Efficient removal of dyes from seawater using as biosorbent the dead and living biomass of the microalga *Phaeodactylum tricornutum*: equilibrium and kinetics studies

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Abstract

Dyes are used in many applications with their consequent discharge into aquatic environments. Alternative methods of pollutant removal, such as biosorption, are currently necessary to achieve an efficient, low-cost, and environmentally friendly process. The biomasses, living and dead, from the microalga *Phaeodactylum tricornutum* were studied to determine their ability to remove three dyes, Methylene Blue, Crystal Violet and Safranin from seawater. The removal properties were determined as a function of contact time, initial dye concentrations, and pH, and characterized with the determination of the point of zero charge (pH_{PZC}) and FTIR. The highest removal capacity was obtained with crystal violet (66.4 mg g^{-1}), without significant differences between both biomasses and following a pseudo-fourth order kinetics, indicating a high affinity for said dye. Maximum removal capacity for methylene blue was 18.9 mg g^{-1} with significant differences between both biomasses until the concentration of 10 mg L^{-1}, the dead biomass being more effective since this dye would present difficulties to enter the living cells. The kinetics was of pseudo-third order. Safranin obtained a removal capacity of 19.6 mg g^{-1} with also significant differences between both biomasses up to a concentration < 10 mg L^{-1}, but with living biomass being more effective and a sigmoidal kinetics, indicating that this dye would more easily enter living cells. Photodegradation of these dyes and isotherms were also analyzed to properly characterize the process. The results demonstrated that the biomass of *P. tricornutum* eliminated efficiently these dyes from a solution with high ionic load (seawater).

Keywords Methylene Blue · Crystal Violet · Safranin · Microalga · Bioremediation · Biosorption

Introduction

Environmental pollution has become in one of the main problems of the modern world. Among the large number of compounds that contribute to this problem, dyes stand out because of their continuous production, applications, and discharge into water streams. Dyes are used in several applications: cosmetic, printing, stationer, plastics, textile, food processing, and dye manufacturing industries. For this reason, the production of these compounds is increasing (Grand View Research I 2017), and therefore, the risk of discharges to natural media. This discharge of dyes in the waters includes a series of drawbacks, mainly due to the recalcitrant nature of these compounds or their perception even at concentrations less than 1 mg L^{-1} (Singh and Iyengar 2007). Precisely, color is one of the main problems because apart from causing an unaesthetic issue, it reduces sunlight penetration, influencing the photosynthetic activity and eliminating the food source for aquatic organisms (Banat et al. 1996). Since most of dyes are complex organic molecules with poor biodegradability, they can generate a high chemical oxygen demand but a low biochemical oxygen demand (the biodegradability index (BOD/COD) decreases). Dyes can also decrease the amount of dissolved oxygen due to the thin layer that dyes can form on the surface of the water (Lellis et al. 2019; Singh 2014). Finally, some studies have evidenced that some dyes cause respiratory toxicity, carcinogenesis, or mutagenesis in fish and also, dysfunction of the brain, liver, reproductive system, and kidney in human beings (Lellis et al. 2019; Zusková et al. 2007). Hence, there

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is a clear urgency to develop an effective and economic way to remove these pollutants.

Numerous treatments have been used to eliminate dyes from aqueous solutions. Among them are oxidative processes, ion exchange, flocculation/coagulation, precipitation, ozonation, or sorption. These methods have limited versatility, expensive equipment, generation of sludge or dissolved solids, and, in some cases, low efficiency due to the chemical stability of the dyes (Kaushik and Malik 2009; van der Zee and Villaverde 2005). Of these methods, sorption is one of the most widely used methods with less drawbacks (Asgher 2012; Fomina and Gadd 2014; Gadd 2009; Yagub et al. 2014). In fact, many sorbents have been assayed for the removal of dyes, like activated carbon, charcoal, coal, peat, plant products, chitosan, clays, agricultural waste, industrial waste, soil, or nanoparticles (Salem et al. 2016; Sanghi and Verma 2013; Shariati et al. 2011). Although the materials used as sorbents can have a very diverse origin, nowadays, the materials of biological origin are being better considered and accepted because of their effectiveness, affordability in terms of costs, lower production of sludge, and they are considered eco-friendly. For these reasons, there is a growing interest in applying this type of materials as sorbents; and this technology is known as biosorption (Torres 2020).

Biosorption is a promising alternative for the removal of dyes (or other pollutants) from aquatic systems because with a biological material as sorbent, the generation of by-products can be avoided, and it is considered a more economical and eco-friendlier alternative. In this context, the search for new effective and cheap biosorbents is essential for the development of this type of methodologies. The use of biomass from microorganisms for its application as biosorbents has become a great option due to the number of studies in which its effectiveness has been proven (Huang et al. 2020; Priyadarshani et al. 2011; Santaeufemia et al. 2019). In particular, the use of microalgal biomass is gaining greater attention due to its high capacity to remove pollutants and, in addition, these microorganisms are considered promising biosorbents (Daneshvar et al. 2018; Lee et al. 2019; Ubando et al. 2020). For these reasons, biomass from the marine microalga *Phaeodactylum tricornutum* has been used in this work to evaluate its capacity to remove different dyes, Methylene Blue (MB), Crystal Violet (CV), and Safranin O (S). According to the literature, this biomass has demonstrated satisfactorily its ability to remove pollutants (Santaeufemia et al. 2016, 2018; Torres et al. 1998). It is important to consider that for this study, seawater was used as aqueous solution. Most of the studies focus on laboratory tests using distilled water as an aqueous solution to assess the effectiveness of different biosorbents and there are hardly any studies to validate the use of biosorbents to remove pollutants from seawater (Santaeufemia et al. 2016; 2018; 2019). Thus, this procedure would validate the use of

this microalgal biomass in seawater, with high ionic load, which requires the use of biomasses with a high affinity for pollutants to avoid interference from salts.

Furthermore, living and dead biomass were used in this study to determine which one is more effective and convenient for its application. In fact, in applications of this type, and in particular with microorganisms, the use of living or dead biomass is feasible. However, both have advantages and disadvantages. Toxic wastes do not have impact in the dead biomass, the requirements of nutrients are nonexistent, and the ability to regenerate and reuse during various cycles is more feasible than with living biomass. These properties make this form of biomass preferable as biosorbent (Michalak et al. 2013). However, living biomass may be more suitable (especially when this biomass is less susceptible to the toxic effect of the pollutant) due to the conservation of biological activity that allow to increase the intracellular content of pollutants (bioaccumulation) or transforming them in less active forms (biotransformation). This leads to an increase in the performance of removal since these processes are added to the simple biosorption (Peng et al. 2019; Reddy and Osborne 2020; Sedlakova-Kadukova et al. 2019; Xu et al. 2020). Therefore, the state of biomass is another important factor to consider for the development of new biosorbents.

Finally, it is necessary to consider the possible photodegradation of the dyes. Since for the culture of microalgae (living biomass) lighting is required, and light can affect the integrity of the dyes (Shoueir et al. 2018; Weyermann et al. 2009), this effect has been quantified to adequately study the ability of this biomass to eliminate the dyes studied.

Material and methods

Species used

Phaeodactylum tricornutum Bohlin (CCAP 1055/1), a marine microalga (diatom), was used for the experiments. The culture of this species to obtain biomass was carried out in the laboratory with the following conditions: ALGAL as culture medium (Herrero et al. 1991), filtered air continuously bubbled (10 L min^{-1}), temperature of 18 ± 2 °C, and illumination of 68 µmol photons m^{-2} s^{-1} (12:12 h light/dark).

The living and dead biomass of this diatom was used for their comparison. Dead biomass was obtained as follows: centrifugation of the culture at 4500 × g (15 min, 4 °C); then, to eliminate salts, a resuspension in 0.15 M ammonium formate at pH 8; and finally, a recentrifugation with the pellet lyophilized and placed in a desiccator. To obtain the living biomass (with a dry weight equivalent to that of dead biomass) it was necessary to determine the volume to be taken from the culture considering the dry weight of the

cells. Previously, the cell density of the culture was obtained by counting an aliquot in a Neubauer chamber. Taking into account this data and the dry weight, the volume that was necessary to take from the microalgal culture was calculated to obtain a dry weight equivalent to that of the dead biomass in the experiments.

Chemicals

Three dyes purchased from Sigma (USA) were used in this work, Methylene Blue (MB), Crystal Violet (CV), and Safranin O (S). A stock solution of each of these dyes was prepared in methanol (1 mg mL^{-1}).

Natural seawater used in the experiments was firstly passed through a filter (0.22-μm pore size/nitrocellulose, Millipore Iberica, Spain) and then through activated carbon with the aim of eliminating organic compounds. A pH = 8.2 and a salinity of 35‰ were the characteristics of this seawater after its sterilization in autoclave at 120 °C for 15 min.

Biosorption assays

Experiments of biosorption were performed with the following conditions: axenic environment using sterile seawater in Kimax glass tubes placed on an orbital shaker at 200 rpm for 11 h, at 18 ± 2 °C, and with 68 μmol photons m^{-2} s^{-1} light intensity. Seawater with an appropriate volume of the stock solution of dye was included in the tubes to obtain final concentrations of 0.5, 1, 2.5, 5, 10, 15, 20, and 40 mg L^{-1}. Then, one or the other biomass was added to the tubes at a concentration equivalent to 0.4 g L^{-1} of dry biomass. The living biomass was taken directly from the algal culture using the volume calculated as indicated above, and the dead biomass by weighing the suitable amount. Two controls without biomass but with dye were included, one under lighting and the other in darkness (in this latter case the tubes were covered with aluminum foil). Samples were taken at different times, 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 5, 7, 8, and 11 h, and centrifuged at 13,000 × g for 15 min. Afterwards, the supernatants were kept at −20 °C until their subsequent analysis in a UV/Vis spectrophotometer PharmaSpec UV-1700 (Shimadzu Corporation, Japan). Experiments were carried out in triplicate.

