Potential for bifenthrin removal using microalgae from a natural source

Leticia Weis, Rosana de Cassia de Souza Schneider, Michele Hoeltz, Alexandre Rieger, Schirley Tostes and Eduardo A. Lobo

ABSTRACT

The accumulation of environmental pesticides can cause problems in aquatic ecosystems and adverse effects in humans. These compounds can be found in water due to runoff from agricultural, industrial and domestic applications. In Southern Brazil, tobacco cultivation is one of the most important economic agricultural activities. The bifenthrin pesticide, classified as having moderate toxicity (class II), is commonly used as an insecticide in this culture. In this context, the present research aimed to study the performance of microalgae-induced bioremediation processes. Microalgae were isolated from a natural water source in the city of Santa Cruz do Sul, RS, Brazil, which is an artificial reservoir used for public water supply. For this purpose, biodegradation, biosorption, influence of pH, percentage of inoculum and photoperiod were evaluated in batch experiments for 20 cultivation days. After the phycoremediation process, the bifenthrin pesticide (m/z = 181) was quantified by gas chromatography with mass spectrometry (GC-MS). The results indicated that microalgae isolated from the water of the lake were able to contribute to the removal of approximately 99% of bifenthrin through biodegradation and biosorption processes. Photodegradation was identified (>77%) and the best condition for the phycoremediation was 20% inoculum with a photoperiod of 18:6 h.

Key words | bifenthrin, bioremediation, microalgae, pesticide, phycoremediation, residual waters

Leticia Weis
Rosana de Cassia de Souza Schneider
  (corresponding author)
Michele Hoeltz
Eduardo A. Lobo
Environmental Technology Post-Graduation
  Program,
University of Santa Cruz do Sul (UNISC),
Santa Cruz do Sul, RS,
Brazil
E-mail: rosana@unisc.br

Rosana de Cassia de Souza Schneider
Michele Hoeltz
Alexandre Rieger
Schirley Tostes
Center of Excellence in Oilchemistry and
  Biotechnology,
University of Santa Cruz do Sul, UNISC,
Santa Cruz do Sul, Rio Grande do Sul,
Brazil

INTRODUCTION

Toxic chemicals from industrial, agricultural and domestic activities continuously contaminate the aquatic environments. In recent decades, the necessity to increase agricultural production and food quality for the general population has been propelling new agro-technologies that still heavily depend on the use of pesticides. The continuous uncontrolled usage of these substances is a constant concern, regarding not only impacts to human health but also environmental impact through the contamination of water sources and soil.

According to the World Health Organization (WHO), pesticides are chemical compounds that are used to kill pests, such as insects that can vector diseases of public health concern and cause damage to agricultural crops. In recent decades, pesticide production has increased, and there is little knowledge about the toxicity and effects on human health and the environment (Rousis et al. 2017). The pollution of water surfaces by pesticides results mainly from agricultural storm-water discharge and water runoff. Propagation occurs through the leaching of soil caused by heavy rains and winds that pick up and distribute pesticides, depositing them into lakes, rivers, coastal waters and wetlands (Weston et al. 2015).

Tobacco production is one of the most important agricultural products in southern Brazil and is practiced in 574 cities (>731,390 tons of tobacco leaves), including the city of Santa Cruz do Sul, which is the fifth largest producer in Rio Grande do Sul state (Carvalho et al. 2019). Due to the cultivation, which can occur near rivers, there is a great concern about these compounds in the water. The presence of pesticide molecules in the water bodies near tobacco crops is an indicator of the improper and incorrect usage of pesticides.

Among the pesticides used in southern Brazil, we high-lighted bifenthrin (2-methylbiphenyl-3-ylmethyl-(Z)-(1RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propane carboxylate) in this study. Bifenthrin is a pyrethroid insecticide with a strong environmental persistence (Li *et al.* 2017) and is classified as a toxicity class II, moderately hazardous compound, according to the WHO. Residues are often detected in different zones, including water and sediment. Bifenthrin is generally more toxic than other pyrethroids for terrestrial and aquatic insects; according Pamanji *et al.* (2015) it induces hatching rate in zebrafish embryos, until in lower concentrations due to membrane weakening or enzymatic activity induction; and, in general, pyrethroids and their metabolites also interact as endocrine disruptors (Ligocki *et al.* 2019).

Therefore, pesticide contamination is a problem that requires new alternatives for prevention and remediation. As an alternative, biological agents present appropriate characteristics to decrease environmental contaminants. Bacteria, fungi and microalgae are the most commonly employed biological agents. Microalgae are microscopic photosynthetic organisms that are found in aquatic environments (fresh and marine). There are many factors that influence the growth, such as light (quality and quantity), temperature, nutrient concentration, O_2, CO_2, pH and salinity, and other organisms (biotic factors), such as fungi, bacteria and viruses. Competition with other species in natural environments is also a relevant factor (Grobbelaar & Bornman 2004).

Microalgae are generally more efficient than plants in converting solar energy to biomass due to an abundant access to water, CO_2 and nutrients. Moreover, microalgae can have autotrophic, heterotrophic and mixotrophic metabolic profiles (Hamouda *et al.* 2016). Microalgae can be influenced by operational factors, such as shear from mixing, depth, harvest frequency and dilution rate, and the influence of these factors can be positive or negative, depending on the quantity and quality as it occurs. Another beneficial characteristic is that algae commonly uptake organic compounds, increasing the removal efficiency from water (Ding *et al.* 2017).

