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ABSTRACT

The Chlorophyta *Picocystis* sp. isolated from a Tunisian household sewage pond appears promising for effective removal of Bisphenol A (BPA). Efficient and cost-effective technology for contaminants remediation relies on a tradeoff between several parameters such as removal efficiency, micro-organism growth, and its tolerance to contaminant toxicity. This article demonstrates the optimum conditions achieving the highest removal rates and the minimal growth inhibition in batch cul-tures of *Picocystis* using response surface methodology. A central composite face-centered (CCF) design was used to determine the effects on removal and growth inhibition of four operating parameters: temperature, inoculum cell density, light intensity, and initial BPA concentration. Results showed that the maximal BPA removal was 91.36%, reached the optimal culture conditions of 30.7 °C, 25 × 10^5 cells ml^{-1} inoculum density, 80.6 µmol photons m^{-2} s^{-1} light intensity, and initial BPA concentration of 10 mg l^{-1}. Various substrate inhibition models were used to fit the experimental data, and robustness analysis highlighted the Tessier model as more efficient to account for the interaction between *Picocystis* and BPA and predict removal efficiency. These results revealed how *Picocystis* respond to BPA contamination and suggest that optimization of experimental conditions can be effectively used to maximize BPA removal in the treat-ment process.

KEYWORDS Chlorophyta; bioremedi-ation; optimization; bisphenol A; central composite face-centered design (CCF); removal

Introduction

The sharp industrial evolution during the last decades entailed a huge increase in goods production and natural resource consumption. This trend resulted in an increase of pollutants discharged in natural ecosystems and consequently, a considerable change in the composition of the atmosphere, soil, and especially water was noticed (Kjellstrom *et al.* 2006; Earnhart 2013). Indeed, the high pollutant amount in open seas and water streams may disrupt the self-purifying system of natural water bodies affecting badly the proper functioning of the aquatic ecosystem (Whitehead and Lack 1982; Earnhart 2013). Among water pollutants, endocrine disruptors such as Bisphenol A (BPA) are one of the very common types (Pookpoosa *et al.* 2014).

BPA is an organic compound extensively used to produce epoxy resins and polycarbonate plastics. This chemical has only recently gained attention and became of great concern because of its toxicogenic properties. BPA is currently recognized as a xenoestrogen associated with many diseases among which cancer, diabetes, liver, kidney, and brain function disorders, early sexual maturation in females, obesity prevalence, and immune disorders (Rezg *et al.* 2014). According to recent studies, the worldwide annual production of BPA was estimated to 60,000 tons, from which about 100 tons are released into the environment (Inadera 2015), further raising concerns about exposure of humans and effects on ecosystems. In this regard, the effective removal of BPA from water has become mandatory to maintain public human health and to achieve environmental protection.

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A considerable amount of literature reported the efficiency of microalgae in BPA removal from aqueous solution in static conditions (Hirooka *et al.* 2005; Gattullo *et al.* 2012). However, successful application of this technology is often limited by environmental, physical, and chemical factors that affect microalgae growth and BPA fate, such as temperature, light intensity, pollutant toxicity, and inoculum cell density (Boopathy 2000; Hammed *et al.* 2016). Nowadays, a particular interest is given to the use of extremophilic microalgae in bioremediation systems (Varshney *et al.* 2015). Such species are assumed to have physiological adaptation and genetic modifications that allow them to tolerate harsh environmental conditions and high anthropogenic pollution and further could be more efficient in the fast contaminant removal from wastewaters (Seckbach *et al.* 2007; Varshney *et al.* 2015). Consequently, efficient bioremediation requires optimization of these factors to achieve the maximal BPA removal while maintaining the microalgae growth. Several methodologies have been proposed to define optimal parameters to achieve intended uses (Gupta *et al.* 2017).

The response surface methodology (RSM) is an efficient tool widely applied in the engineering process for optimization purposes (Mezhoud *et al.* 2014; Kermet-Said and Moulai-Mostefa 2015; Azzaz *et al.* 2017). Such methodology allows the identification of optimal conditions by considering the interactive effects of independent factors while minimizing the number of experiments required for a selected response. RSM has been successfully applied to define optimal yields of microbial consortia (Vasseur *et al.* 2012) for bioenergy purposes.