Effect of pH

To investigate the effect of pH on the removal of these dyes, both living and dead biomass were tested. In addition, control tubes with dye but without biomass, both in light and in darkness, at the same pHs were included. Several pHs (2–10) were tested in these experiments for 11 h, at a dye concentration of 5 mg L^{-1} for Methylene Blue and Safranin and 40 mg L^{-1} for Crystal Violet. The biomass concentration

was equal to 0.4 g L^{-1}. The pH of the seawater solution was adjusted with sodium hydroxide or hydrochloric acid. pH was measured with a Basic 20 pH meter (Crison).

Biosorbent characterization

Point of zero charge (pH$_{PZC}$) and Fourier transform infrared spectroscopy (FTIR) were used to characterize the *P. tricornutum* biomass.

Point of zero charge (pH$_{PZC}$) determination The point of zero charge was determined both in the living and dead biomass. The pH drift test was used for this determination. Twelve pH solutions (2–13) containing 20 mL of distilled water with 0.6-M NaCl were used in the test. The pH of each solution was adjusted to these initial pHs using NaOH or HCl. Twenty milligrams of microalgal biomass was added to each of the pH solutions. In the case of living biomass, the 20 mg of biomass were obtained as follows: a volume of the microalga stock culture enough to obtain a number of cells equivalent to 20 mg of dry weight was centrifuged at 4500 × g for 15 min. The cells obtained were washed in the 0.6 M NaCl solution. After a new centrifugation, the collected biomass was resuspended in each pH solution. In the case of dead biomass, 20 mg of lyophilized biomass was washed with the 0.6 M NaCl solution and centrifuged at 4500 × g for 15 min to obtain the biomass, which was resuspended in each pH solution. The biomasses in these solutions were stirred for 3 h, and the final pH was recorded.

Fourier transform infrared spectroscopy (FTIR) analysis FTIR was used to recognize functional groups on the biomass, before and after the biosorption of the dyes. These data were obtained applying the attenuated total reflection (ATR) mode on the FTIR spectrometer (Thermo Scientific Nicolet iS10). The specifications were 64 cumulative scans, a range of 525–4000 cm^{-1}, and resolution spectrum of 4 cm^{-1}. Both biomasses were dried and ground before these analyses.

Analytical methods

The concentration of the dyes was measured in a UV/Vis spectrophotometer at the λ$_{max}$ of each dye (666, 590, and 532 nm for Methylene Blue, Crystal Violet, and Safranin, respectively).

The percentage of each dye removed at time t (P_t) was:

$$P_t = \frac{(C_i - C_t) \times 100}{C_i} \tag{1}$$

where C_i and C_t are the initial dye concentration and the dye concentration in the tubes with biomass and light at time t (mg L^{-1}), respectively.

The amount of dye eliminated per unit of biomass at time t (q_t) (mg g^{-1}) was determined as:

$$q_t = \frac{(C_c - C_t) \times V}{m} \qquad (2)$$

where C_c is the dye concentration in solution in the control tubes without biomass and with light at time t (mg L^{-1}), C_t is the same as in Eq. (1), V is the volume of solution (L), and m is the mass of the sorbent (g).

Sorption kinetics

Seven kinetic models were used: pseudo-first order, pseudo-second order, pseudo-third order, pseudo-fourth order, pseudo-fifth order, intraparticle diffusion, and sigmoidal. Details are presented in Table S1.

Biosorption isotherms

Four isotherm models were used: Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich. Details are presented in Table S1.

Statistical tools

Data is represented as mean ± standard error of three independent experiments. Non-linear regression was used to fit the biosorption data to the equations of the different models using SigmaPlot version 12.5 for Windows (Systat Software, Inc.). The statistical significance ($\alpha = 0.05$) of the comparisons was studied by Student's t test (two groups) or two-factor ANOVA with Tukey test (more than two groups) (normality of the data and homogeneity of the variances were previously verified, meeting these requirements). The SPSS 26 (IBM, Spain) statistics package was used to perform these analyses.

Results

Photodegradation of the dyes

Since all the experiments were performed under illumination, the amount of photodegraded dye, without considering the action of the microalgal biomass, was quantified. A comparison was made at the end of the experiments between the tubes exposed to light and the tubes in darkness, both without biomass. The results of the statistical analysis are shown in Table S2. Previously, in the dark tubes, no significant differences were observed in the concentration of the dyes, which indicated that the three dyes remained stable in the seawater during the 11 h of the experiments. In the case of Methylene Blue and Crystal Violet there were no significant differences between the tubes with and without light, and therefore, no photodegradation was observed during the time of the experiments and with the experimental conditions used. However, significant differences in the amount of photodegraded dye were obtained with Safranin. Around 13% of Safranin was eliminated after 11 h due to the illumination effect.

Effects of contact time and type of biomass

Figure 1 represents the amount of each dye per unit of volume of the solution removed during the time. The amount of removed dye increased quickly at the start of the experiment for the three dyes (except for Safranin, although only using living cells). This behavior was more evident in the case of Crystal Violet where the removal rate was higher than that of the other two dyes. After this initial phase, an equilibrium was observed in most of the cases, but the time needed to reach this equilibrium was different for the different dyes. In the case of Crystal Violet, the equilibrium was reached in less than 2 h for both biomasses. However, with Methylene Blue, 5 h were necessary using both dead and living biomass, but in the latter case only at dye concentrations higher than 2.5 mg L^{-1}. No equilibrium was observed at low dye concentrations with this living biomass during the time of the experiment. Safranin was the dye that showed continuous photodegradation; therefore, an equilibrium was not reached in the total elimination process. However, with this dye and when only the amount of dye removed by the biomass was considered, there were differences between the two biomasses. In the case of living biomass, the equilibrium was reached in less than 4 h at the lowest concentrations; but no equilibrium was observed at high concentrations. With dead biomass, the equilibrium was reached within 4 h.

The two-factor ANOVA (dye concentration and type of biomass) and Tukey test (Table S3) indicated that, with Methylene Blue, there were differences between both biomasses up to the concentration of 2.5 mg L^{-1}. This dye was more removed by the dead biomass in these concentrations. When the concentration was equal or superior to 5 mg L^{-1}, both biomasses removed a similar amount of dye. The opposite occurred in the case of Safranin, significant differences were also observed between living and dead biomass, and in addition, there was interaction between both factors (Table S3). However, in this case, the dye was more removed by living biomass when the concentration was below 10 mg L^{-1}. Living biomass would be more efficient for the removal of low concentrations of Safranin. With Crystal Violet, no significant differences were observed between living and
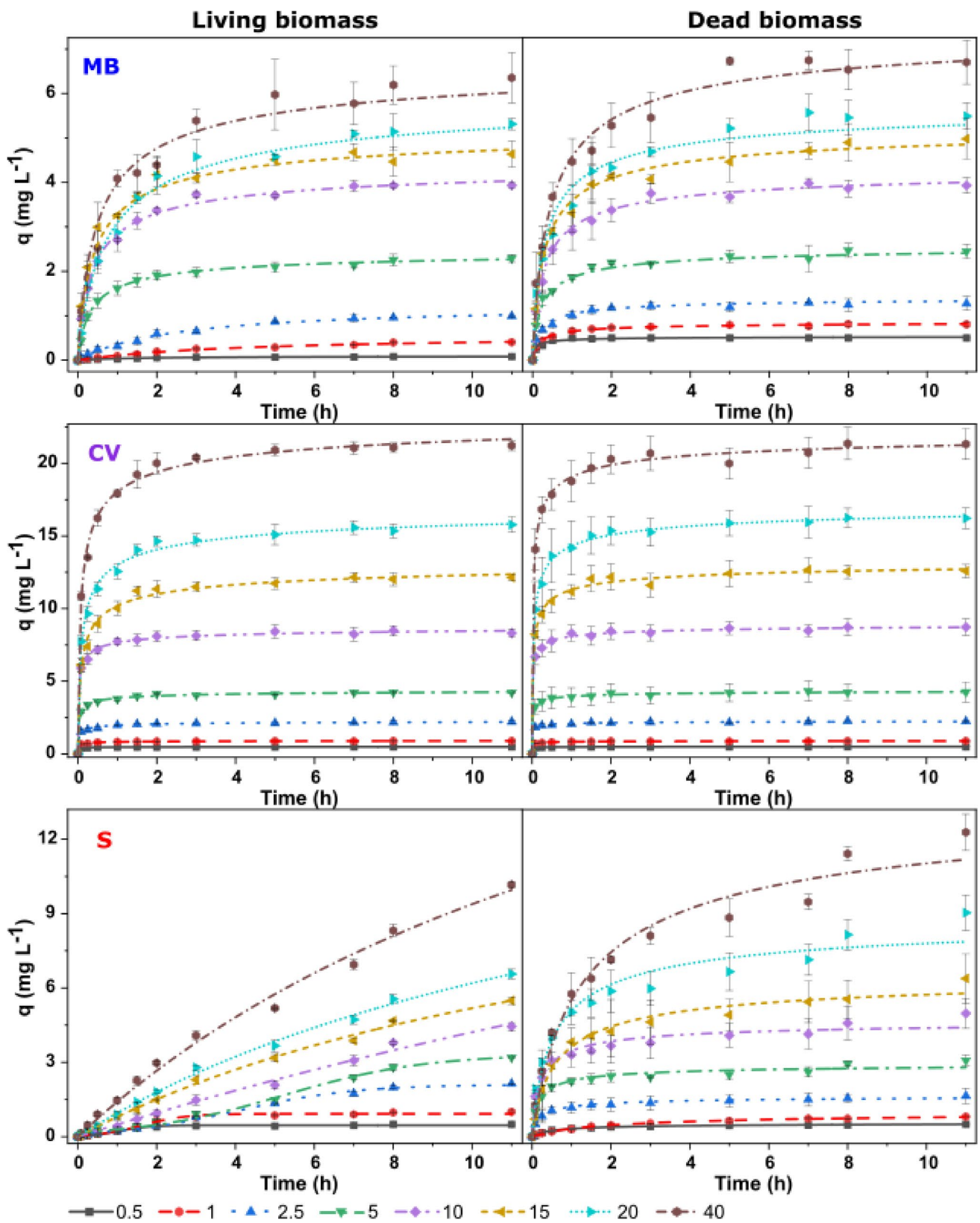
Fig. 1 Kinetics and effect of contact time on the total amount of Methylene Blue, Crystal Violet, and Safranin eliminated per unit of volume using living and dead biomass of *P. tricornutum*

dead biomass in the concentrations tested, both biomasses removed the same amount of dye, and there was no interaction between both factors.