Therefore, bioremediation is an attractive option to reduce the environmental impact caused by pesticides. In general, the process consists of reducing pollutants from the environment using living organisms, such as microalgae, which have the ability to degrade organic pollutants directly (Gao & Chi 2015). Several studies have focused on the capacity of microalgae to remove compounds from water, such as pharmaceutical compounds (Xiong *et al.* 2016),

metals (Li *et al.* 2015), polycyclic aromatic hydrocarbons (García de Llasera *et al.* 2018), nutrients (Ansari *et al.* 2017) and pesticides (González-Barreiro *et al.* 2006; Dosnon-Olette *et al.* 2010; Zhang *et al.* 2011; Ardal 2014; Matamoros & Rodriguez 2016).

Table 1 summarizes some studies performed using the phycoremediation process on pesticide-contaminated waters. Different species of microalgae are being studied due to their ability to remove organic pollutants present in contaminated waters with different types of pesticides. Removal rates range from 0 to 95% due to the variables used in each study being different, such as pesticide type, pesticide concentration, microalgae species, photoperiod, temperature and light intensity. The studies were performed in the laboratory scale.

Likewise, in the Pardinho River hydrographic basin, where there is intense agricultural production of tobacco by family farms, there is the possibility for river water contamination with pesticides. The Pardinho River passes through the city of Santa Cruz do Sul, and along its extension, agriculture is practiced near its banks, such as tobacco cultivation and other plant cultures. Its waters are dammed in Dourado Lake (29.4349°S, 52.2739°W), which is the water reservoir for the city of Santa Cruz do Sul and supplies more than 129,000 citizens. As this artificial lake receives contaminants, it produces microalgal blooms during several periods of the year. The microalgae technology can improve bioremediation processes in regions such as rivers, lakes and agricultural effluent treatment. It is relevant to study the same microalgae for bifenthrin removal.

Therefore, considering the importance of maintaining the quality of the reservoirs for public supply and the potential of native microorganisms in organic pollutant remediation, the aim of this study was to evaluate the performance of microalgae induced bioremediation processes from a natural source on the removal of bifenthrin insecticide.

MATERIALS AND METHODS

Microalgae cultivation conditions

The microalgae were obtained from the freshwater reservoir in the city of Santa Cruz do Sul during a microalgal bloom. The experiments were performed under *ex situ* conditions. The cultivation was conducted in a closed-system photo-bio-reactor using an aquatic pump for stirring, with a total volume of 70 L. A $3 \, g \, L^{-1}$ commercial fertilizer solution containing 17% nitrogen (NO_3^-), 11% phosphorus (P_2O_5)

Table 1 | Studies carried out for the removal of pesticides using microalgae

Pesticide group	Pesticide and approximately removal percentage (%)	Concentration tested	Incubation time (days)	Temperature (°C)	Photoperiod (light:dark h)	Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	Microalgae	Reference
Herbicide	Atrazine (6), simazine (10) and alachlor (51)	10 µg L^{-1}	8 (hydraulic retention times) continuous feed reactors	23 ± 5	12:12	150	*Chlorella* sp. and *Scenedesmus* sp.	Matamoros & Rodriguez (2016)
Insecticide	Diazinon (39), chlorfenvinphos (49) lindane (41), malathion (95), chlorpyrifos (84) and endosulfan (89)							
Fungicide	Pentachlorobenzene (84)							
Fungicide	Dimethomorph (24) and pyrimethanil (10)	600 µg L^{-1}	1–4	23 ± 2	24:0	65	*Scenedesmus obliquus* and *Scenedesmus quadricauda*	Dosnon-Olette et al. (2010)
Herbicide	Isoproturon (54)	10 µg L^{-1}						
Fungicide	Metalaxyl (0), cyprodinil (0), propamocarb (50) and mandipropamid (0)	2 µg L^{-1}	4	20	16:8	100	*Chlorella vulgaris*	Ardal (2014)
Herbicide	Fluroxypyr (56)	0.5 mg L^{-1}	5	20–25	14:10	80	*Chlamydomonas reinhardtii*	Zhang et al. (2011)
Herbicide	Atrazine (80) and terbutryn (90)	0.75 mol L^{-1}	1	18 ± 1	12:12	70 ± 2	*Synechococcus elongatus* and *Chlorella vulgaris*	González-Barreiro et al. (2006)

and 18% potassium (K_2O) (NPK) was added to intensify microalgal growth. The solid fertilizer was purchased from Yara (Brazil), and the minerals nitrogen, phosphorus, potassium and other micronutrients, composes each granule. This cultivation system was developed under these conditions to ensure the growth process similar to that in a natural lake environment with solar temperatures and radiations. The microalgae multiplied and the inoculum formation process took 7 days.

An aliquot of the inoculum was taken to the laboratory, and the acclimatization conditions were conducted using a 1 L glass container with an aquatic pump for stirring that was intended to avoid sedimentation and flotation of the microalgae, in addition to bubbling. An NPK solution ($3\,\mathrm{g\,L^{-1}}$) was used to maintain the inoculum every 14 days, with a ratio of 30% inoculum to 70% NPK solution. The photoperiod was 24 h (light) supplied with cool-white fluorescent lamps, with a light intensity of $25\,\mu\mathrm{mol\,m^{-2}\,s^{-1}}$, using a digital light meter, and the temperature was maintained at $25 \pm 2\,^{\circ}\mathrm{C}$.

