9–271.8 mJ cm^{-2}), or UVC (1.2–18.1 min; 4.2–63.0 mJ cm^{-2}). Following the treatment, all of the samples were placed in the culture chamber (each UV dose was the content of two Petri plates treated subsequently and placed in borosilicate flasks). Two different incubations were performed: i) untreated culture and treated cultures that were immediately exposed to light and ii) untreated culture and treated cultures that were covered for five days with aluminium foil to imitate the storage in the ballast tanks after the treatment (the day of exposure to light was considered as day 0 of the growth). Comparing the untreated sample covered for five days with the untreated sample immediately exposed to light, the effect due to only the darkness was calculated.

2.3. Determining the concentration of viable organisms after the treatment

Cell concentration was monitored throughout the post-treatment incubation with chlorophyll fluorescence measurements according to the linear correlation between them and the cell concentration measured by microscopy and the Neubauer chamber determined in previous studies [45]. The chlorophyll fluorescence along the post-treatment incubation was measured with a Microplate Fluorescence Reader (Tecan infinite F200; software Tecan i-control, 1.6.19.2; plate Corning 96 Flat Bottom White Polystyrol; excitation at 360 nm, emission at 670 nm, 25 flashes, 20 μs integration time). Three different measurements were made for each sample.

Growth curves were obtained by plotting chlorophyll fluorescence measurements (in logarithmic scale) since the detection of exponential growth versus exposure time to environmental light. The growth of the cultures followed a Verhulst logistic model [46,47] with an initial phase without significant changes in the cell concentration, a second phase with exponential growth, and a final phase with no significant increases in the cell concentration as the culture became closer to the carrying capacity. A simplification of the logistic model used in previous studies ([48], 2021; [17]) to the exponential model (Eq. (1); in which N_v: concentration of viable organisms [cells mL^{-1}] at the time equal to t [d]; N_{v0}: initial concentration of viable organisms [cells mL^{-1}]; r: growth rate [d^{-1}]) was done considering that only the points of the exponential phase are those that influence the calculation of the number of viable cells after treatment.

$$N_v\ (t) = N_{v0} \cdot e^{rt} \quad (1)$$

Survival (S) was calculated as the quotient between the Nv_0 of the treated samples and Nv_0 of the untreated sample immediately exposed to light of the same experiment. Inactivation curves were determined by representing the values of Log (S) against the UV dose. The GInaFiT tool for MS Excel [49] was used to fit the inactivation curves to the best inactivation model and to obtain the corresponding inactivation kinetics parameters. The D_n parameter (UV dose required to achieve n log-reductions) was utilized to compare the inactivation effect of the different UV LEDs.

2.4. Determining the effect of UV irradiation combined with persulfate salts

2.4.1. Determination of the test concentration

Two persulfate salts were selected to be combined with ultraviolet light: peroxymonosulfate salt (PMS; HSO_5^-) (Oxone©, Sigma-Aldrich; $KHSO_5{\cdot}0.5KHSO_4{\cdot}0.5K_2SO_4$,) and peroxydisulfate salt (PDS; $S_2O_8^{2-}$) (PanReac AppliChem 98 %; $Na_2S_2O_8$). A toxicity assay was performed to determine the initial concentration to use for each persulfate salt. The formula proposed by Hampel et al. [50] was employed to determine the concentration that causes the minimum amount of damage. This was established as causing 10 % of the inhibition and allows quantifying possible inactivation enhancement by the combination of the persulfate salts and the UV irradiation. Concentrations between 0.34 and 6.78 μM were tested in the PMS, and 1.66 μM was determined as the concentration to be applied in PMS/UV experiments. Concentrations up to 0.430 mM were tested in PDS, and no inactivation was observed; therefore it was decided to select 0.043 and 0.430 mM as the concentrations to be applied in PDS/UV experiments.

2.4.2. Determination of the UV wavelengths combinations

To decide which UV wavelengths would be combined with the PMS and PDS, a UV–Vis spectrum was obtained with a spectrophotometer (Jenway 7315) for each salt solution (Fig. 2). Both persulfate salts have considerable absorbance in the UVC range, but only the PMS has some absorbance in the UVB and UVA range. Therefore, the PDS was only combined with UVC wavelength while the PMS was combined with the three UV wavelengths.