Effect of the initial dye concentration

The initial concentration of the three dyes had a significant effect on the amount of dye removed (Fig. 1). An increase in the initial dye concentration caused an increase in the concentration of dye eliminated in both biomasses. The eliminated amounts were 0.081 ± 0.004, 0.489 ± 0.016, and 0.500 ± 0.025 mg L^{-1} for Methylene Blue, Crystal Violet, and Safranin, respectively, with an initial dye concentration of 0.5 mg L^{-1} and using living biomass. In the case of dead biomass, the eliminated amounts were 0.497 ± 0.005, 0.495 ± 0.014, and 0.509 ± 0.026 mg L^{-1} for Methylene Blue, Crystal Violet, and Safranin, respectively. These values increased to 6.353 ± 0.565, 21.224 ± 0.347, and 10.161 ± 0.098 mg L^{-1}, respectively, for the initial dye concentrations of 40 mg L^{-1} and living biomass. The amounts obtained for dead biomass and 40 mg L^{-1} of dye were 6.694 ± 0.491, 21.325 ± 1.060, and 12.284 ± 1.223 mg L^{-1} for Methylene Blue, Crystal Violet, and Safranin, respectively.

However, when the removed amount of dye was expressed as percentage of the added initial amount (Table 1), the percentage decreased when the initial concentration of dye increased. These three dyes were almost removed in 11 h when the initial concentration was 0.5 mg L^{-1} and using the dead biomass (dead and living biomass in the case of Safranin). As the initial concentration of these dyes increased, the percentage of elimination decreased due to the saturation of the biomass. Despite this, the total percentage eliminated by dead biomass in the highest concentration of dye was 16.2, 52.9 and 30.7% for Methylene Blue, Crystal Violet, and Safranin, respectively. The removed percentage by both biomasses was similar in all the concentrations in the case of Crystal Violet. Nevertheless, regarding Methylene Blue, this percentage was much higher with dead biomass in the lowest concentrations. As the concentration of Methylene Blue increased, the percentage of elimination between living and dead biomass was equalized. The opposite occurred in the case of Safranin, and the percentage removed was higher with living biomass in the lower concentrations (below 10 mg L^{-1}).
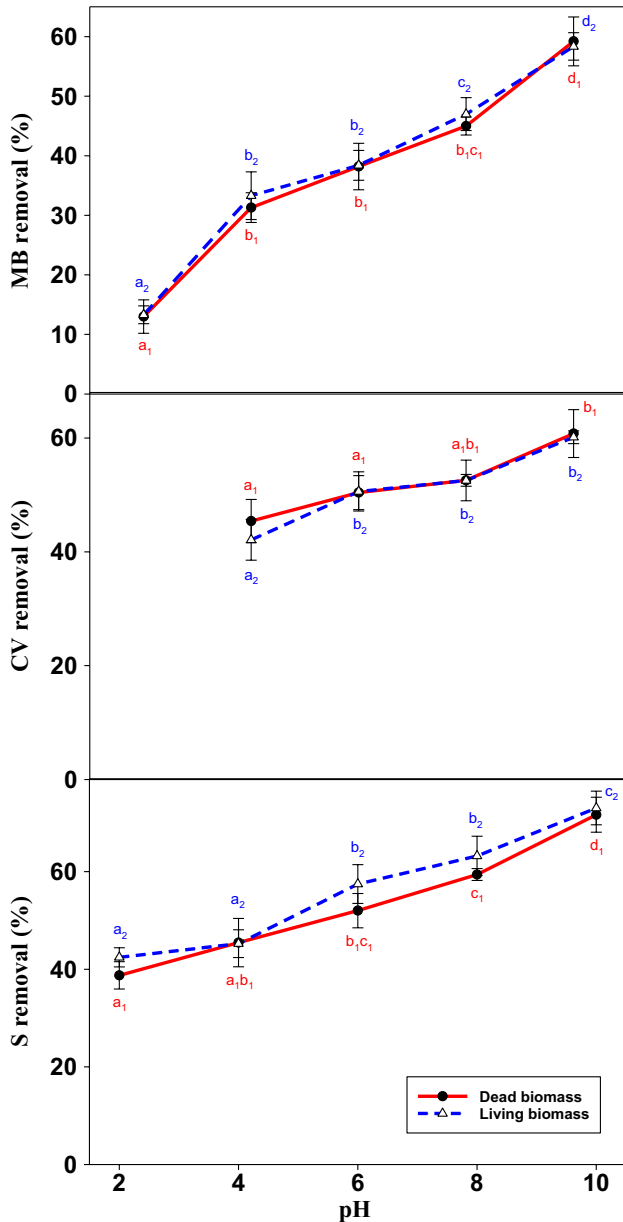
Effect of pH

The three dyes remained stable in the range of pHs tested during the biosorption time because there were no significant differences in the concentration of dyes in the tubes that remained in darkness at time 11 h with respect to time 0 h (Table S4). Likewise, none of the dyes showed

Table 1 Percentage removed P_t (%) of each dye depending on the initial concentration

Type of biomass	Dye	Dye concentration (mg L^{-1})							
		0.5	1	2.5	5	10	15	20	40
Living biomass	MB	16.3	39.9	39.7	45.5	39.1	30.8	26.5	15.9
	CV	97.6	89.2	88.7	84.3	83.0	80.7	78.8	52.8
	S	100	98.3	85.5	63.3	43.9	36.2	32.8	25.2
Dead biomass	MB	100	79.5	50.7	47.9	39.1	33.1	27.5	16.2
	CV	98.8	88.3	87.4	84.7	86.0	82.3	79.7	52.9
	S	99.9	80.9	65.8	59.6	49.4	41.9	44.6	30.7

significant variations in photodegradation in relation to the different pHs after 11 h. Although Safranin was the dye that experienced the highest photodegradation, it also did not show significant differences among pHs. The effect of the different pHs on the biosorption properties of the *P. tricornutum* biomass in relation to the three dyes is shown in Fig. 2. The percentage removed of each dye increased as the pH increased and was statistically significant (Table S5).