Selective pressure was performed on the culture over time, which was established under the new *ex situ* conditions, for the predominant species identified by molecular techniques. Microalgae were isolated following serial dilutions, and samples were taken from cultures containing isolated species. The DNA was extracted using the cetyl trimethylammonium bromide (CTAB) protocol. For species identification, three regions of rDNA were amplified and sequenced: 18S rDNA; internal transcribed spacer (ITS1-ITS2); and D1-D2. Polymerase chain reaction (PCR) conditions were the same for all primers, starting with an initial denaturation at $96\,^{\circ}\mathrm{C}$ for 5 min, followed by 35 cycles with denaturation at $94\,^{\circ}\mathrm{C}$ for 30 seconds, annealing temperature at $52\,^{\circ}\mathrm{C}$ for 30 seconds and extension at $72\,^{\circ}\mathrm{C}$ for 1 min, including a final extension at $72\,^{\circ}\mathrm{C}$ for 7 min. The purified products obtained were subjected to sequencing using the Sanger sequencing method, which was conducted on the ABI 3500 Genetic Analyzer automated DNA sequencer from Life Technologies – Applied Biosystems using the Sequencing Analysis Software v. 6.

The Regional Scientific and Technological Center located at University of Santa Cruz do Sul carried out the sequencing. It was performed using the same primers described for PCR reactions using 5 pmol for each primer. The amplicons were labeled using $1\,\mu\mathrm{L}$ of BigDye Terminator Reagent v. 3.1 Cycle Sequencing Kit (Applied Biosystems) for $4.5\,\mu\mathrm{L}$ of purified PCR product in a final volume of $10\,\mu\mathrm{L}$. The labeling reactions were conducted in a Veriti 96-Well Termal Cycler (Applied Biosystems) with

the following conditions: denaturation at $96\,^{\circ}\mathrm{C}$ for 1 min, followed by 35 cycles with $96\,^{\circ}\mathrm{C}$ for 15 seconds, $50\,^{\circ}\mathrm{C}$ for 15 seconds and $60\,^{\circ}\mathrm{C}$ for 4 min. After labeling steps, the samples were purified using Ethanol/EDTA precipitation method and the samples were electro injected in the automated sequencer. The sequences were submitted to the Basic Local Alignment Search Tool-BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for comparisons against deposited sequences at the GenBank. The samples were analyzed and compared using the E-value, the identity and the maximal score obtained from GenBank. To confirm the species, the deposited sequence with the minimum E-value and the maximum identity and score values was chosen.

Considering that the inoculum was produced from an environmental source and that the experiments were not performed aseptically, the microbial population of fungi and bacteria were quantified in the samples in the beginning of the experiments (t_0) and after 20 days of incubation (t_{20}), without pH adjustment and with a photoperiod of 12:12 h. To evaluate the colony forming units ($\mathrm{CFU\,mL^{-1}}$), the standard plate count technique was applied from the serial dilutions.

Chemicals and reagents

Bifenthrin analytical standards (purity >97%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany); acetonitrile (HPLC grade) and methanol (HPLC grade) were from J. T. Baker (USA), and ethyl acetate (HPLC grade) was from Vetec (Brazil). Stock solutions of bifenthrin containing $1,000\,\mathrm{mg\,L^{-1}}$ were prepared in acetonitrile and stored at approximately $-4\,^{\circ}\mathrm{C}$. A commercially-available product, Talstar 100 EC, from FMC (Brazil) contains $100\,\mathrm{g\,L^{-1}}$ (10% m/v) of the active ingredient bifenthrin and $810\,\mathrm{g\,L^{-1}}$ (81% m/v) of other ingredients, such as emulsifiers and solvents. Talstar was used in all phycoremediation experiments. Deionized water was prepared using a Milli-Q system (Gradient A10, Millipore, USA). Sodium hydroxide was obtained from Synth (Brazil), and the Strata C18-E (500 mg/6 mL) solid phase extraction (SPE) cartridges were obtained from Phenomenex (Torrance, CA, USA).

Phycoremediation experiment

The pH, photoperiod and percentage of inoculum were controlled to study the effects of microalgae on the removal of bifenthrin in water. Three different groups of samples were established, microalgae and bifenthrin (sample), microalgae

only (blank) and bifenthrin only (control), using a closed system with a photoheterotrophic mode of nutrition.

Experiment setups included a Marconi (Brazil) light incubator shaker at 190 rpm with temperature controlled at 29 ± 3 °C. During the light photoperiod, the light intensity was approximately $20 \, \mu mol \, m^{-2} \, s^{-1}$ using cool-white fluorescent lamps. The experiments were run simultaneously for a 20-day cultivation period and were repeated three times in a volume of 160 mL. The controls and blanks were carried out in parallel. A commercial formulation containing bifenthrin was used to prepare the reactors with a starting concentration of $5 \, mg \, L^{-1}$ and containing $1 \, g \, L^{-1}$ of NPK in each reactor.

A pH adjustment in the neutral pH range and pH monitoring was performed, as pH is an important factor that influences microalgal growth. The pH was adjusted using buffered solutions in each reactor, in triplicate, with the pH set at 6.5, 7.0 and 7.5, and results were compared to experiments without pH adjustment. Experiments were carried out in glass shake flask containers using a 12:12 h (light/dark) photoperiod and were inoculated with approximately 10% of inoculum (González-Barreiro *et al.* 2006; Zhang *et al.* 2011; Matamoros & Rodriguez 2016). Sodium hydroxide at $1 \, mol \, L^{-1}$ and $0.01 \, mol \, L^{-1}$ were used to adjust the pH value every 2 days.

The adjusted pH values did not present a significant difference in the percentage of bifenthrin removal in the first experiment. Therefore, the second set of experiments, which included a factorial experimental, was designed without pH adjustment. A two-factorial experimental design was carried out to evaluate the influence of two variables: percentage of inoculum (10 and 20) and photoperiod (12:12 and 18:6 h), without pH adjustment.

Analytical methodology

Biomass concentration

Total biomass was removed from the aqueous phase by centrifugation process, at 2,500 rpm for 15 min and rinsed with deionized water. The biomass concentration was estimated from dry weight measurements, up to constant weight, using an analytical scale. The biomass was dried, using an oven at 60 °C, to not cause compounds degradation.