2.4.3. Experimental setup and persulfate salts decay monitoring

During the experiments, the addition of the PMS and PDS was performed simultaneously with the initiation of UV irradiation. In both cases, the corresponding volume of each persulfate salt solution was added in a single dose to the Petri plate where the culture was being irradiated. After the treatment, the concentration of the PDS was measured by spectrophotometric determination (352 nm) with an iodometric titration method [51] to determine their consumption. In the case of the PMS, this was not done due to the low concentration that was tested (1.66 μM).

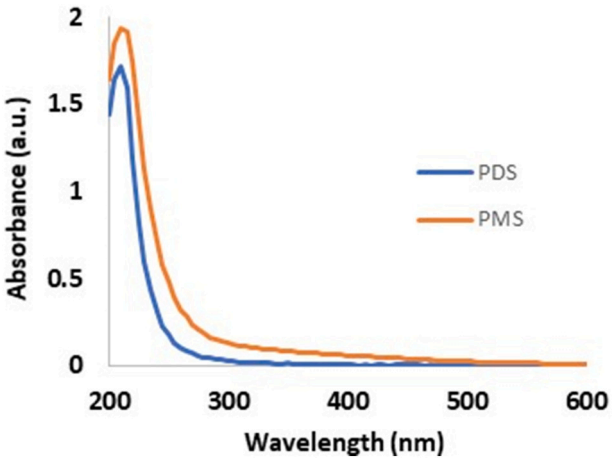

Fig. 2. UV–Vis spectrum from PDS and PMS. The concentration of both persulfate salts was 1000 ppm in marine water matrix.

3. Results and discussion

3.1. Modelling of the growth curves

Growth curves were obtained by representing the logarithm of the chlorophyll fluorescence versus the incubation time under ambient light. The delay in reaching the exponential phase was proportional to the UV dose that was applied. The growth curve of one of the experiments with UVC is shown as an example in order to understand the modelling of the growth curves (Fig. 3). As was mentioned in Section 2.3, only the data from the exponential phase were considered for the modelling. Considering that photorepair occurs in the first hours after the irradiation [52], the delay in reaching the exponential phase in treated samples was due to a decrease in the initial concentration of viable organisms caused by the UV irradiation.

The growth curves of the different samples of each experimental series were analyzed together ("Further ANOVA for variables in the order fitted") with Statgraphics Centurion XVI software (16.1.03) to ascertain their corresponding intercept and slope values as well as determining if there were significant differences between their slopes (growth rate [*r*]). Since there were none in practically all of the cases, it was decided to assume the same *r* for all of the growth curves of the same experimental series. In addition, no relevant changes were observed in the slopes due to UV wavelength (UVA, UVB or UVC) or post-treatment conditions (light immediately after treatment or five days of darkness). This absence of influence of the treatment on the *r* was already observed in previous studies [45,48]. The growth rate "*r*" (mean ± SE) from all of the experiments was 0.74 ± 0.04 d^{-1} (n = 19). Intercept values decreased according to the UV dose that was applied and were used to determine the values of Nv_0.

3.2. Inactivation curves and kinetic parameters

Due to the characteristics of each of the wavelengths, the same UV exposure times result in very different dose values. To better understand the differences between the three wavelengths, inactivation curves were obtained by confronting Log (*S*) versus exposure time. Samples with a dark post-treatment avoid photorepair processes due to darkness while samples exposed to light immediately after irradiation undergo these processes that can repair part of the DNA damage [25].

The UVA inactivation curve described a log-linear inactivation characterized by maintaining the inactivation rate (*k*) constant throughout the different doses (Eq. (2); in which *S*: survival of organisms; S_0: initial survival of organisms; *k*: inactivation rate [min^{-1}]; *t*: exposure time [min]) [53]. The UVC and UVB inactivation curves described a log-linear with shoulder inactivation characterized by a first stage with practically no disinfection (up to a threshold dose) followed by a log-linear disinfection stage (Eq. (3); in which *S*: survival of organisms; S_0: initial survival of organisms; *k*: inactivation rate [min^{-1}]; *sl*: shoulder length [min]; *t*: exposure time [min]) [54] (Fig. 4). The kinetic constants values of the models are indicated in Table 3.

$$S(t) = S_0\, e^{-k\cdot t} \tag{2}$$

$$S(t) = S_0 \frac{e^{-k\cdot t}\cdot e^{k\cdot sl}}{1 + (e^{k\cdot sl} - 1)\cdot e^{-k\cdot t}} \tag{3}$$

To compare with literature results, UV doses for each of the wavelengths to achieve one (D_1), two (D_2), and three log-reductions (D_3) were calculated. Practically no inactivation was observed for the UVA wavelength, therefore, the dose required for the different log reductions could not be calculated based on the data that were obtained (Table 4).