Biomass characterization

Point of zero charge
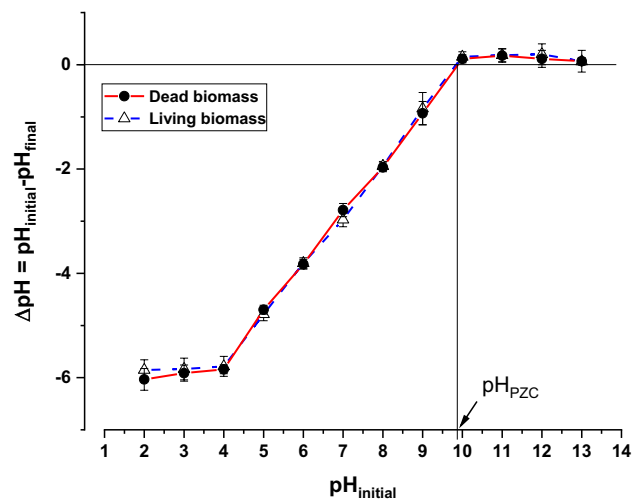
Figure 3 shows the pH drift tests for both biomasses. This figure represents the plot of $\Delta pH = pH_{initial} - pH_{final}$ against $pH_{initial}$. The pH of the point of zero charge (pH_{pzc}) corresponds to the point where the difference (ΔpH) would be zero. There were no significant differences ($p < 0.05$) in the data obtained for both biomasses. The pH of the point of zero charge was estimated at 9.9 ± 0.03.
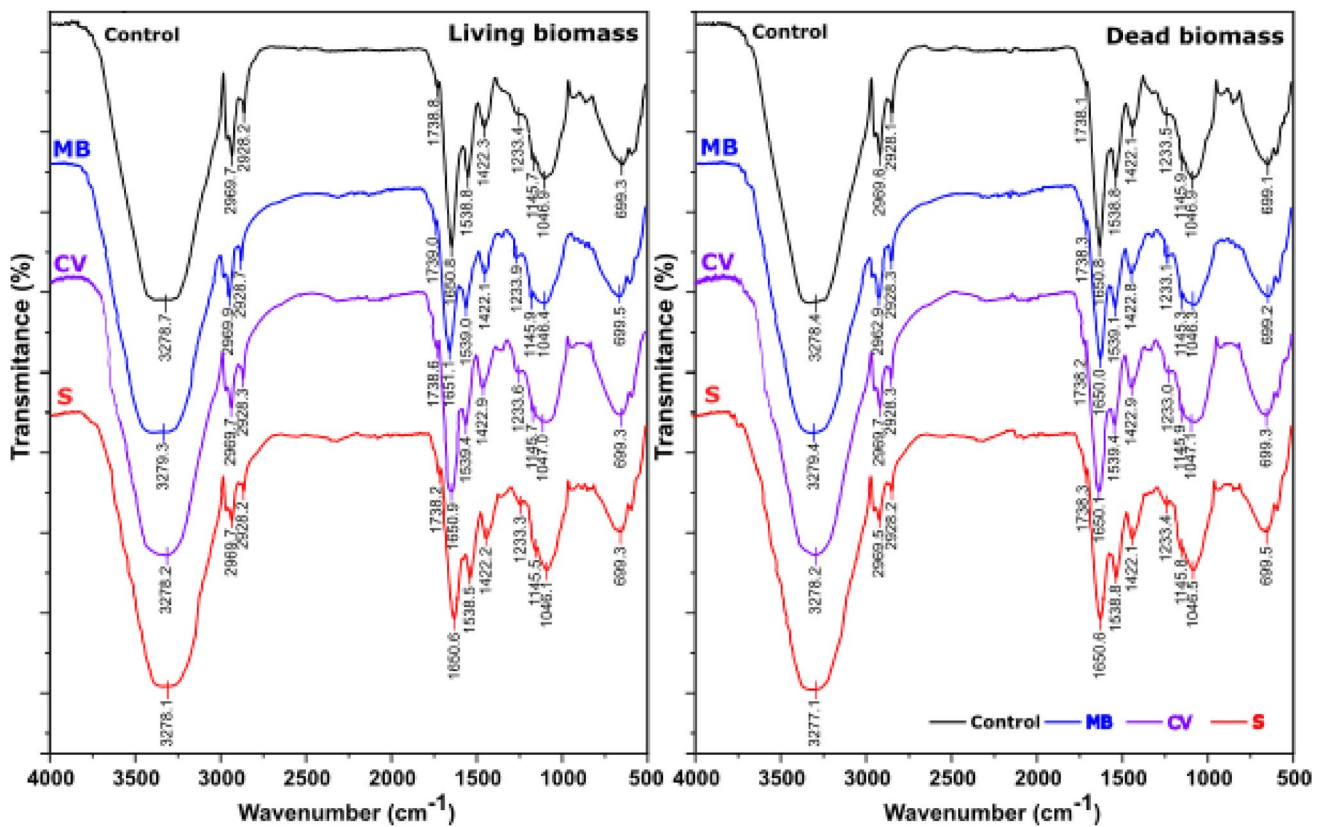
FTIR studies

Figure 4 represents the FTIR spectra of both biomasses obtained before and after the sorption process of each dye. The obtained spectra display bands around 1045, 1535, 1650, 1735, 2928, 2970, and 3279 cm^{-1}. The peak observed at 1045 is characteristic of diatoms, corresponding to a stretching of Si–O–Si. Peaks at 1535 and 1650 are attributed to the proteins. The bands in the range 1650 to 1735 are due to the stretching of C=O bonds (phospholipids correspond to the peak at 1735). Peaks around 2970 reflect asymmetric and symmetric C-H stretching modes. Finally, the O–H stretching vibrations correspond to the peak near to 3279. Both biomasses had a similar spectrum. The FTIR peaks not changed, and no new peaks were observed (in all dyes) after sorption experiments.

Sorption kinetics

To study the kinetics, it is necessary to differentiate two processes, one would be the total process in which the amount

Fig. 2 Effect of pH on the percentage of dye removed after 11 h considering the type of biomass and using a dye concentration of 5 mg L^{-1} for Methylene Blue and Safranin and 40 mg L^{-1} for Crystal Violet. Different letters indicate significant differences with $\alpha = 0.05$

Fig. 3 Point of zero charge (pH_{PZC}) determination of both biomasses of *P. tricornutum*

Fig. 4 FTIR spectra of living and dead biomass after 11 h of sorption of each dye

of dye removed (mg L^{-1}) is determined by photodegradation and removal by biomass, and the other in which only the amount of dye removed by the biomass (mg g^{-1}) is considered. Since in the case of Methylene Blue and Crystal Violet the photodegradation was not significant, the type of kinetic model of the total process was not different from the type of kinetics model that the biomasses had (compare Tables 2 and 3). However, this was different in the case of Safranin since the photodegradation was significant. For this reason, the kinetic model of the total process was different from the kinetic model considering only the biomass.

The sorption kinetics obtained was different for each dye (Figs. 1 and 5). The kinetic parameters are shown in Tables 2 and 3. The r^2 values obtained for the fits to the tested kinetic models are shown in the Tables S6–S11. Regarding Crystal Violet, the removal was very fast; therefore, the data of both biomasses had to be fitted to a pseudo-fourth order kinetics (dead biomass was a little faster than live one). In the highest concentration of this dye, dead biomass removed 52.9% of dye in 11 h and 41.7% was removed in just 0.25 h. This seems to indicate a high affinity of this biomass for this dye.

For the data obtained with Methylene Blue, the best fit was obtained with a pseudo-third order kinetics using dead biomass. Although it was a high sorption rate, it was lower than that obtained with Crystal Violet. In addition,

with Methylene Blue, a significant difference was obtained between the two biomasses up to the concentration of 2.5 mg L^{-1}. In these concentrations, as seen in Table 3, the removal rate of living biomass was lower than that of dead biomass, and the best fit of the living biomass only obtained a pseudo-second order kinetics. However, from the concentration of 5 mg L^{-1}, the two biomasses had a similar kinetics (pseudo-third order).

With Safranin and with respect to the total process with dead biomass (dead biomass + photodegradation), the best fit (Tables 2 and S10) was obtained with a pseudo-third order kinetics, while considering only dead biomass the kinetics were pseudo-second order. However, the kinetics of living biomass was different since the best fit (up to the concentration of 5 mg L^{-1}) was obtained with a sigmoidal kinetics. From this concentration, the kinetic model that best fitted was pseudo-second order kinetics when the total process is considered (Table 2) and pseudo-first order when considering only biomass (Table 3). Nevertheless, during the contact time, an equilibrium was not reached in these dye concentrations; therefore, a sigmoidal adjustment cannot be ruled out.

Finally, an intraparticle diffusion model was applied to determine the rate-limiting step. Figure 6 shows the fit of the data of the three dyes and both biomasses to this model in its linear form. It can be observed that in the three dyes, there

Table 2 Kinetic parameters obtained with the best fit for the removal of dyes considering jointly the effect of photodegradation and the removal by the biomasses of the microalga *P. tricornutum*

[Initial dye] (mg L⁻¹)	Type of biomass	Methylene blue				Crystal violet					Safranin O			
		Pseudo-second order		Pseudo-third order		Pseudo-fourth order		Sigmoidal			Pseudo-second order		Pseudo-third order	
		q_e ($mg\,L^{-1}$)	k_2 ($L\,mg^{-1}\,h^{-1}$)	q_e ($mg\,L^{-1}$)	k_3 ($L^2\,mg^{-2}\,h^{-1}$)	q_e ($mg\,L^{-1}$)	k_4 ($L^3\,mg^{-3}\,h^{-1}$)	q_e ($mg\,L^{-1}$)	k_S (h^{-1})	t_c (h)	q_e ($mg\,L^{-1}$)	k_2 ($L\,mg^{-1}\,h^{-1}$)	q_e ($mg\,L^{-1}$)	k_3 ($L^2\,mg^{-2}\,h^{-1}$)
0.5	Living	0.09±0.004	6.70±1.13	-	-	0.51±0.004	3587±846	0.46±0.01	0.39±0.05	1.06±0.07	-	-	0.60±0.02	5.39±1.05
	Dead	-	-	0.55±0.01	48.4±11.4	0.51±0.003	6370±1235	-	-	-	-	-	-	-
1	Living	0.58±0.03	0.40±0.07	-	-	0.96±0.008	129±21	0.92±0.02	0.56±0.07	1.64±0.09	-	-	1.12±0.03	0.39±0.05
	Dead	-	-	0.88±0.01	8.00±0.81	0.93±0.007	350±69	-	-	-	-	-	-	-
2.5	Living	1.25±0.04	0.32±0.04	-	-	2.38±0.03	4.63±0.89	2.10±0.08	1.48±0.14	4.06±0.24	-	-	1.70±0.03	1.88±0.25
	Dead	-	-	1.45±0.01	2.60±0.25	2.33±0.02	25.97±6.12	-	-	-	-	-	-	-
5	Living	-	-	2.58±0.03	0.48±0.03	4.59±0.04	0.77±0.11	3.33±0.15	1.80±0.14	5.21±0.27	-	-	3.04±0.08	0.76±0.18
	Dead	-	-	2.66±0.04	0.70±0.08	4.52±0.04	1.88±0.38	-	-	-	-	-	-	-
10	Living	-	-	4.61±0.07	0.14±0.01	9.15±0.09	0.10±0.02	-	-	-	29.81±10.95	$7\times10^{-4}\pm6\times10^{-4}$	6.91±0.22	0.03±0.006
	Dead	-	-	4.46±0.05	0.19±0.02	9.23±0.08	0.23±0.04	-	-	-	-	-	-	-
15	Living	-	-	5.25±0.09	0.14±0.02	14.15±0.24	0.005±0.001	-	-	-	13.32±1.23	0.005±001	-	-
	Dead	-	-	5.44±0.10	0.12±0.02	13.87±0.17	0.02±0.004	-	-	-	-	-	-	-
20	Living	-	-	6.44±0.10	0.03±0.01	18.17±0.27	0.002±0.0004	-	-	-	15.97±1.42	$0.004\pm9\times10^{-4}$	6.88±0.24	0.04±0.008
	Dead	-	-	6.37±0.02	0.06±0.01	18.01±0.16	0.007±0.001	-	-	-	-	-	9.26±0.50	0.02±0.008
40	Living	-	-	7.67±0.03	0.02±0.01	24.65±0.29	0.001±0.0002	-	-	-	25.42±3.72	$0.002\pm8\times10^{-4}$	14.58±0.83	0.004±0.001
	Dead	-	-	7.78±0.24	0.04±0.01	23.05±0.22	0.005±0.001	-	-	-	-	-	-	-