Total carbon and nitrogen in biomass

Carbon and nitrogen content in biomass were determined using a CHNS Elemental Analyzer from Perkin Elmer

(2400 Series II CHNS/O System). CHN mode was used, with the combustion temperature set at 925 °C and the reduction temperature set at 640 °C. The calibration was performed before, respecting the k-factor stipulated by the manufacturer. The dry biomass was weighed in a range from 2 to 3 mg before starting the analysis.

Bifenthrin extraction

Bifenthrin concentrations were determined in dry biomass by adding 5 mL ethyl acetate to approximately 10 mg of dry biomass with sonication for 15 min. This extraction process was repeated three times. The extracts were evaporated using temperature under reduced pressure. Bifenthrin was determined through gas chromatography with mass spectrometry (GC-MS). The recovery of the extraction process was $96 \pm 4\%$.

An additional analysis was performed to remove bifenthrin residue that was deposited on the reactor (retained on glass). Ethyl acetate was added to the empty reactor to remove the bifenthrin. The extraction was performed in a shaker for 15 min (250 rpm). The procedure was repeated two times. The extract was evaporated at 60 °C. Bifenthrin concentration was determined through GC-MS. The extraction process was optimized by injecting each extract individually until concentrations below the limit of detection to determine the number of extractions and minutes needed to remove the compound.

All water samples (aqueous phase) were percolated through previously preconditioned SPE cartridges with 3 mL of methanol immediately after the experiment. The flow rate used was approximately one drop per second. Interferers were removed with deionized water and then air-dried. Bifenthrin was eluted with 3 mL of ethyl acetate and determined through GC-MS. The SPE extraction with ethyl acetate used for the stability studies yielded extraction recoveries of $98 \pm 2\%$ ($n = 3$) for bifenthrin at a concentration of $10 \, mg \, L^{-1}$.

Bifenthrin concentration was determined using a Shimadzu GC-2010 gas chromatograph coupled with a QP2010 Plus mass spectrometer. Analyses were performed with a DB-5MS ($30 \, m \times 0.25 \, mm \times 0.25 \, \mu m$) capillary column. Helium was used as the carrier gas at a flow rate of $1 \, mL \, min^{-1}$. The injection volume was $1 \, \mu L$ in split mode at 250 °C. The oven temperature was programmed as follows: conditioned at 100 °C; first ramp $25 \, °C \, min^{-1}$ to 250 °C; second ramp $3 \, °C \, min^{-1}$ to 260 °C, then 3 min at 260 °C. The electron impact (EI) source (70 eV) was maintained at 300 °C. The solvent delay was set to 5 min. The selected ion-monitoring mode (SIM) was set to the

bifenthrin most abundant fragment (181 m/z) to improve the sensitivity.

The linearity of the calibration curve was evaluated using Cochran's test ($\alpha = 0.05$), and the outlier presence was evaluated by Grubbs' test ($\alpha = 0.05$). For quantification purposes, a calibration curve for the concentration range from 0.1 to 25 mg L^{-1} was prepared in acetonitrile. The r-square value for the regression line was $r^2 = 0.999$. The determination was performed with six calibration points. The limit of detection (LOD) was determined as three times the signal-to-noise ratio (10 μg L^{-1}). The limit of quantification (LOQ) was determined as ten times the signal-to-noise ratio (33 μg L^{-1}).

Acute toxicity tests using the micro crustacean *Daphnia magna* were performed after the phycoremediation process in aqueous medium, without pH adjustment, following the Brazilian guideline. The EC50 of 48 h was estimated using the nonparametric statistical method of Trimmed Spearman-Karber, which corresponds to the concentration of a substance that causes adverse effects (immobility or mortality) on 50% of the test population after 48 h of exposure.

Statistical analysis

Statistical analysis was carried out using GraphPad InStat software (version 3, 1997). Cochran's and Grubbs' tests were evaluated for calibration curves ($\alpha = 0.05$). The results for each parameter were expressed as the mean ± standard deviation. Significant differences were determined by Kruskal-Wallis test with Dunn's posttest, and $p < 0.05$ was considered significant. The experiments were conducted in triplicate. For duplicate results, significant differences were determined by Mann-Whitney test.

Chemoface software (version 1.61, 2016) was used to evaluate the effect of the variables in the two-factorial experimental design, using a Pareto diagram. A 95% confidence interval was used ($p < 0.05$).

RESULTS AND DISCUSSION

Microalgae identification

Molecular identification of the main species was performed from the inoculum with the presence of microalgae reproduced from water reservoir. The species isolated and identified were *Lobochlamys segnis* (class Chlorophyceae) and *Parachlorella kessleri* (class Trebouxiophyceae). The medium was composed of a diversity of microalgae, of which *P. kessleri* was observed as the most predominant organism.

Biomass yield and percentage of remaining nutrients

Table 2 shows the results of dry biomass and elemental analysis. There was no significant difference ($p > 0.05$) between the samples with different pH values adjusted for these analyses, by buffered solutions.

In this experiment (Table 2), no biomass was observed in the controls. The blank (microalgae only) dry biomass content was 418.75 ± 119.73 mg L^{-1}, with no significant difference ($p > 0.05$) when compared to the samples. There was no significant difference ($p > 0.05$) between the four samples comparing the percentage of C, H and N. These results show similarities between the compositions of the biomass obtained with the different pH values.