3.2.1. UVA wavelength

Practically no inactivation of *P. tricornutum* was observed with the UVA doses tested in this study ($2{\cdot}10^3$–$4{\cdot}10^4$ mJ cm^{-2}). In addition, no increase in inactivation was observed due to the five day dark post-treatment (Table 3).

The results obtained were compared with those of previous studies using different organisms. The dose response (mJ cm^{-2} per log-reduction) at UVA irradiation of 365 nm for *E. coli* with $13.8{\cdot}10^3$ mJ cm^{-2} [28], $55.3{\cdot}10^3$ mJ cm^{-2} [55] or $260.0{\cdot}10^3$ mJ cm^{-2} [56] or for other organisms like *Salmonella enteritidis* with $148.3{\cdot}10^3$ mJ cm^{-2} at 365 nm [55] or Mesophilic bacteria with 12.5 mJ cm^{-2} at 365 nm or 88 mJ cm^{-2} at 405 [57] indicates a notably greater resistance by

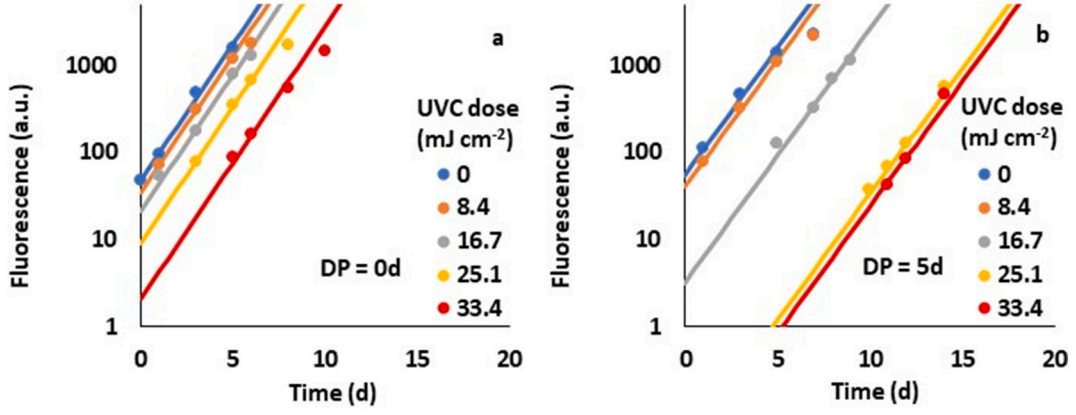

Fig. 3. Growth curves of the samples without dark post-treatment (a) and with dark post-treatment (b) in one of the UVC experiments. Only data of the exponential phase are represented. DP = dark post-treatment.

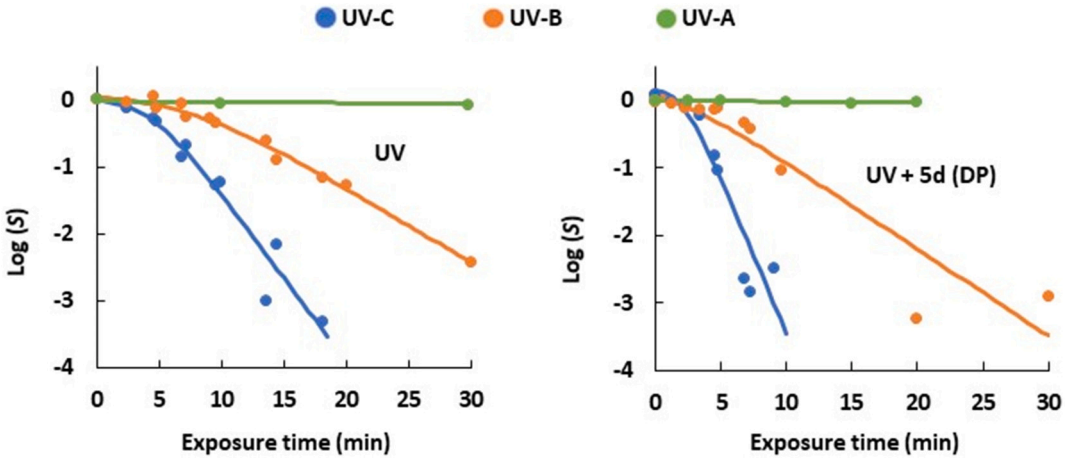

Fig. 4. UVA, UVB and UVC inactivation curves. DP: dark post-treatment.