Table 3 Kinetic parameters obtained with the best fit for the removal of dyes considering only the biomass of *P. tricornutum*

| [Initial dye] ($mg\,L^{-1}$)	Type of biomass	Methylene blue				Crystal violet		Safranin O						
		Kinetic model with best fit												
		Pseudo-second order		Pseudo-third order		Pseudo-fourth order		Sigmoidal			Pseudo-first order		Pseudo-second order	
		q_e ($mg\,g^{-1}$)	k_2 ($g\,mg^{-1}\,h^{-1}$)	q_e ($mg\,g^{-1}$)	k_3 ($L^2\,mg^{-2}\,h^{-1}$)	q_e ($mg\,g^{-1}$)	k_4 ($L^3\,mg^{-3}\,h^{-1}$)	q_e ($mg\,g^{-1}$)	k_S (h^{-1})	t_c (h)	q_e ($mg\,g^{-1}$)	k_1 (h^{-1})	q_e ($mg\,g^{-1}$)	k_2 ($g\,mg^{-1}\,h^{-1}$)
0.5	Living	0.22±0.01	2.92±0.45	-	-	1.26±0.01	229.55±54.20	1.02±0.02	0.34±0.03	0.96±0.04	-	-	-	-
	Dead	-	-	1.33±0.01	7.18±0.69	1.26±0.01	407.57±79.06	-	-	-	-	-	1.12±0.01	2.41±0.21
1	Living	1.41±0.08	0.16±0.03	-	-	2.38±0.02	8.71±1.49	2.06±0.04	0.51±0.05	1.55±0.06	-	-	-	-
	Dead	-	-	2.23±0.03	1.37±0.14	2.25±0.02	24.03±5.14	-	-	-	-	-	1.84±0.04	0.37±0.03
2.5	Living	3.07±0.10	0.13±0.02	-	-	5.91±0.07	0.32±0.06	4.38±0.12	1.34±0.11	3.79±0.18	-	-	-	-
	Dead	-	-	3.38±0.07	0.50±0.09	5.84±0.05	1.87±0.44	-	-	-	-	-	2.96±0.02	1.87±0.11
5	Living	-	-	6.37±0.06	0.08±0.01	11.42±0.10	0.05±0.008	6.56±0.24	1.68±0.13	4.98±0.23	-	-	-	-
	Dead	-	-	6.30±0.10	0.13±0.02	10.13±0.10	0.13±0.003	-	-	-	-	-	5.69±0.07	1.44±0.15
10	Living	-	-	11.34±0.18	0.02±0.002	22.75±0.23	0.007±0.001	-	-	-	18.13±4.94	0.05±0.02	-	-
	Dead	-	-	10.91±0.17	0.03±0.004	22.18±0.19	0.016±0.003	-	-	-	-	-	8.05±0.09	0.88±0.07
15	Living	-	-	13.01±0.23	0.02±0.003	35.15±0.60	$0.0004\pm4\times10^{-5}$	-	-	-	10.82±0.49	0.16±0.01	-	-
	Dead	-	-	13.65±0.25	0.02±0.003	33.43±0.42	$0.001\pm1\times10^{-4}$	-	-	-	-	-	11.13±0.10	0.37±0.02
20	Living	-	-	15.79±0.40	0.006±0.001	45.15±0.69	$0.0002\pm1\times10^{-5}$	-	-	-	11.94±0.44	0.17±0.01	-	-
	Dead	-	-	15.83±0.43	0.01±0.002	42.52±0.41	$0.0002\pm2\times10^{-5}$	-	-	-	-	-	13.87±0.16	0.30±0.02
40	Living	-	-	18.53±0.44	0.008±0.001	61.09±0.78	$0.00008\pm1\times10^{-5}$	-	-	-	12.47±0.42	0.22±0.02	-	-
	Dead	-	-	17.71±0.48	0.009±0.002	60.33±0.55	$0.0003\pm6\times10^{-5}$	-	-	-	-	-	17.56±0.36	0.12±0.01
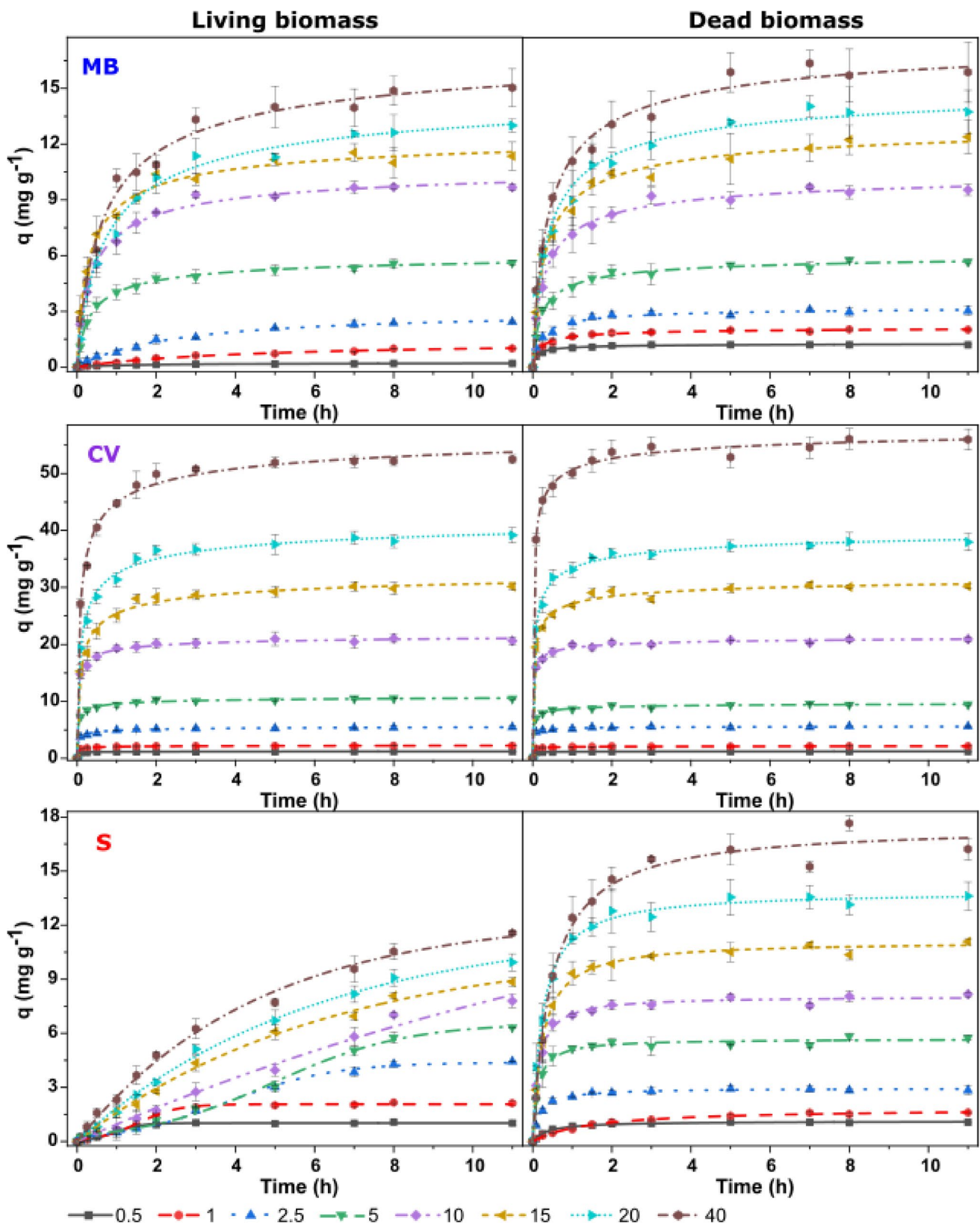
Fig. 5 Evolution of the amount of Methylene Blue, Crystal Violet, and Safranin eliminated per unit of biomass throughout the contact time and considering only the living and dead biomass. Points represent the means of three replicates and bars indicate the standard deviation
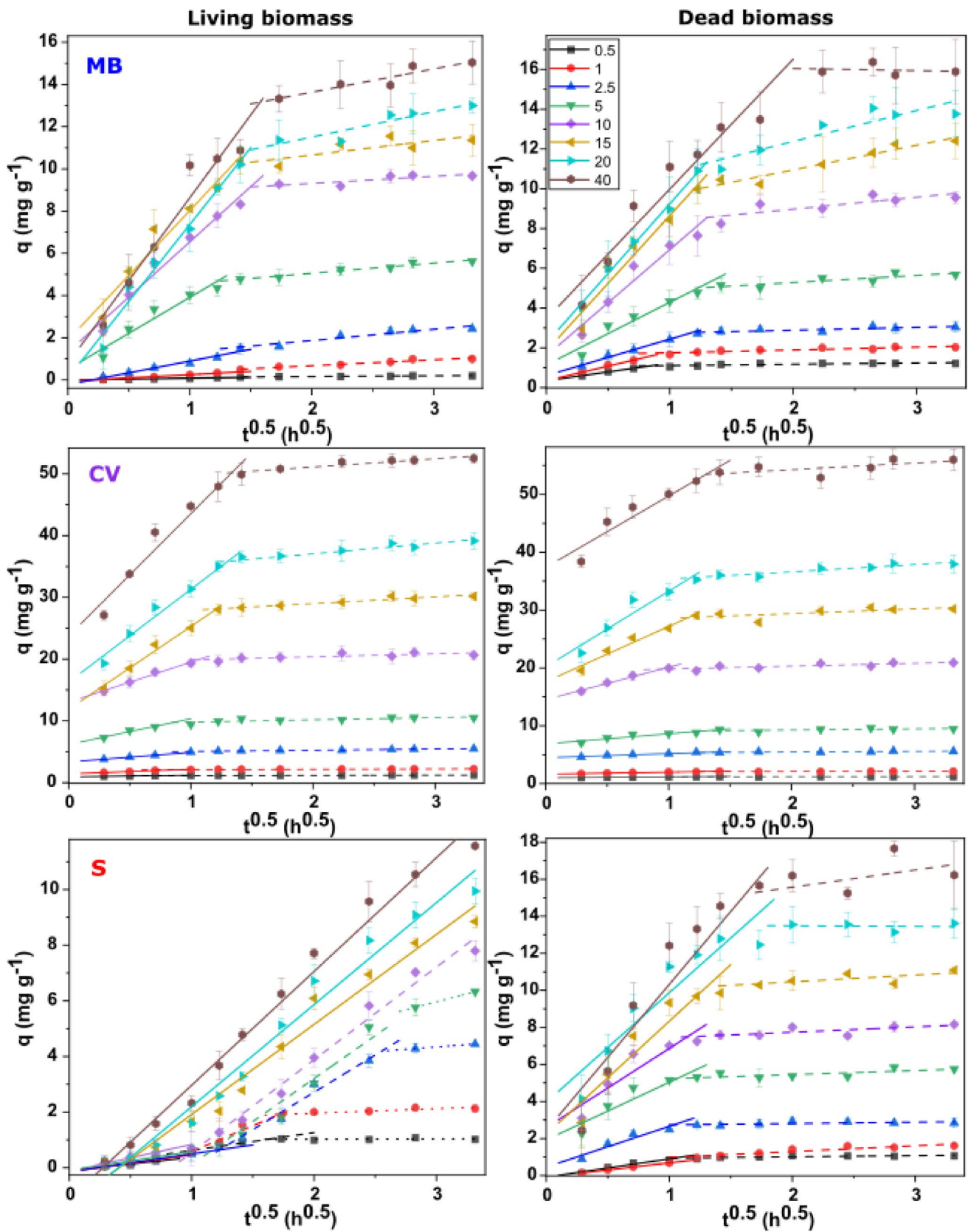
Fig. 6 Intraparticle diffusion kinetic model in its linear form for each dye with dead and living biomass