In our previous research, an extremophilic microalga belonging to the genus *Picocystis* sp., isolated from a Tunisian household sewage pond, has shown a great tolerance and removal efficiency regarding BPA (Ben Ouada *et al.* 2018). In the actual research, an experimental design through RSM was planned for the optimization of culture conditions aiming at increasing BPA removal efficiency by this Chlorophyta species. Four main variables namely temperature, light intensity, inoculum cell density, and initial BPA concentration were selected as factors that may potentially have an effect on algal growth and BPA removal. Kinetic modeling was carried out using various substrate inhibition models. Data from this study may contribute to system design approaches for improving BPA-containing water treatment and further could be incorporated into the pilot and full-scale applications.

Materials and method

Algal strains and culture conditions

The Chlorophyta *Picocystis* sp. CINS 23 was isolated from household sewage "Essed valley" located in Center East of Tunisia (35°59′23″N, 10°30′10″E). The strain has been deposited in the National Institute of Marine Sciences and Technology Collection (INSTM, Monastir, Tunisia). *Picocystis* was cultivated in batch culture under sterile conditions in Zarrouk medium (Zarrouk 1966) at optimal growth conditions temperature of 30 °C and light intensity of 75 µmol m^{-2} s^{-1} under 16/24-h illumination cycle as established by preliminary laboratory experiments (Ben Ali *et al.* 2017; Ben Ouada *et al.* 2018). Experimental cultures were carried out in photo-bioreactors consisting of 500 ml sterilized flasks containing 200 ml medium and equipped with a device for aseptic removal of samples. Experiments were conducted under controlled light in a temperature-programmable room with continuous illumination by Phyto-Claude halogen lamps (400 W). The intensity of incident light was measured using a Delta Ohm (Caselle di Selvazzano, Italy) light sensor HD 8366. For all designed experiments, exponentially growing cultures were inoculated at different initial cell densities (12, 24, and 36×10^5 cells ml^{-1}) and exposed separately to different temperatures (20, 30, and 40 °C), light intensities (20, 70, and 120 µmol photons m^{-2} s^{-1}) and BPA concentrations (10, 30, and 50 mg l^{-1}) as shown in Table 1. The range of each tested parameter was defined according to preliminary experiments carried out to determine the optimal conditions for *Picocystis* growth (unpublished data), in which results showed that *Picocystis* cells continued to grow under tested conditions (temperature and light intensity). This taxon has been previously shown to tolerate high temperature and a large range of light intensity and water salinity (Bernard *et al.* 2019; Pálmai *et al.* 2020). Blank controls, experiments without algae inoculum, were performed for the determination of the BPA abiotic removal.

Chemical test

A stock solution of 10 g l^{-1} BPA (Sigma–Aldrich) was prepared in anhydrous ethanol and properly preserved at 4 °C for further analysis. Dilutions to the desired concentrations were prepared aseptically in Zarrouk medium. Ethanol concentration in the medium including the control groups did not exceed 0.5% (v/v), which showed nontoxic effects on *Picocystis* sp.

Experimental design

A full factorial central composite face-centered (CCF) design was used in order to investigate the influence of the experimental factors and their interactions on BPA removal and microalgae growth. The CCF design was chosen because it provides relatively high-quality predictions over the entire design space and does not require using points outside the original factor range (Rajakumar *et al.* 2010). This design was used for the optimization of various treatment processes (Azzaz *et al.* 2017).

The independent variables selected for optimization were Temperature (X_1), light intensity (X_2), BPA concentration (X_3), and inoculum cell density (X_4). The independent variables were studied at three different levels chosen based on the available literature and preliminary experiments. The factors levels were coded as −1 (low), 0 (central point) and +1 (high) (Table 1). The responses were Y_1 (% of BPA removal) and Y_2 (Growth inhibition percentage). 27 experiments were carried out in this work, including three

Table 1. Factors level and design matrix selected for the study of growth inhibition and BPA removal by *Picocystis* using the central composite design approach.