Table 3
Kinetic parameters (mean ± SE) of the inactivation curves. The last column represents the increase in the inactivation rate due to the avoidance of the photoreactivation. *k*: inactivation rate. *sl*: shoulder length. DP: dark post-treatment.

UV wavelength	DP (d)	k (min^{-1})	*sl* (min)	R^2	↑ *k* (%)
UVA	0	0.003 ± 0.001	–	0.711	–
	5	0.003 ± 0.001	–	0.565	0
UVB	0	0.256 ± 0.014	7.7 ± 1.0	0.989	–
	5	0.296 ± 0.047	2.2 ± 3.7	0.891	13.5
UVC	0	0.573 ± 0.069	4.3 ± 1.6	0.948	–
	5	1.053 ± 0.154	2.1 ± 1.0	0.924	45.6

Table 4
Doses of each UV light needed to reach the first three log-reductions. DP: dark post-treatment. D_{max}: maximum dose applied.

UV light	DP (d)	D_1 (mJ cm^{-2})	D_2 (mJ cm^{-2})	D_3 (mJ cm^{-2})
UVA	0	$>D_{max}$	$>D_{max}$	$>D_{max}$
	5	$>D_{max}$	$>D_{max}$	$>D_{max}$
UVB	0	152.5	236.6	318.5
	5	95.1	166.6	237.1
UVC	0	28.4	42.9	56.9
	5	15.9	23.7	31.4

P. tricornutum for which doses up to $4{\cdot}10^4$ mJ cm^{-2} at 365 nm caused practically no inactivation.

The minimal amount of damage produced by the UVA irradiation is consistent with the low energy of the wavelength and being outside the absorption spectrum of DNA ranging from 200 nm to 300 nm [58–60]. On the other hand, the lack of increase in disinfection by the five-day dark post-treatment (Table 3) is consistent with the absence of absorbance of UVA radiation by the DNA and, therefore, there is no photoreparable damage [31,55]. Although some studies show substantial damage to some pathogenic and non-pathogenic bacteria with UVA [28,55], they are based on very high doses and long exposures that make it an ineffective, especially in the case of ballast water.

3.2.2. UVB wavelength

In this study, there was considerable damage to *P. tricornutum* by the UVB irradiation, and a five day dark post-treatment increased the efficacy of the treatment by preventing photoreactivation with an increase of 13.5 % on the *k* (Table 3).

Inactivation data from other organisms of the literature were compared with the results from this study. The dose response from *E. coli* with different UVB wavelengths with 94.8 mJ cm^{-2} at 310 nm [30], 5.5 mJ cm^{-2} at 285 nm [56], and 26.9 mJ cm^{-2} at 300 nm [61] and from other organisms with 300 nm with 23.4 mJ cm^{-2} for *Pseudomonas aeruginosa*, 4.8 mJ cm^{-2} for *Legionella pneumophila*, 38.4 mJ cm^{-2} for the bacteriophage Qβ and 525.0 mJ cm^{-2} for *Bacillus subtilis* spores [61] indicate a higher resistance from *P. tricornutum* (152.5 mJ cm^{-2} at 300 nm; D_1 from Table 4) with the exception of the *Bacillus subtilis* spores. Compared to the D_1 of other marine microalgae such as *Tetraselmis* sp. treated with UVB LEDs (516 mJ cm^{-2} at 280 nm and more than 2000 mJ cm^{-2} at 300 nm) [58], *P. tricornutum* has more sensitivity to UVB irradiation (152.5 mJ cm^{-2} at 300 nm; D_1 from Table 4).