was multilinearity. In the case of Methylene Blue and Crystal Violet, both biomasses presented a similar kinetics with two steps, a quick first step of joining the dye to the external surfaces, followed by a much slower step of intraparticle diffusion until the equilibrium was reached. However, Safranin showed differences between both biomasses and with respect to the other dyes. Dead biomass, as in the other dyes, presented two steps, but living biomass with low concentrations of this dye (< 10 mg L^{-1}) presented three steps and without multilinearity at the highest concentrations.

Biosorption isotherms

The analysis of the biosorption isotherms is an effective approach to evaluate the sorption capacity of a sorbent. Table 4 shows the parameters derived from the models studied and the accuracy of these models (r^2). Graphs with their fits are plotted in Fig. 7. All biosorption isotherms fit well with all experimental data for the dead and living biomasses and the three dyes. However, the Langmuir model, with a higher r^2, described better the sorption process in comparison with the other models.

Attending to the Langmuir model, maximum sorption capacity obtained for Methylene Blue was 18.50 ± 1.1 and 18.90 ± 1.1 mg g^{-1} for living and dead biomass, respectively, and without significant differences. However, it is important to clarify (as indicated above) that at low concentrations (< 5 mg L^{-1}), the living biomass was less efficient. In the case of Crystal Violet, the maximum sorption capacity was the highest of the three dyes, 62.30 ± 2.5 and 66.19 ± 2.1 mg g^{-1} for living and dead biomass, respectively, also without significant differences between both biomasses.

Finally, the maximum sorption capacity for Safranin was 19.60 ± 1.4 and 10.31 ± 0.9 mg g^{-1} for dead and living biomass, respectively, in this case with significant differences. Although these values indicate a superiority of dead biomass, living biomass was more efficient at concentrations below 10 mg L^{-1} (the opposite of what happened with Methylene Blue).

The Freundlich model also provides information to characterize the sorbent. K_F is related to the affinity and sorption capacity of this microalgal biomass. The values obtained were high for all dyes, and even higher for Crystal Violet, indicating a better affinity of this dye for the *P. tricornutum* biomass (Table 4). The *1/n* parameters obtained were between 0.21 and 0.46 for both biomasses and dyes, indicating a very favorable and better sorption properties, and a sorbent more heterogeneous. The b_T values from the Temkin model were superior to 0 which implies that the biosorption processes for the three dyes and both biomasses were exothermic. Finally, the obtained values of E_D in the Dubinin-Radushkevich model were < 8 kJ mol^{-1}, involving the predominance of a physisorption process in all cases.

Discussion

Today, microalgae are considered emerging biosorbents for the treatment of pollutants (Xiong et al. 2018). In fact, biomass derived from microalgae has long been considered a highly effective biomass in biosorption of metals (Ubando et al. 2020); however, there are fewer studies that use this type of biomass for the removal of organic pollutants (Daneshvar et al. 2018; Pathak

Table 4 Parameters of the isotherms and error function for the elimination of the dyes

| Isotherm model	Constants and error function	Methylene blue		Crystal violet		Safranin O	
		Type of biomass					
		Living	Dead	Living	Dead	Living	Dead
Langmuir	q_{max} (mg g^{-1})	18.50 ± 1.05	18.90 ± 1.11	62.30 ± 2.49	66.19 ± 2.14	10.31 ± 0.89	19.60 ± 1.41
	K_L (L mg^{-1})	0.15 ± 0.02	0.17 ± 0.03	0.32 ± 0.03	0.31 ± 0.03	1.18 ± 0.61	0.17 ± 0.03
	r^2	0.988	0.985	0.993	0.995	0.900	0.982
Freundlich	$1/n$	0.46 ± 0.07	0.39 ± 0.05	0.41 ± 0.06	0.43 ± 0.05	0.21 ± 0.01	0.40 ± 0.04
	K_F (mg$^{1-(1/n)}$ L$^{1/n}$ g^{-1})	3.38 ± 0.72	4.45 ± 0.61	16.73 ± 2.19	16.85 ± 2.09	5.67 ± 0.27	4.26 ± 0.48
	r^2	0.912	0.955	0.931	0.941	0.986	0.971
Temkin	A_T (L mg^{-1})	2.00 ± 0.28	164.50 ± 292.06	20.23 ± 13.38	28.20 ± 24.65	1856.26 ± 2629.89	738.17 ± 1875
	b_T (g J mg^{-1} mol^{-1})	661.61 ± 38.30	1676.7 ± 473	327.30 ± 62.1	350.66 ± 81.7	2651.45 ± 439.49	2109.47 ± 717.4
	r^2	0.977	0.625	0.794	0.716	0.892	0.525
D-R	q_{max} (mg g^{-1})	13.40 ± 0.69	14.14 ± 1.10	48.91 ± 3.83	49.93 ± 4.05	9.17 ± 0.82	13.70 ± 1.37
	B_D (mol^2 J^{-2})	$1.5 \times 10^{-6} \pm 3 \times 10^{-7}$	$1.5 \times 10^{-6} \pm 5 \times 10^{-7}$	$6.8 \times 10^{-7} \pm 2 \times 10^{-7}$	$5.7 \times 10^{-7} \pm 1 \times 10^{-7}$	$8.2 \times 10^{-8} \pm 4 \times 10^{-8}$	$1.2 \times 10^{-6} \pm 5 \times 10^{-7}$
	E_D (kJ mol^{-1})	0.58	0.58	0.86	0.94	2.46	0.64
	r^2	0.960	0.902	0.940	0.933	0.785	0.850
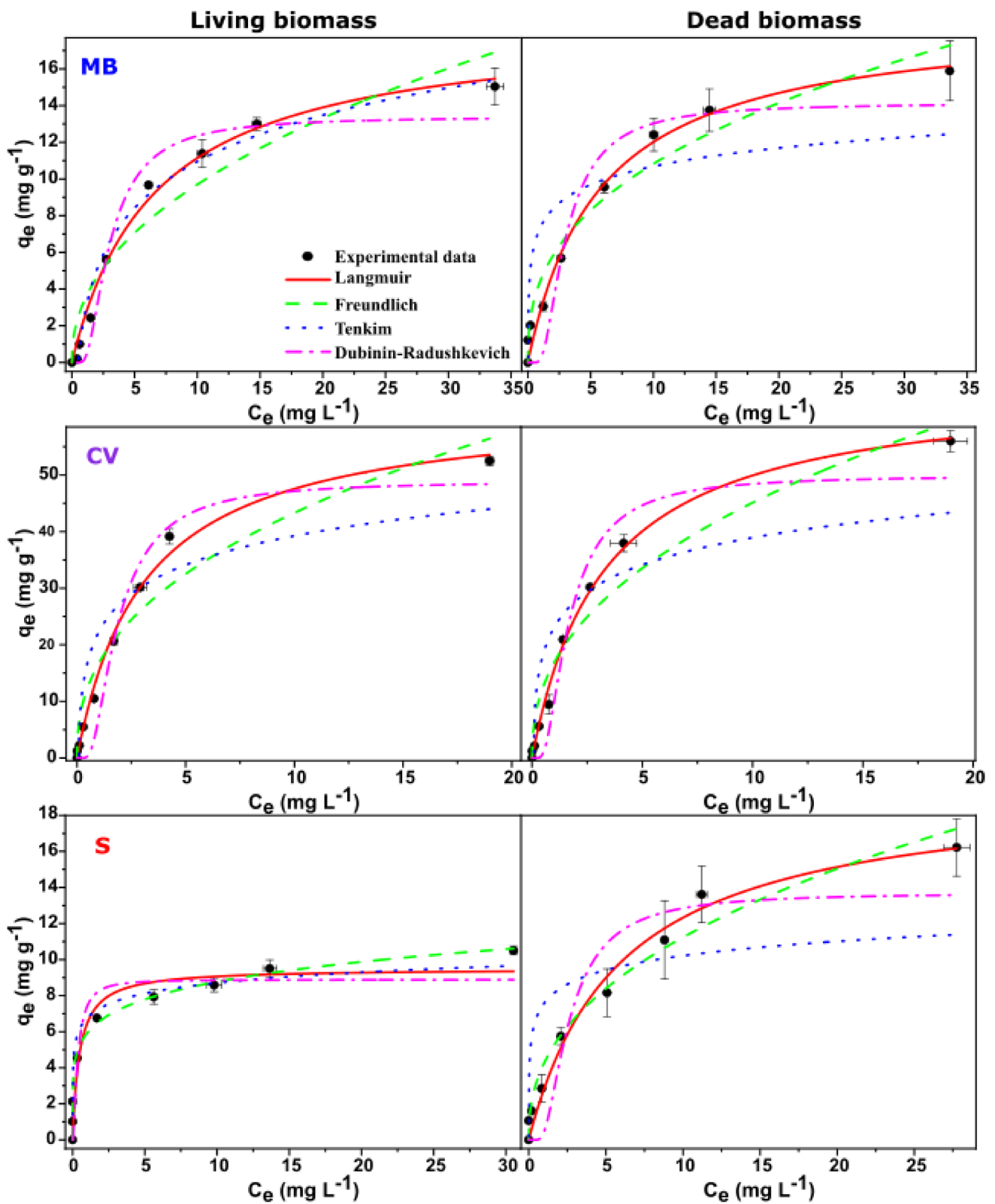
Fig. 7 Sorption isotherms for Methylene Blue, Crystal Violet and Safranin using living and dead biomass. Points represent mean values ± standard deviations