Patidar *et al.* (2015) observed, in a lagoon rich in nutrients from industries, urban sewage and agricultural runoff, the biomass composition of microalgae during different seasons. During the rainy and winter seasons, the carbon percentages were 16.47 ± 0.02 and 12.31 ± 0.06, respectively. Nitrogen percentages were between 1.33 and 3.08 during the seasons, without fertilizer. These carbon results, when compared with the present study, were similar. Otherwise, the nitrogen percentages

Table 2 | Total biomass and percentage of nutrients remaining in biomass with pH monitoring and adjustment

Condition	Biomass (mg L^{-1})	Elemental analysis (CHN)		
		C (%)	H (%)	N (%)
Without pH adjustment	470.67 ± 203.87	17.63 ± 3.35	4.01 ± 0.48	10.00 ± 1.79
pH 6.5	500.00 ± 122.06	13.93 ± 5.28	3.33 ± 0.53	9.07 ± 1.25
pH 7.0	390.48 ± 115.00	10.53 ± 3.71	2.93 ± 0.29	9.32 ± 0.49
pH 7.5	295.24 ± 41.00	16.44 ± 2.38	3.14 ± 0.48	7.11 ± 1.21

$n = 3$; $p > 0.05$.

were higher. The difference could be explained due to the NPK concentration, approximately $1\,g\,L^{-1}$, added to the experiments.

Bifenthrin removal

Microalgal biomass has the ability to biosorb organic compounds. The amount of bifenthrin encountered in biomass ranged from 14 to 26% (Figure 1(a)), in samples with different pH values, and no significant difference was observed ($p > 0.05$).

The sorption of organic compounds is a passive process involving the partition of a chemical compound into the hydrophobic biomass. Based on the partition coefficient of bifenthrin (log K_{ow} 6) (National Center for Biotechnology Information 2020), it has hydrophobic characteristics, which explains the significant percentage of pesticide found in the biomass of the samples. González-Barreiro *et al.* (2006) showed that for terbutryn with an intermediate partition coefficient (log K_{ow} 3.49), this characteristic was favorable for removing the compound from the medium by the algal cells.

Samples with the presence of microalgae were compared to controls (absence of microalgae), to study the advantages of microalgae for the removal of pesticides from water, confirming the bifenthrin removal in the medium by the microalgae action. Bifenthrin degradation undergoes the action of other sources as shown in Figure 1(b) (controls), since there is no microalgae in this experiment and the pesticide degradation may involve other processes such as photolysis and still

be influenced by abiotic factors. The photodegradation was a process observed under simulated photoperiod conditions, stimulated bifenthrin degradation. It is understood that, factors such as concentration, solvent systems, time of irradiation, light source and light intensity can interfere with acceleration of photodegradation. Besides that, the photodegradation in aquatic systems is an excellent dissipation route of pesticides (Vryzas 2018).

On the other hand, there was a difference in degradation between the controls (Figure 1(b)) and the samples (Figure 1(a)) in the removal of bifenthrin. The percentage of degradation in the controls was 15–37% lower than the removal rates from samples in the same pH set.

Chen *et al.* (2012) conducted a study with bifenthrin in which no significant degradation was observed in the noninoculated control. The biodegradation of bifenthrin was in liquid medium, without light presence, using mineral salt medium as the nutrient solution. However, the experiments from our study used cool-white fluorescent lamps with approximately $20\,\mu mol\,m^{-2}\,s^{-1}$. This lamp emits some radiation, which likely contributes to bifenthrin degradation in control reactors. As the focus was the study of photosynthetic microorganism effect in the degradation, the light was essential. Table 3 shows that control 8 presents 27.33% more degradation than control 3, due to 6 h more exposure to the light during cultivation. In addition, photo-acclimation is a very important process to microalgae cultivation (Nie *et al.* 2020) and as indicated in this study. Therefore, we did not need to test the culture in the total absence of light, since there
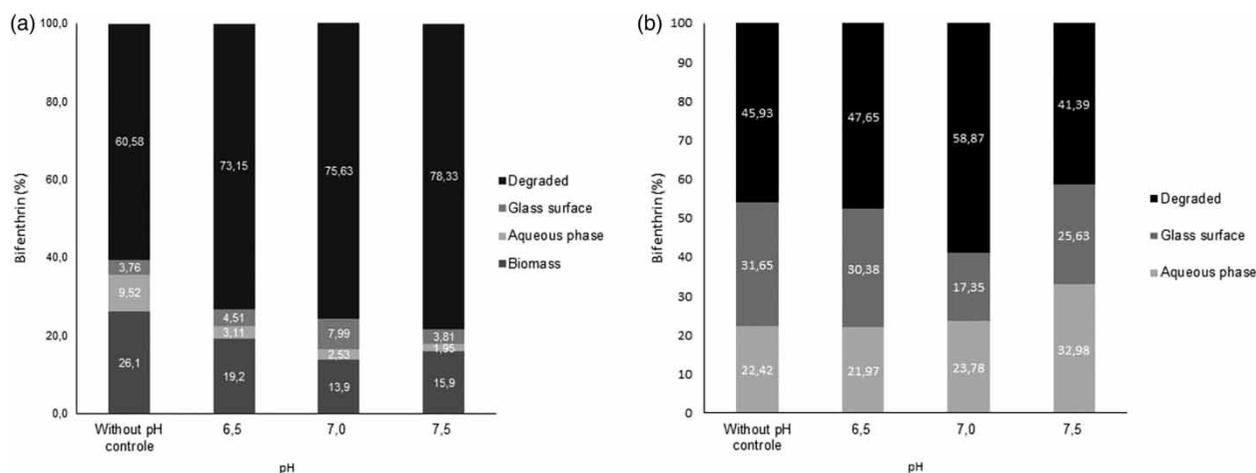

Figure 1 | Degradation levels of bifenthrin in samples (a) and controls (b) ($n = 3$ and $p > 0.05$).