In the case of the UVB wavelength (280–315 nm), some of it is absorbed by the DNA, and thymidine dimers are formed [62] which would explain the higher inactivation of UVB irradiation at the same dose compared to UVA irradiation (Table 4). The lower *k* in the samples without a dark post-treatment (Table 3) is consistent with the reparation of the damage throughout the photorepair processes [56,63]. More radiation is absorbed by the DNA when the wavelength in the UVB region is closer to the DNA absorption peak (260 nm) [26], and higher increases in inactivation could be achieved in these cases if photoreactivation is avoided.

3.2.3. UVC wavelength

In this study, *P. tricornutum* was significantly damaged by the UVC irradiation, and a five day dark post-treatment increased the efficacy of the treatment by preventing photoreactivation with an increase of 45.6 % on the *k* (Table 3).

Comparing with the dose response of other type of organisms at different UVC wavelengths with LEDs, *P. tricornutum* showed higher resistance (28.4 mJ cm^{-2} at 275 nm; D_1 from Table 4) in practically all of the cases with the exception of the Adenovirus 5 ATCC VR5 and, in some studies, the bacteriophages MS2 and Qβ (Table 5). In this experiment, the emission peak of the UVC LEDs was 275 nm, and the inactivation of *P. tricornutum* would probably have been different at other wavelengths as demonstrated by the inactivations for the same organism at various wavelengths (Table 5).

To compare inactivations and photoreactivation in microalgae between LEDs and mercury lamps (low [LP] and medium [MP] pressure), the D_2 parameter (dose required to reach two log reductions) was used. This parameter was selected considering that two log reductions were needed to achieve the minimum concentration of organisms between 10 and 50 μm in discharged ballast water (10 cells ml^{-1}) from the minimum concentration established by the BWMC (10^3 cells ml^{-1}) to test the BWMS [14].

The D_2 without a dark post-treatment for *P. tricornutum* with UVC LEDs (42.9 mJ cm^{-2}) was significantly lower than the D_2 with UVC LP mercury lamps (82.7 mJ cm^{-2}) ($p = 0.031$; analysis of covariance

Table 5
Dose response from various organisms at different UVC wavelengths with UV LEDs. Dose response is the dose required per log inactivation.

Microorganism	Wavelength (nm)	Dose response (mJ cm^{-2})	Reference
E. coli K12 IFO 3301	285	6.4	[64]
E. coli K12 IFO 3301	280	3.5	[30]
E. coli CGMCC 1.3373	280	3.4	[65]
E. coli K12 ATCC 29425	280	3.0	[66]
E. coli IFO 3301	280	2.3	[61]
E. coli ATCC 11229	275	2.4	[67]
E. coli CGMCC 1.3373	275	4.1	[29]
E. coli CGMCC 1.3373	267	2.9	[29]
E. coli K12 IFO 3301	265	2.7	[30]
E. coli IFO 3301	265	1.5	[61]
E. coli CGMCC 1.3373	265	2.4	[65]
E. coli ATCC 11229	265	2.3	[56]
E. coli K12 ATCC 29425	260	3.3	[66]
E. coli ATCC 11229	255	3.3	[67]
Coliphage Qβ ATCC 23631 B1	285	27.0	[64]
Bacteriophage Qβ ATCC 15597 B1	280	4.1	[61]
Coliphage Qβ	280	28.7	[68]
Bacteriophage Qβ ATCC 15597 B1	265	2.4	[61]
Coliphage Qβ	255	12.5	[68]
MS2 ATCC 15597-B1	285	34.5	[64]
MS2 ATCC 15597-B1	280	19.3	[66]
MS2 ATCC 15597-B1	275	28.6	[67]
MS2 ATCC 15597-B1	260	15.2	[66]
MS2 ATCC 15597-B1	255	26.1	[67]
MS2	255	12.8	[68]
φX174	280	2.8	[68]
φX174	255	1.7	[68]
T7 coliphage	275	4.3	[67]
T7 coliphage	255	5.1	[67]
Bacillus subtilis spores	282	8.3	[69]
Bacillus subtilis spores ATCC 6633	280	10.4	[61]
Bacillus subtilis spores	269	6.8	[69]
Bacillus subtilis spores ATCC 6633	265	6.2	[61]
Bacillus subtilis spores	250	19.7	[70]
Pseudomonas aeruginosa	280	1.4	[61]
Pseudomonas aeruginosa	265	1.5	[61]
Legionella pneumophila	280	0.5	[61]
Legionella pneumophila	265	0.3	[61]
Adenovirus 5 ATCC VR5	285	43.5	[64]