Factors					Levels		
					Low (−1)	Central (0)	High (+1)
X_1: Temperature (°C)					20	30	40
X_2: Light intensity (µmol photons m^{-2} s^{-1})					20	70	120
X_3: Initial BPA concentration (mg l^{-1})					10	30	50
X_4: Initial inoculums cell density (10^5 cells ml^{-1})					12	24	36
Run	X_1	X_2	X_3	X_4		Y_1	Y_2
1	−1	−1	−1	−1		27.74	28.4
2	+1	−1	−1	−1		35.57	47.24
3	−1	+1	−1	−1		40.1	43.5
4	+1	+1	−1	−1		70.57	74.1
5	−1	−1	+1	−1		35.5	18.4
6	+1	−1	+1	−1		66.25	32.01
7	−1	+1	+1	−1		50.59	37.03
8	+1	+1	+1	−1		86.5	53.81
9	−1	−1	−1	+1		36.45	30.2
10	+1	−1	−1	+1		43.12	52.32
11	−1	+1	−1	+1		50.5	56.07
12	+1	+1	−1	+1		80.21	83.1
13	−1	−1	+1	+1		47.5	21.12
14	+1	−1	+1	+1		66.16	35.21
15	−1	+1	+1	+1		60.12	43.23
16	+1	+1	+1	+1		88.21	64.01
17	−1	0	0	0		22.27	54.24
18	+1	0	0	0		44.35	68.4
19	0	−1	0	0		24.9	51.23
20	0	+1	0	0		42.12	80.11
21	0	0	−1	0		5.42	92.12
22	0	0	+1	0		24.56	72.65
23	0	0	0	−1		19.54	69.14
24	0	0	0	+1		25.54	73.25
25 (C)	0	0	0	0		11.1	82.5
26 (C)	0	0	0	0		9.31	84.23
27 (C)	0	0	0	0		7.9	79.11

Y_1: growth inhibition percentage; Y_2: BPA removal percentage; (C): center point.

replicates at the central point to establish the experimental errors. The relationship of the independent variables and the responses were calculated by the second-order polynomial equation (Eq. (1)):

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} x_i x_j \qquad (1)$$

The coefficient values of Eq. (1) were calculated and tested for their significance. Model fitting and graphical simulations were obtained using Modde-11 software (Umetrics). Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). This analysis included Fisher's F test (overall model significance), its associated probability values, and the coefficient of determination R^2 which measures the goodness of fit of the regression model.

Growth inhibition test

Picocystis growth was followed spectrophotometrically by measuring the daily changes in OD 680 nm of both control and treated cultures during five days of BPA exposure. The control groups were cultivated in the optimal growth conditions at a temperature of 30 °C, a light intensity of 75 µmol photons m^{-2} s^{-1} initial cell density of 5×10^5 cells ml^{-1} and without BPA (as was optimized in the Lab).

The regression equation (Eq. (2)) between cell density (10^5 cell ml^{-1}) and OD_{680nm} was calculated as:

$$\text{Cell density } (10^5 \text{cells ml}^{-1}) = \text{OD}_{680\ \text{nm}} \times 30\ (R^2 = 0.99) \qquad (2)$$

Growth curves of the microalgae population were obtained by plotting cell density as a function of incubation time. The areas under each growth curve were evaluated using Origin 8.5 software (OriginLab, Northampton, USA). Inhibition of algal activity was expressed as the percentage inhibition (PI%) defined according to de Orte *et al.* (2013) by the following equation (Eq. (3)):

$$\text{PI\%} = \left[\frac{\text{Control area} - \text{Treated area}}{\text{Control area}}\right] \times 100 \qquad (3)$$

Where the Control area and Treated area are the integrated areas under the growth curve of control and treated culture, respectively.