Table 3 │ Bifenthrin factorial experimental design results

Samples	Photoperiod	Inoculum (%)	Biomass (mg L^{-1})	GC-MS bifenthrin (%) Biomass	Aqueous phase	Glass deposits	Degradation
Sample 1	12:12	10	212.50 ± 16.54	3.76 ± 0.71	0.87 ± 0.35	1.09 ± 0.10	94.28 ± 0.70
Sample 2	12:12	20	418.75 ± 43.75	4.79 ± 1.06	0.95 ± 0.28	1.54 ± 0.60	92.72 ± 0.56
Control 3	12:12	0	<LOD	<LOD	39.73 ± 1.51	9.96 ± 0.18	50.32 ± 1.49
Blank 4	12:12	10	210.00 ± 20.34	<LOD	<LOD	<LOD	<LOD
Blank 5	12:12	20	387.67 ± 34.87	<LOD	<LOD	<LOD	<LOD
Sample 6	18:6	10	341.67 ± 3.61	3.42 ± 1.05	0.90 ± 0.19	0.71 ± 0.03	94.96 ± 1.20
Sample 7	18:6	20	435.42 ± 23.66	2.90 ± 0.78	0.58 ± 0.30	0.33 ± 0.05	96.19 ± 0.76
Control 8	18:6	0	<LOD	<LOD	15.07 ± 2.69	7.28 ± 2.25	77.65 ± 1.24
Blank 9	18:6	10	339.00 ± 5.60	<LOD	<LOD	<LOD	<LOD
Blank 10	18:6	20	440.65 ± 17.56	<LOD	<LOD	<LOD	<LOD

$n = 3$; $p > 0.05$; LOD = limit of detection.

would be a high probability of a change in the metabolism of microalgae.

Pyrethroids are strongly adsorbed into the containers' walls, made from organic matter and used as collection and storage containers. Some factors, such as sample concentration, container material (glass) and exposure time can decrease the analyte loss (Albaseer *et al.* 2011). The quantity of bifenthrin deposited on the wall of the reactor differed between the controls and the samples. The percentage of microalgae and wall interaction found in the controls was 2–8 times greater than the samples in all media with different pH values. In the presence of microalgae, this interaction was smaller and concentration of pesticide was determined in the biomass.

The samples were compared to the controls in order to verify an advantage in the use of these microorganisms in the removal of bifenthrin from water. The results between the different pH conditions tested did not show significant differences ($p > 0.05$) for the comparison between controls (Figure 1(b)), and comparison between the samples (Figure 1(a)).

Based on the positive differences observed in the percentage of degradation (>60%), microalgae reproduced from a natural source increased the percentage of bifenthrin degradation. Bifenthrin was removed with a percentage above 85% in both the processes of biodegradation (liquid medium) and biosorption (biomass). A significant percentage present in the biomass is characteristic of log K_{ow} 6, showing great affinity for the organic phase.

Experimental design

Table 3 shows the results of dry biomass and percentage of bifenthrin determined after the phycoremediation process using a factorial experimental design, considering the percentage of inoculum (10 and 20%) and photoperiod (12:12 and 18:6 h) variables. In the controls, no biomass was observed, as expected. There was no significant difference ($p > 0.05$) in the biomass obtained between the blanks when compared to the samples in both photoperiods tested. As the inoculum increases, with a higher cell density and turbidity there is a limitation in the light passage (Nie *et al.* 2020), and consequently in the pesticide degradation.

The variables were tested and the effects were evaluated based on the bifenthrin degradation percentage, the Pareto diagram (Figure 2) shows that there was a significant difference ($p < 0.05$) in the photoperiod variable and in the combination of the two variables. However, with the inoculum variable, there was no significant difference ($p > 0.05$) between 10% and 20% added at the beginning of the phycoremediation.