et al. 2015). The results obtained with the present work showed the efficacy in the removal of dyes (Crystal Violet, Methylene Blue and Safranin) by the biomass (both living and dead) from the microalga *P. tricornutum*. Although most biosorption studies focus on the use of dead biomass (Torres 2020), the use of living biomass may be more advantageous in certain circumstances as demonstrated in the present study. The living biomass of *P. tricornutum* removed more Safranin at dye concentrations below 10 mg L^{-1}. Living biomass would have a better performance in the removal of low concentrations of this dye. The opposite occurred in the case of Methylene Blue. In this case, the use of living biomass for the removal of low concentrations of this dye would not be an efficient process. This result demonstrates the different behavior towards cells of these dyes. The Methylene Blue dye would scarcely enter the cell interior when the cell is alive; the removal would only be by sorption to the cell surface. When the cell dies because the toxic concentration of the dye is reached, the cell loses its integrity, and the dye can gain access to the cell interior and the amount of dye removed increases. Possibly the toxicity of Safranin to the living cells of this microalga was low, and the cells remained alive, but in this case, the dye could enter the cell, increasing the removal efficiency. In fact, when this dye was tested with the living cells of this alga, the sorption kinetics was different at low concentrations with respect to the other dyes, and a sigmoidal model was the one that best allowed to fit the data (Tables S10 and S11). Furthermore, the intraparticle diffusion model showed in this case the appearance of a more marked additional step before reaching the last step towards equilibrium. This step, which is related to internal diffusion, could be determined by the entry of this dye into living cells. A first step would be the binding of the dye to the external surfaces of the cell and its entry through the cell wall, and finally its diffusion through the cell interior; this last step would be the most limiting. This three-step model was also observed in the biosorption of other compounds (Chen et al. 2011; Coelho et al. 2020). From all this, it can be deduced that when cells are more resistant to the toxic effect of the pollutant, living biomass can, in some cases, be superior to dead biomass. However, if the concentration of the pollutant is toxic and the cell loses its integrity, there will be no difference in the amount of pollutant removed between both types of biomass. This can be applied to the case of Crystal Violet, no significant differences were observed between dead and living biomass in the concentrations tested, both biomasses removed the same amount of dye, and there was no interaction between both factors. Possibly all the tested concentrations of this dye were toxic

for the living cells of this alga and its removal capacity was similar to that of the dead biomass.

The initial concentration of the three dyes had a significant effect on the amount of dye removed (Fig. 1). An increase in the initial dye concentration led to an increase in the removed concentration by both biomasses. However, this increase was gradually less as the initial concentration of the dyes increased, which is in accordance with other studies found in literature for sorption of dyes (Bouzikri et al. 2020; Silva et al. 2019). This smaller increase was best observed when the removed amount of dye was expressed as percentage of the added initial amount (Table 1). This percentage decreased when the initial concentration of dye increased, which is due to the saturation of the biomass. As the initial concentration of these dyes increased, the percentage of elimination decreased. Despite this, this biomass managed to remove almost 53% of Crystal Violet in the highest concentration tested (40 mg L^{-1}).

pH is one of the important factors influencing a biosorption process since it alters the surface charge of the sorbent material and the degree of ionization and speciation of sorbates. The sign of the sorbent surface charge depends on an important property of the material, the point of zero charge (pH_{pzc}), which is the pH at which the surface charge of the material is zero. At pH below this point, the charge would be positive and above negative. In the case of *P. tricornutum* biomass, the charge would be negative at pHs above 9.9. Furthermore, the three dyes are considered basic, and therefore, positively charged when the pH is higher than the respective pK_as (3.14 for Methylene Blue, 0.8 for Crystal Violet and 5.28 for Safranin). As a result of this, increasing the pH increases the negative charge of the microalgal biomass allowing a higher electrostatic interaction between the positively charged dye molecules and the negatively charged molecules of the biomass. This explains why increasing the pH increased the efficiency in the removal of these dyes by the biomass of this alga (Fig. 2). This is a common result in basic dyes removal processes (Albadarin and Mangwandi 2015; Zhou et al. 2011). Once the pH of the zero-charge point is exceeded, the amount of dye removed is usually kept constant (Bouzikri et al. 2020; Georgin et al. 2020). For this reason, it should be noted that the biomass obtained from *P. tricornutum* has a high zero-charge point value, which would allow to increase the amount of dye removed increasing the pH to that point. In this way, the process can be optimized. Taking this into account, the biomass of *P. tricornutum* would be suitable for treating solutions of these dyes with high pH. Despite this, in this work, the pH of 8.2 was maintained in the tests to keep the living biomass in suitable conditions.

This effect of pH would indicate that the main sorption mechanism of these dyes would be electrostatic attraction. This was confirmed by the FTIR studies. FTIR spectra not changed after sorption experiments, in all dyes; this suggests that the sorption process took place mostly by ion exchange. Furthermore, the low values obtained for the constant E_D of the Dubinin-Radushkevich model (Table 4) are also indicative of a sorption process of a physical nature. It is interesting to observe that the highest value of this constant was obtained with Safranin and living cells of the microalga, and the lowest value with Methylene Blue and living cells. This greater need for energy could be related to the incorporation of Safranin into the cell interior, while that in the case of Methylene Blue, the energy requirement was lower because this dye enters living cells with greater difficulty.

In biosorption studies, it is important to determine the biosorbent that has the best characteristics to optimize the process; for this reason, it is interesting to compare with other sorbents. Table 5 shows some examples of sorbents used for the removal of these dyes. Nevertheless, it is important to note that this comparison may be questioned because (as can be seen in the Table) most studies of this type found in the bibliography were performed in solutions of low ionic strength (mostly distilled water), in contrast with this study that was carried out in seawater (natural, and even more similar to a wastewater). Anyway, the comparison between different sorbents is interesting to assess the suitability of this microalgal material. To facilitate these comparisons, the dead biomass of this alga reached a maximum removal amount of 252.54 ± 30.57, 437.26 ± 34.13, and 281.66 ± 37.91 mg g^{-1} for Methylene Blue, Crystal Violet, and Safranin, respectively, when the experiments were carried out in distilled water (data not shown). These data show the good properties of this biomass and show the influence that ionic strength can have on biosorption processes of some pollutants and, therefore, the need to carry out these studies in more realistic conditions. According to Table 5, the biomass of *P. tricornutum* was even superior in conditions of high ionic load, to other biomasses in distilled water, and even much higher in the case of Crystal Violet. For instance, the removal capacity obtained in this work for Crystal Violet was 66.4 mg g^{-1} (437.26 mg g^{-1} in distilled water), an amount of elimination greater than some sorbents found in the literature, such as coniferous *Pinus* bark (Ahmad 2009), sugarcane fiber (Parab et al. 2009), sawdust (Khattri and Singh 2000), or *Calotropis procera* (Ali and Muhammad 2008). Biomass from microorganisms offers better performance. This is the case of the cyanobacterium *Spirulina* (Guler et al. 2015); the elimination was higher (101.87 mg g^{-1}), although after modification with

hexadecyltrimethylammonium bromide (HDTMA-modified *Spirulina* sp.), unlike the biomass of this work in which no modification was carried out. Something similar occurs in the case of Methylene Blue and Safranin; even under conditions of high ionic load, the biomass of *P. tricornutum* was superior to other materials (Malarvizhi and Ho 2010; Sharma and Upadhyay 2009). Moreover, when compared under similar conditions, this biomass can compete with materials as efficient as activated carbons (Ait Ahsaine et al. 2018; Kumar et al. 2005). It is evident that there are materials with greater efficiency; however, microorganisms offer better biotechnological possibilities. In this sense, *P. tricornutum* is a well-known and easy-to-grow microalga (Sabir et al. 2018); therefore, knowing its ability to eliminate these dyes is interesting.