BPA measurement

After 5 days of exposure, 150 ml of BPA-treated algal cultures were centrifuged at 10,000 × g for 10 min. The supernatant was filtered through pre-LC-MS 0.2 µm PTFE filters (Whatman, Puradisc) and used for the determination of the residual BPA in the medium. The same extraction procedure

Table 2. MRM parameters for BPA analyses.

Retention time (min)	Precursor ion (m z^{-1})	Collision energy (eV)	Capillary voltage (V)
1.36	227 > 212	20	25

was applied for media without algae cells exposed to the same culture conditions and under different BPA concentrations, to determine the abiotic removal of BPA.

BPA analysis was carried out using a Waters (Aquity UPLC) liquid chromatographic system coupled to a mass spectrometer detector (Quattro Premier; Micromass) equipped with an electrospray ionization source. Chromatographic separation was performed on a BEH-C18 chromatographic column (100 mm × 2.1 mm ID; 1.7 µm). LC elution was performed with 100% acetonitrile as mobile phase A and an ultrapure water 9:1 acetonitrile (v/v) as mobile phase B. Gradient elution consisted of a solution of 40% A started from 0 to 1.5 min, followed by an increase to 95% A in 3 min; held for 1 min; finally, from 5.5 to 7 min, mobile phase A decreased back to 40% and held for 3 min. Nitrogen was used as the collision and nebulizing gas. The injection volume was 5 µl and the flow rate was 0.4 ml min^{-1}. The temperature in the column compartment was set at 45 °C. The analysis was performed using ESI negative and BPA was detected and quantified using the multiple reaction monitoring (MRM) mode. Complete calibration curves were performed at the beginning and the end of the sample set, the mean slope value of these curves was used for BPA quantification. The detection limit (LOD) and the quantification limit (LOQ) were 2.131 and 6.458 mg l^{-1}, respectively. The retention time and MRM parameters are summarized in Table 2. The analytical device was controlled by Micromass MassLynx 4.1 software.

BPA removal percentage was calculated according to the following equation (Eq. (4)):

$$\text{BPA removal } (\%) = \frac{(\text{initial BPA concentration} - \text{Final BPA concentration})}{\text{Initial BPA concentration}} \times 100 \quad (4)$$

Results and discussion

Experimental design analysis

The application of statistical design strategy for screening and optimization of process parameters enables quick identification of the key factors and interactions between them with the least number of experiments (Mezhoud *et al.* 2014; Zili *et al.* 2015; Azzaz *et al.* 2017). In this study, a three-level, four factors central composite face (CCF) design was used to assess the effect of the experimental culture conditions on the BPA removal yields (Y_1 (%)) and *Picocystis* growth inhibition (Y_2 (%)). Four independent variables were included (Table 1): temperature (X_1; °C), light intensity (X_2; µmol photons m^{-2} s^{-1}), BPA concentration (X_3; mg l^{-1}), and inoculums cell density (X_4; 10^5 cells ml^{-1}). The design matrix and the corresponding responses are summarized in Table 1. The modeled BPA removal (Y_1) and the growth inhibition (Y_2) yields were highly variable, ranging between 5.42 and 88.21 and 18.4 and 92.12%, respectively. Consequently, the optimization process was proven important for further enhancement of BPA removal efficiency. By using multiple regression analysis (MLR), both BPA removal and growth inhibition yields were correlated with the four design factors using the following second-order polynomial equations.

$$\begin{aligned} Y_1\ (\%) = {} & 179.627 - (10.0901 \times X_1) - (0.978279 \times X_2) \\ & + (0.308682 \times X_3) - (1.59701 \times X_4) \\ & + (0.177335 \times X_1^2) + (0.00717341 \times X_2^2) \\ & - (0.00146621 \times X_3^2) + (0.0483578 \times X_4^2) \\ & + (0.00753375 \times X_1X_2) + (0.0121031 \times X_1X_3) \\ & - (0.0113698 \times X_1X_4) - (0.00178063 \times X_2X_3) \\ & + (0.000323959 \times X_2X_4) - (0.00342447 \times X_3X_4) \end{aligned} \quad (5)$$