In relation to the total biomass obtained (Figure 3), there was a significant difference ($p < 0.05$) in the inoculum and photoperiod variables and the combination of both. The highest effect observed was the inoculum percentage used. The effect was positive and showed that 20% of inoculum added at the beginning of the phycoremediation process increased the amount of biomass obtained at the end by 27% in the photoperiod 18:6 h and 100% in the photoperiod 12:12 h when compared with 10% of inoculum added.
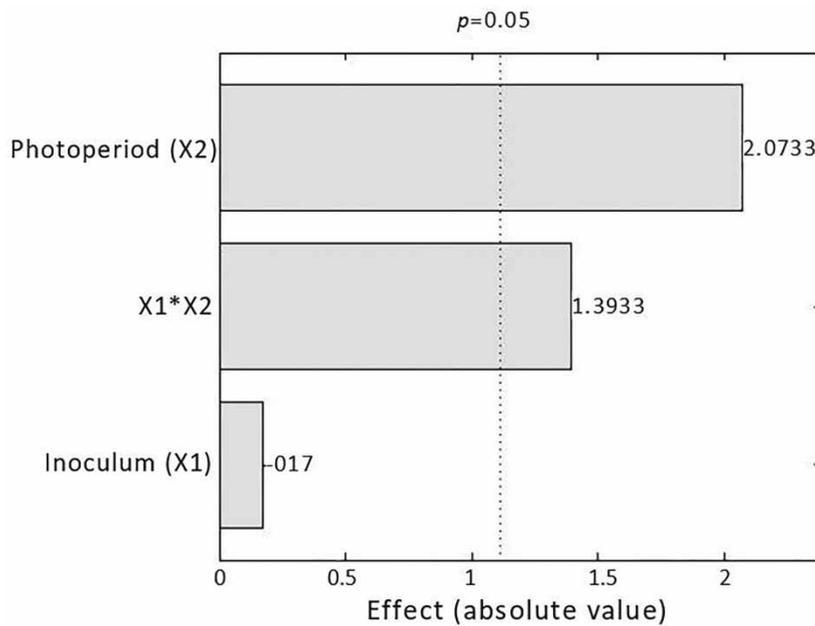
Figure 2 │ Pareto diagram of the effects of the photoperiod and inoculum variables on total biomass (mg L^{-1}).
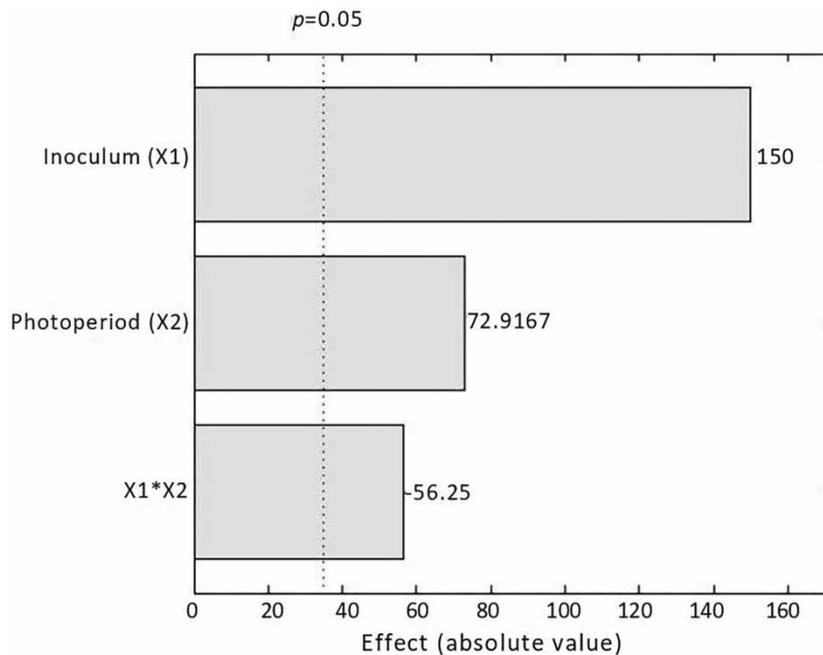

Figure 3 │ Pareto diagram of the effects of the photoperiod and inoculum variables on bifenthrin degradation (%).

In the presence of microalgae, bifenthrin had a high percentage of biodegradation, above 90%. The percentage present in the biomass ranged from 2.90 to 4.79% with all variables. Due to the high affinity for the organic phase (log K_{ow} 6), the low biosorption percentage may have occurred during the 20 days of incubation. Long days of incubation may increase the detachment of the compound in biomass. More bifenthrin quantified in biomass was expected.

Toxicity results

The acute toxicity was evaluated for blanks nonremediated (NRS) and remediated samples (RES), and the results indicated an EC50 of $1.06\,\mu g\,L^{-1}$ for NRS, which corresponds to a toxic condition. The RES showed a decrease in the toxicity by approximately 50%, with an EC50 of $2.04\,\mu g\,L^{-1}$ after 48 h. However, this value is within the range variation for toxic conditions, suggesting that the remaining bifenthrin was responsible for maintaining the toxicity in the treated sample. This probably is due to the presence of metabolites that may have been generated during the biodegradation process but were not identified in this study by GC-MS. Chen *et al.* (2012) identified the presence of five total metabolites of bifenthrin degradation; however, two of these metabolites were from the initial degradation: cyclopropane-carboxylic acid and 2-methyl-3-biphenyl methanol.

The literature shows different EC50 results from *D. magna* for bifenthrin pesticides with a high purity (>90%) if compared with the present study. Concentrations such as $0.86\,\mu g\,L^{-1}$ (Brausch *et al.* 2010) and $12.40\,\mu g\,L^{-1}$ (Ye *et al.* 2004) were evidenced. These studies evaluated bifenthrin at different concentrations and exposure times to verify the growth, reproduction and survival capacity of *D. magna*.

CONCLUSIONS

We identified, evaluated and controlled parameters that allowed the removal of the bifenthrin pesticide achieving acceptable removal percentages and higher percentages than those obtained for other pesticides under similar conditions. The set at pH values was not important for optimizing the efficiency of the remediation system.

Phycoremediation using native microalgae from natural source proved to be a promising process for bifenthrin removal, with the predominant microalgae species being *P. kessleri*, mediating the biosorption and biodegradation process. Bifenthrin showed a maximum percentage of removal up to 99% using 20% of inoculum at the beginning of the experiment with a photoperiod of 18:6 h and without pH adjustment. The photodegradation process also contributes to the bifenthrin removal rate. The controls (absence of microalgae) in the same condition showed a percentage of removal around 77%. The remediated samples showed a decrease in toxicity by approximately 50% compared to the nonremediated blanks; however, this value is within the range variation for toxic conditions.

REFERENCES

Albaseer, S. S., Mukkanti, K., Rao, R. N. & Swamy, Y. V. 2011 Analytical artifacts, sample handling and preservation methods of environmental samples of synthetic pyrethroids. *TrAC Trends in Analytical Chemistry* **30** (11), 1771–1780.

Ansari, F. A., Singh, P., Guldhe, A. & Bux, F. 2017 Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. *Algal Research* **21**, 169–177.

Ardal, E. 2014 *Phycoremediation of Pesticides Using Microalgae*. Available from: https://stud.epsilon.slu.se/7343/ (accessed 27 January 2020).

Brausch, K. A., Anderson, T. A., Smith, P. N. & Maul, J. D. 2010 Effects of functionalized fullerenes on bifenthrin and tribufos toxicity to *Daphnia magna*: survival, reproduction, and growth rate. *Environmental Toxicology and Chemistry* **29** (11), 2600–2606.