[ANCOVA]) under the same experimental conditions [45], indicating a higher efficiency of UVC LEDs. This dose with the UVC LEDs was also lower than the D_2 with UVC (LP and MP) mercury lamps applied to other microalgae like *T. suecica* with values between 83.8 and 636.4 mJ cm^{-2} [33,71,72] or *Synechococcus* sp. and *Anabaena* sp. with values of 240.7 and 111.8 mJ cm^{-2}, respectively [45]. This improvement in the inactivation from the UVC LEDs over UVC mercury lamps agrees with data from studies with other organisms [58,65,73]. On the other hand, comparing with UVC LEDs' inactivation at different wavelengths for other marine microalgae like the diatom *Asterionellopsis glacialis* (256, 262, 268 and 274 nm) or the chlorophyta *Tetraselmis* sp. (265 and 280 nm) [58,74] indicated a lower resistance by *P. tricornutum* that was consistent with its high sensitivity to UV radiation [45].

Photoreactivation makes it possible, in the presence of light, to repair DNA damage caused by UV irradiation. Within the UVC region, although it is less energetic with a higher wavelength and the damage should be lower, the photoreactivation is also lower, thus the treatment may end up being more effective if this is considered [29,30,65]. In *P. tricornutum*, there is an increase in the D_2 due to the photoreactivation from 23.7 to 42.9 mJ cm^{-2} (factor of 1.8) when it is irradiated with UVC LEDs and from 37.5 to 82.7 mJ cm^{-2} (factor of 2.2) when it is irradiated with a UV LP mercury lamp [45]. The lower photoreactivation in the case of the UVC LEDs used in this study (275 nm) compared with LP mercury lamps (254 nm) was expected due to its greater distance to the absorption peak of the DNA (260 nm) and is consistent with other results from the literature for *E. coli* [29,65,75]. On the other hand, the absence of differences observed by [65] between photoreactivation with UVC LEDs at 265 nm and LP mercury lamps at 254 nm in *E. coli* could be related to the fact that they are practically at the same distance from the absorption peak of the DNA (260 nm).

The UVC wavelength is the most energetic one, and its entire spectrum (200–280 nm) is absorbed by the DNA. Additionally, the peak of absorption from the DNA (260 nm) [26] is in its region. For these reasons, the UVC has been the one most used for disinfection. The data from this experiment agree with those from the literature where UVC irradiation has the most germicidal effect and the higher photoreactivation [29,30,56,61].

3.2.4. Application of the three different wavelengths in the ships

When implementing a UV-based BWMS, the differences in inactivation and subsequent photoreactivation of the various wavelengths must be considered. The results of this experiment seem to indicate a higher resistance to UV radiation at the three wavelengths (UVA, UVB, and UVC) of phytoplanktonic organisms than that for viruses and bacteria. This is consistent with UVC data from the literature [44].

UVA LEDs alone would not be of interest for ballast water treatment specially focused on microalgae inactivation because of the enormous doses that are needed. Additionally, no photoreactivation is associated with the UVA irradiation, therefore, the possibility of increasing the efficacy of the treatment by avoiding it (treating at the ballasting procedure and subsequent storage in darkness in the ballast tanks) is not possible.

Although the inactivation with UVB LEDs is higher than with UVA LEDs, they are less effective for inactivating microalgae compared with mercury lamps that are the most used at present. The D_2 parameter has been used to compare the differences between both UV treatments applied to *P. tricornutum*. Without a dark post-treatment, UVB LEDs are 65 % less efficient than a UVC LP mercury lamp while UVB LEDs are 77.6 % less efficient with a five day dark post-treatment (Table 6) [45].

In the case of UVC LEDs, the D_2 parameter was also used to compare with the other UV treatments applied to *P. tricornutum*. It was observed that UVC LEDs were the most efficient of all of the UV LEDs tested in this study. Without a dark post-treatment, UVC LEDs were 74.2 % more efficient than UVB LEDs and 48.1 % more efficient than UVC LP mercury lamps (Table 6). With a five day dark post-treatment, the UVC LEDs were 90 % more efficient than UVB LEDs and 36.8 % more efficient than UVC LP mercury lamps (Table 6). Considering the possibility of avoiding photoreactivation by dark storage in ballast tanks, it might be interesting to develop UVC LEDs with emission peaks closer to the absorbance peak of DNA. In these cases, the combination of UVC irradiation and subsequent storage in ballast tanks would further enhance inactivation.