$$\begin{aligned} Y_2\ (\%) = {} & -167.877 + (11.5699 \times X_1) + (0.83571 \times X_2) \\ & - (0.498857 \times X_3) + (2.62407 \times X_4) \\ & - (0.175883 \times X_1^2) - (0.00529533 \times X_2^2) \\ & + (0.00869168 \times X_3^2) - (0.0535648 \times X_4^2) \\ & + (0.00331625 \times X_1X_2) - (0.0104156 \times X_1X_3) \\ & + (0.00218232 \times X_1X_4) - (0.000454375 \times X_2X_3) \\ & + (0.00262188 \times X_2X_4) - (0.00159635 \times X_3X_4) \end{aligned} \quad (6)$$

To apprehend the importance of the estimated coefficients of the model's terms for Y_1 and Y_2, namely linear, square, and interaction patterns, probability tests were established. Results are presented in Table 3. A confidence level of 95% was set and the insignificant terms ($p > 0.05$) were excluded to further improve the fitting of the models.

Considering the latter results, the empirical equations could be simplified as:

$$\begin{aligned} Y_1\ (\%) = {} & 192.231 - (10.2624 \times X_1) - (1.01454 \times X_2) \\ & + (0.0138782 \times X_3) - (1.96231 \times X_4) \\ & + (0.17566 \times X_1^2) + (0.00710638 \times X_2^2) \\ & + (0.047942 \times X_4^2) + (0.00753375 \times X_1X_2) \\ & + (0.0121031 \times X_1X_3) \end{aligned} \quad (7)$$

Table 3. *p*-Values and coefficients (scaled and centered) of linear quadratic and interactive effects of the factors on growth inhibition (Y1) and BPA removal (Y2) percentage.

	Y_1		Y_2	
	Coeff. SC	*p*	Coeff. SC	*p*
Constant	13.5299	8.91821e−007	79.9211	1.36437e−017
X_1	11.6761	3.38128e−008	9.88945	3.76239e−009
X_2	10.3183	1.3323e−007	12.1572	3.47622e−010
X_3	7.53945	3.75564e−006	−7.19889	1.33201e−007
X_4	3.63611	0.00227419	3.04889	0.000572554
X_1X_1	17.7335	1.22564e−005	−17.5883	3.17566e−007
X_2X_2	17.9335	1.0971e−005	−13.2383	6.24064e−006
X_3X_3	−0.586482	0.817992	3.47667	0.0688018
X_4X_4	6.96352	0.0162524	−7.71333	0.000814661
X_1X_2	3.76688	0.00267761	1.65812	0.0348967
X_1X_3	2.42062	0.0322076	−2.08313	0.0113293
X_1X_4	−1.36437	0.197277	0.261878	0.713802
X_2X_3	−1.78063	0.100127	−0.454375	0.526935
X_2X_4	0.194375	0.849057	1.57313	0.043524
X_3X_4	−0.821873	0.426948	−0.383125	0.592786

X_1: temperature (°C); X_2: light intensity (μmol photons m^{-2} s^{-1}); X_3: initial BPA concentration (mg l^{-1}); X_4: initial inoculums cell density (10^5 cells ml^{-1}).

Table 4. Analysis of variance (ANOVA) for central composite design of growth inhibition and BPA removal percentage.

	Growth inhibition percentage					BPA removal percentage				
	DF	SS	MS	*F*	*P*	DF	SS	MS	*F*	*p*
Total	27	61310.4	2270.75			27	98134	3634.59		
Constant	1	46633.7	46633.7			1	86329.8	86329.8		
Total corrected	26	14676.7	564.489			26	11804.2	454.007		
Regression	9	14392.1	1599.12	95.5154	0.000	10	11673	1167.3	142.366	0.000
Residual	17	284.614	16.742			16	131.189	8.19928		
Lack of fit	15	279.47	18.6314	7.24382	0.128	14	117.622	8.40157	1.23858	0.534
Pure error	2	5.14407	2.57203			2	13.5665	6.78324		
	$n = 27$	$Q^2 = 0.950$				N = 27	$Q^2 = 0.970$			
	DF = 17	$R^2 = 0.981$				DF = 16	$R^2 = 0.989$			
		R^2 Adj. = 0.970					R^2 Adj. = 0.982			