Carvalho, F. S., Fornasier, F., Leitão, J. O. M., Moraes, J. A. R. & Schneider, R. C. S. 2019 Life cycle assessment of biodiesel production from solaris seed tobacco. *Journal of Cleaner Production* **230** (1), 1085–1095.

Chen, S., Luo, J., Hu, M., Geng, P. & Zhang, Y. 2012 Microbial detoxification of bifenthrin by a novel yeast and its potential for contaminated soils treatment. *PLoS ONE* **7** (2), e30862.

Ding, T., Lin, K., Yang, B., Yang, M., Li, J., Li, W. & Gan, J. 2017 Biodegradation of naproxen by freshwater algae *Cymbella* sp. and *Scenedesmus quadricauda* and the comparative toxicity. *Bioresource Technolology* **238**, 164–173.

Dosnon-Olette, R., Trotel-Aziz, P., Couderchet, M. & Eullaffroy, P. 2010 Fungicides and herbicide removal in *Scenedesmus* cell suspensions. *Chemosphere* **79** (2), 117–123.

Gao, J. & Chi, J. 2015 Biodegradation of phthalate acid esters by different marine microalgal species. *Marine Pollution Bulletin* **99** (1–2), 70–75.

García de Llasera, M. P., León Santiago, M., Loera Flores, E. J., Bernal Toris, D. N. & Covarrubias Herrera, M. R. 2018 Mini-bioreactors with immobilized microalgae for the removal of benzo(a)anthracene and benzo(a)pyrene from water. *Ecological Engineering* **121**, 89–98.

González-Barreiro, O., Rioboo, C., Herrero, C. & Cid, A. 2006 Removal of triazine herbicides from freshwater systems using photosynthetic microorganisms. *Environmental Pollution* **144** (1), 266–271.

Grobbelaar, J. U. & Bornman, C. H. 2004 Algal biotechnology: real opportunities for Africa. *South African Journal of Botany* **70** (1), 140–144.

Hamouda, R. A. E. F., Sorour, N. M. & Yeheia, D. S. 2016 Biodegradation of crude oil by *Anabaena oryzae, Chlorella kessleri* and its consortium under mixotrophic conditions. *International Biodeterioration & Biodegradation* **112**, 128–134.

Li, T., Lin, G., Podola, B. & Melkonian, M. 2015 Continuous removal of zinc from wastewater and mine dump leachate by a microalgal biofilm PSBR. *Journal of Hazardous Materials* **297**, 112–118.

Li, L., Yang, D., Song, Y., Shi, Y., Huang, B., Yan, J. & Dong, X. 2017 Effects of bifenthrin exposure in soil on whole-organism endpoints and biomarkers of earthworm *Eisenia fetida*. *Chemosphere* **168**, 41–48.

Ligocki, I. Y., Munson, A., Farrar, V., Viernes, R., Sih, A., Connon, R. E. & Calisi, R. M. 2019 Environmentally relevant concentrations of bifenthrin affect the expression of estrogen and glucocorticoid receptors in brains of female western mosquitofish. *Aquatic Toxicology* **209**, 121–131.

Matamoros, V. & Rodriguez, Y. 2016 Batch vs continuous-feeding operational mode for the removal of pesticides from agricultural run-off by microalgae systems: a laboratory scale study. *Journal of Hazardous Materials* **309**, 126–132.

National Center for Biotechnology Information 2020 PubChem Database. Bifenthrin, CID = 5281872, Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Bifenthrin (accessed 28 January 2020).

Nie, J., Sun, Y., Zhou, Y., Kumar, M., Usman, M., Li, J., Shao, J., Wang, L. & Tsang, D. C. W. 2020 Bioremediation of water containing pesticides by microalgae: mechanisms, methods, and prospects for future research. *Science of The Total Environment* **707**, 136080.

Pamanji, R., Yashwanth, B., Bethu, M. S., Leelavathi, S., Ravinder, K. & Rao, J. V. 2015 Toxicity effects of profenofos on embryonic and larval development of Zebrafish (*Danio rerio*). *Environmental Toxicology and Pharmacology* **39** (2), 887–897.

Patidar, S. K., Mishra, S. K., Bhattacharya, S., Ghosh, T., Paliwal, C., Goel, S. & Mishra, S. 2015 Naturally floating microalgal mat for in situ bioremediation and potential for biofuel production. *Algal Research* **9**, 275–282.

Rousis, N. I., Zuccato, E. & Castiglioni, S. 2017 Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. *Environment International* **99**, 213–220.

Vryzas, Z. 2018 Pesticide fate in soil-sediment-water environment in relation to contamination preventing actions. *Current Opinion in Environmental Science & Health* **4**, 5–9.

Weston, D. P., Chen, D. & Lydy, M. J. 2015 Stormwater-related transport of the insecticides bifenthrin, fipronil, imidacloprid, and chlorpyrifos into a tidal wetland, San Francisco Bay, California. *Science of The Total Environment* **527–528**, 18–25.

Xiong, J. Q., Kurade, M. B., Abou-Shanab, R. A., Ji, M. K., Choi, J., Kim, J. O. & Jeon, B. H. 2016 Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate. *Bioresource Technolology* **205**, 183–190.

Ye, W. H., Wen, Y. Z., Liu, W. P. & Wang, Z. Q. 2004 Effects of bifenthrin on *Daphnia magna* during chronic toxicity test and the recovery test. *Journal of Environmental Sciences (China)* **16** (5), 843–846.

Zhang, S., Qiu, C. B., Zhou, Y., Jin, Z. P. & Yang, H. 2011 Bioaccumulation and degradation of pesticide fluroxypyr are associated with toxic tolerance in green alga *Chlamydomonas reinhardtii*. *Ecotoxicology* **20** (2), 337–347.