3.3. Combination of UV LEDs with PMS and PDS

As described in Section 2.4.2, the UV–Vis spectra of the two persulfate salts was obtained for determining the best UV emission wavelength (if the absorption and emission spectra coincide, a possible photolysis of the compound is ensured and therefore greater efficiency). Accordingly,

Table 6
Doses needed to reach the D_2 (two log reductions) in *P. tricornutum*. DP: dark post-treatment. UV LP mercury lamp data come from [45].

Dark post-treatment	UVB LEDs (emission peak at 300 nm)	UVC LEDs (emission peak at 375 nm)	UV LP mercury lamp (emission peak at 254 nm)
0d	166.6 mJ cm^{-2}	42.9 mJ cm^{-2}	82.7 mJ cm^{-2}
5d	236.6 mJ cm^{-2}	23.7 mJ cm^{-2}	37.5 mJ cm^{-2}

it was decided to combine the PMS with the three UV wavelengths (UVA, UVB, and UVC) and the PDS with the UVC wavelength. The activation of the PMS and the PDS with UVC [32–34] and UVA [76–78] has been demonstrated and tested as a way to increase disinfection. On the other hand, while some activation studies of the PMS with solar light that includes UVA and UVB have been done [79–81], the authors did not find research about PMS combined with only UVB.

3.3.1. *Inactivation curves of UV lights combined with PMS and PDS*

In the case of the PMS, looking for a moderate effect of UV irradiation and based on the results from Section 3.2, an exposure time of 5 min was selected for the UVC, and the same exposure time was considered for the other wavelengths. Five minutes of exposure suppose UV doses of 17.4, 45.3, and 3606.9 mJ cm^{-2} at UVC, UVB, and UVA wavelength, respectively. In the case of PDS, in both concentrations (0.043 and 0.430 mM), exposures times of 10 and 20 min of the UVC wavelength were selected, suppose 34.8 and 69.6 mJ cm^{-2}, respectively. These longer exposure times and thus higher UV doses for PDS were based on its higher stability compared to PMS [34].

The PMS by itself causes significant damage to *P. tricornutum* and, if an increase in inactivation due to its combination with UV irradiation is the desired effect, very low concentrations must be used. In the toxicity assay, it was ascertained that 1.78 μM caused an inhibition of 54.5 % of the initial culture while 3.39 μM caused an inhibition of 98.0 %. Considering the inactivation of the PMS, UVC, and UVB alone with 0.04, 0.31 and 0.07 log reductions, respectively, there was a higher inactivation by combining the PMS with UVC (0.43 log reductions) and UVB (0.21 log reductions) than the simple addition of their inactivations alone. These increases in the inactivation when UVC and UVB were combined with PMS might be related to the wavelengths used (275 nm for UVC LEDs and 300 nm for UVB LEDs) that produce strong absorption into PMS (Fig. 2). UVC irradiation produces more biological damage to microalgae than UVB irradiation. Accordingly, the increase effect due to the combination of the UV/PMS is more evident in the UVB region than the UVC (Fig. 5). No enhancement of inactivation was determined with the combination of UVA and PMS as its inactivation was the same as that with only PMS (Fig. 5a). Probably, higher exposure times would be needed to obtain a more efficient process in the case of UVA. In any case, *P. tricornutum* was found to be very sensitive to the PMS which agrees with literature for other microalgae such as *Microcystis aeruginosa* [82]. Therefore, it would be difficult to correctly quantify the increasing effect by combination with UV because of the lowest PMS doses used.