Df: degrees of freedom; SS: sum of square; MS: mean square; *F*: test for comparing model variance with residual (error) variance; *p*: *p*-value.

$$\begin{aligned} Y_2\ (\%) = &-159.976 + (11.0263\ \times\ X_1) + (0.766452 \times X_2) \\ &- (0.0474759 \times X_3) + (2.31054 \times X_4) \\ &- \left(0.16595 \times X_1^2\right) - \left(0.004898 \times X_2^2\right) \\ &- \left(0.046666 \times X_4^2\ \right) + (0.00331625 \times X_1X_2) \\ &- (0.0104156 \times X_1X_3) + (0.00262188 \times X_2X_4) \end{aligned} \quad (8)$$

To have a better understanding of the statistical significance of the models and the difference between experimental and predicted data, an ANOVA test was performed, and results are presented in Table 4.

The results showed high determination coefficients ($R^2 > 0.98$ and adjusted $R^2 > 0.97$) and validity ($Q^2 > 0.5$) for both Y_1 and Y_2 responses. Besides, the predicted versus observed values plot's for growth inhibition (Figure 1a) and BPA removal percentage (Figure 1b) indicated clear linearity between the experimental data and those predicted by the models with only a small deviation amount around the mean. Furthermore, the lack of fit (LOF) was calculated in order to predict the non-suitability of the design of the experiment for fitting the experimental data. The LOF *p*-value was not significant (LOF > 0.05). These findings indicate a normal error distribution and a well-fitting of both elaborated designs to theoretical data.

Effect of the independent variables on Y_1 and Y_2

The effect plot (Figure 2) displays the change in the response when a factor varies from its low to its high level when all other factors are kept at their averages; a positive effect means that the factor has a synergistic effect on the responses and a negative one shows an antagonistic effect factor (Ermer and Miller 2005). In this plot, the effects are sorted from the largest to the smallest and only the significant terms are presented. It was noticed that the quadratic terms of temperature and light intensity were the most influencing terms with a positive contribution to growth inhibition and a negative contribution to BPA removal percentage. The significant quadratic terms indicate that the optimum point of the factor was inside the design boundary level. The linear terms of both variables showed also a high positive effect on both responses. Furthermore, the linear terms of BPA concentration and the quadratic term of cell density were less marked with a negative effect on BPA removal and a positive effect on growth inhibition percentage. The linear terms of inoculum density have a positive effect on both responses.

The interactive terms were the least significant for the two responses; X_1X_2 showed a synergic contribution in both Y_1 and Y_2, X_1X_3 had a negative effect on BPA removal but a positive effect on growth inhibition and X_2X_4 showed a positive effect on BPA removal.