In addition, many studies use sorbents that require materials with some degree of expensive or complex processing (Shariati et al. 2011). These materials can have, in some cases, a high effectiveness, but at the same time, they have some drawbacks (higher costs, high-tech operations, or skilled personnel). Thus, the *P. tricornutum* biomass had a lower sorption capacity than some of these sorbents that may be considered "artificial" (Table 5), especially for Safranin, where this biomass of *P. tricornutum* was inferior. However, a greater superiority was demonstrated in the case of Crystal Violet and Methylene Blue, not only with a greater capacity but also with a greater affinity, considering the highest values obtained in the Freundlich constant. With this in mind, it can be considered that an additional advantage of this biomass is that it is more natural since it is applied without any modification, which also means a lower cost.

These comparisons show that this microalgal biomass can be very interesting for the elimination of dyes, even from complex solutions, as seawater. In addition, its efficiency can be higher since an increase in the pH of the solution increased the amount of these dyes removed (Fig. 2) due to the high value of the zero-charge point that this biomass has (Fig. 3). It must also be taken into account that, with the exception of Safranin at high concentrations or Methylene Blue at low concentrations, living biomass had a similar or even higher efficiency than dead biomass, and with an additional advantage, living biomass requires less elaboration and lower cost, which allows living biomass to be used in many applications. Thus, living biomass is used when biodegradation is required (Reddy and Osborne 2020), or in immobilization processes (Chen et al. 2020), which are currently being perfected for applications in the field of pollutant removal. Precisely, in this technology, microalgae are gaining prominence (de-Bashan and Bashan 2010).

Table 5 Comparison with some materials used for the removal of these dyes

Dyes	Materials	Aqueous solution used for these studies	$q_{max}^{\dagger}$ $(mg\ g^{-1})$	$K_F^{\dagger\dagger}$ $(mg^{1-(1/n)}\ L^{1/n}\ g^{-1})$	Contact time (h)	[Dye] $(mg\ L^{-1})$	References
MB	Neem leaf powder	Distilled water	3.67–19.61	2.42–9.47	5	25–70	(Bhattacharyya and Sharma 2005)
	Spent rice biomass	Distilled water	4.70–8.98	-	2	25–50	(Rehman et al. 2012)
	Coconut coir	Distilled water	15.59	0.98	2.33	60–100	(Sharma and Upadhyay 2009)
	Orange peel	Distilled water	18.60	1.75	24	10–120	(Gurusamy et al. 2002)
	Banana peel	Distilled water	20.80	1.34	24	10–120	(Gurusamy et al. 2002)
	Wood apple rind-carbon	Distilled water	36.21–40.00	15.2–21.3	2	10–100	(Malarvizhi and Ho 2010)
	Oil palm shell-carbon	Distilled water	156.2–243.9	69.5–99.4	30	50–500	(Tan et al. 2007)
	Fly ash	Distilled water	5.57	4.38	2	20–60	(Kumar et al. 2005)
	Glass fibers	Distilled water	0.02–2.24	0.05–2.12	6	25–50	(Chakrabarti and Dutta 2005)
	Clay	Distilled water	58.20	-	2	10–100	(Gurses et al. 2006)
	Chestnut husk	Distilled water	117.2	19.4	0.67	50–500	(Georgin et al. 2018)
	Hydrogel P(AAm-co-AcA)	Distilled water	39.59	-	24	5–50	(Işikver 2017)
	Fucus vesiculosus	Distilled water	698.48	225.3	24	100–2500	(Lebron et al. 2021)
	Bifurcaria bifurcata	Distilled water	2744.5	189.8	0.25	10–1000	(Bouzikri et al. 2020)
	Cystoseira barbatula	Distilled water	21.91–38.62	39.9–81.8	6	5–100	(Caparkaya and Cavas 2008)
	Brazilian berry seeds (*Eugenia uniflora*)	Distilled water	189.6	34.4	3	25–200	(Georgin et al. 2020)
	Brewer's spent grain	Distilled water	298.35	69.51	7	5–250	(de Araujo et al. 2020)
	Ulva lactuca	Distilled water	3.01–10.99	1.43–1.45	2	5–25	(El-Sikaily et al. 2006)
	Paspalum maritimum	Distilled water	56.18	13.08	0.83	10–50	(Silva et al. 2019)
	Cyanthilium cinereum	Distilled water	76.34	10.07	0.83	10–50	(Silva et al. 2019)
	Sargassum ilicifolium	Distilled water	99.7	-	0.67	1.28–38	(Tabaraki and Sadeghinejad 2017)
	P. tricornutum (unmodified)	Distilled water	286	31.51	11	0.5–160	This work (Data not shown)
	P. tricornutum (unmodified)	Seawater	18.90	4.45	11	0.5–40	This work
CV	Wood apple rind-carbon	Distilled water	15.7–19.8	10.0–12.7	2	10–100	(Malarvizhi and Ho 2010)
	Coniferous pinus bark	Distilled water	10.36–32.78	0.57–0.72	2	10–50	(Ahmad 2009)
	Sugarcane fiber	Distilled water	10.44	4.73	7	20–100	(Parab et al. 2009)
	Sawdust	Distilled water	37.83	14.34	7	20–100	(Parab et al. 2009)
	Coir pith	Distilled water	65.53	24.43	7	20–100	(Parab et al. 2009)
	Rice husk	Distilled water	39.96–44.87	15.44–24.46	3	0–100	(Chowdhury and Das 2011)
	Calotropis procera	Distilled water	4.14	-	1.33	10–50	(Ali and Muhammad 2008)
	Neem sawdust	Distilled water	3.99–4.44	-	0.5	6–12	(Khattri and Singh 2000)
	Chestnut husk	Distilled water	90.8	18.2	0.67	50–500	(Georgin et al. 2018)
	Ricinus communis Pericarp Carbon	Distilled water	48.0	53.37	1	25–100	(Madhavakrishnan et al. 2009)
	HDTMA-modified *Spirulina sp.*	Distilled water	101.87	0.96	3	25–300	(Guler et al. 2015)
	Almond Shell activated carbon	Distilled water	625	214.93	2	80–200	(Ait Ahsaine et al. 2018)
	Annona crassiflora seed powder	Distilled water	300.96	-	2	0–300	(Franco et al. 2020)
	Diaporthe schini	Distilled water	642.3	-	2	0–300	(Grassi et al. 2019)
	P. tricornutum (unmodified)	Distilled water	433	73.01	11	0.5–250	This work (Data not shown)
	P. tricornutum (unmodified)	Seawater	66.19	16.85	11	0.5–40	This work

Table 5 (continued)

Dyes	Materials	Aqueous solution used for these studies	$q_{max}^{\dagger}$ $(mg\ g^{-1})$	$K_F^{\dagger\dagger}$ $(mg^{1-(1/n)}\ L^{1/n}\ g^{-1})$	Contact time(h)	[Dye] $(mg\ L^{-1})$	References
S	HDTMA-modified *Spirulina sp.*	Distilled water	54.05	3.56	3	25–300	(Guler et al. 2015)
	Activated carbon	Distilled water	560–576	106.3–165.5	48	-	(Kumar and Sivanesan 2005)
	Calcined bones	Distilled water	93.1–135.3	6.21–8.51	1.5	20–160	(El Haddad et al. 2013)
	Sugar beet pulp	Distilled water	147	9.50	18.5	100–800	(Malekbala et al. 2012)
	Hydrogel P(AAm-co-AcA)	Distilled water	24.02	-	24	5–50	(Işıkver 2017)
	Fe_3O_4 nanoparticles modified	Distilled water	769	-	1	50–500	(Shariati et al. 2011)
	Dodecylsulfate-hydrotalcites	Distilled water	83.3	17.1	6	5–50	(Bouraada et al. 2008)
	Dodecyl benzene sulfonate-hydrotalcites	Distilled water	40.5	11.8	6	5–50	(Bouraada et al. 2008)
	Spent bleaching earth	Distilled water	250	86.69	6	0–180	(Mana et al. 2007)
	P. tricornutum (unmodified)	Distilled water	260	34.28	11	0.5–160	This work (Data not shown)
	P. tricornutum (unmodified)	Seawater	19.60	4.26	11	0.5–40	This work

Conclusions

It may be concluded that the biomasses (both living and dead) from the microalga *Phaeodactylum tricornutum* have the potential to quick and efficiently remove Methylene Blue, Crystal Violet, and Safranin from aqueous solutions, even solutions of high ionic strength such as seawater. This biomass had a high affinity for these dyes and a higher removal capacity than other materials that have been tested as sorbents for these dyes. The state of biomass had a significant effect on the amount of dye removed and is a factor that should be better consider in the biosorption processes. Thus, the living biomass had a higher performance in the removal of low concentrations of Safranin and the dead biomass in the case of Methylene Blue. Both biomasses had the same performance for Crystal Violet. Therefore, the biomass from *P. tricornutum* is a very versatile biomass because it can be used as live or dead, facilitating its use in possible biotechnological applications, such as biotransformation processes or immobilized cells that may require the use of living biomass.

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