In the case of the combination of the PDS with UVC, a slight detrimental effect was obtained compared with UVC alone. In the present study and according to the literature [82,83], the effect of PDS by itself was negligible, thus the combination with UVC was expected to accelerate the inactivation of *P. tricornutum* due to the photolysis of PDS into sulfate radicals. Absorption of the PDS into 275 nm (UVC from the LEDs of this study) was confirmed by a UV–Vis spectra (Fig. 2). The slight consumption of the PDS after UVC irradiation (0.8 % in the first 20 min) was consistent with that seen in other studies [84]. Although no PDS was detected in the cultures on the last day of the inactivation experiment (day 12) no inactivation enhancement was detected in the tested conditions. Similar experiences have been reported with algal organic matter mineralization [85]. In this scenario, it is important to take into account that specific parameters such as PDS dosage, UV wavelength, or inorganic substances that are present in water can influence the UV activation of PDS [86]. In this case, a complex aqueous matrix could have notably influenced the process due to the significant amount of inorganic/organic compounds inherent in microalgal cultures. The emission wavelength at 275 nm also differs a bit in previous studies that obtain disinfection enhancement under a UV/PDS process [32,88,89] that utilizes 254 nm as an emission source. Additionally, the stability of PDS [83] makes its efficient activation difficult under these conditions. Accordingly, more research must be conducted in the future in order to clarify the effects and performance of UV/PDS on seawater microalgae.

4. Conclusions

UVA, UVB, and UVC LEDs have been tested as methods of inactivating microalgae in ballast water by using the diatom *P. tricornutum* as the target organism. The inactivation efficiency and the level of photoreactivation of the three wavelengths observed in this study follows UVA<UVB<UVC. On the other hand, comparing results from this study and from previous studies that used UVC LEDs and UVC from mercury lamps (low pressure or medium pressure), it was determined that the inactivation with LEDs is higher with the same doses. Finally, the combination of the three UV wavelengths with the PMS and UVC with PDS was tested in *P. tricornutum*. There was a slight increase when the PMS was combined with UVC and UVB but not when it was combined with UVA. The combination of UVB with the PMS was especially relevant since, although there are data on UVC and UVA as a source of PMS activation in the literature, no references to UVB alone as a source of activation were found by the authors. In the case of the PDS, no increase on the inactivation was ascertained with its combination with UVC.

P. tricornutum as a representative of phytoplanktonic organisms has demonstrated higher resistance than viruses and bacteria to UV-based treatments at the three wavelengths which must be considered for future research in testing BWMSs.

Considering the implementation of a BWMS, UVC LEDs would be a promising technology due to their higher efficacy compared to UVC

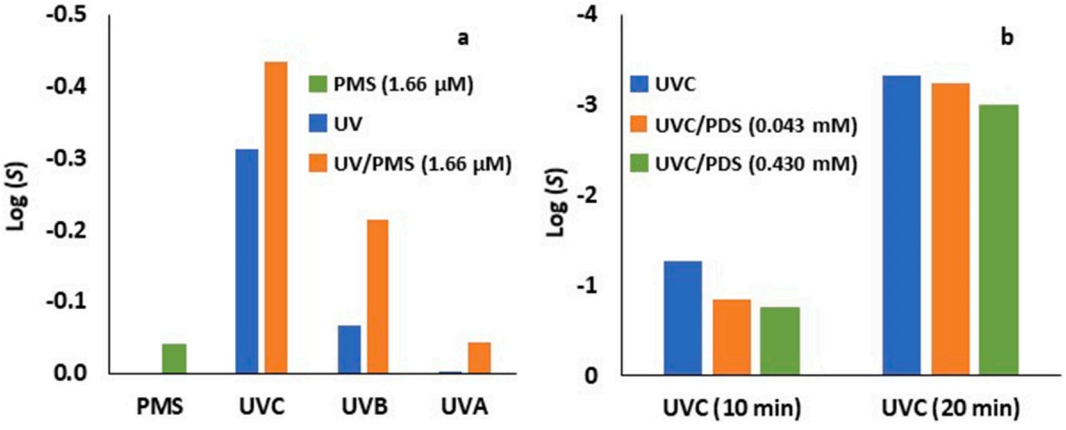

Fig. 5. Survival (*S*) of *P. tricornutum* in the combination of PMS with the three UV wavelengths (a) and PDS with UVC wavelength (b). Radiation time: 5 min for UV/PMS (a) and 10 and 20 min for UVC/PDS (b). PMS represents the inactivation only with the chemical (a).

mercury lamps, although it would be interesting to develop UVC LEDs with greater output power. On the other hand, the combination of UV LEDs with persulfate salts would not enhance the inactivation, therefore, the treatment with only UV irradiation would be safer and less expensive.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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