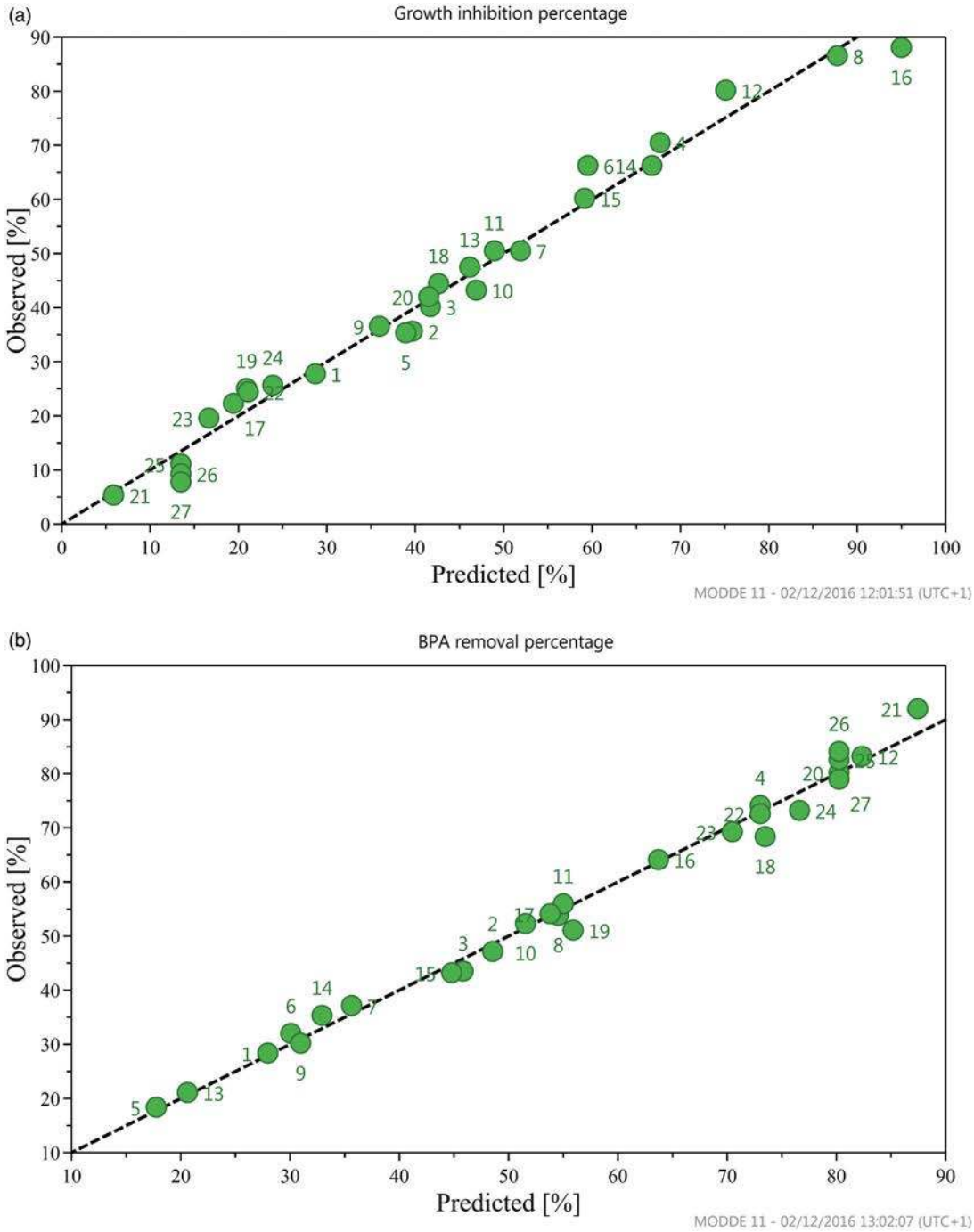

Figure 1. The experimental data versus the predicted data of normalized growth inhibition (a) and BPA removal percentage (b).

In order to investigate the interactions between the independent variables and their corresponding effects on the responses, response surface plots (graphical representations of the regression model) were drawn. The figures are based on Eq. (7) and Eq. (8) with two variables kept constant at their basic levels and varying the other two variables within the experimental range. The most relevant fitted response surface plots of BPA removal and growth inhibition versus significant variables are presented (Figure 3).

Figure 3 showed the combined effect of temperature and light intensity on BPA removal (a) and growth inhibition (b) percentage while keeping the algal cell densities and BPA concentration at zero-level. The graphical clearly depicted an optimum zone of BPA removal at a temperature between 30 and 36 °C and a light intensity between 78 and 112 µmol photons m^{-2} s^{-1}. These results are in agreement with the documented optimal light and temperature conditions for *Picocystis* species, either in culture (Pálmai *et al.* 2020) or in the environment (Bernard *et al.* 2019). It is well known that temperature and light intensity affect BPA removal by modifying its behavior and solubility in water solution. In fact, the increase in temperature could decrease the solution viscosity and thus promotes the mass transfer and diffusion of BPA molecules inducing an increase in its adsorption and removal efficiency (Kim *et al.* 2015). Likewise, the increase in light intensity may increase BPA

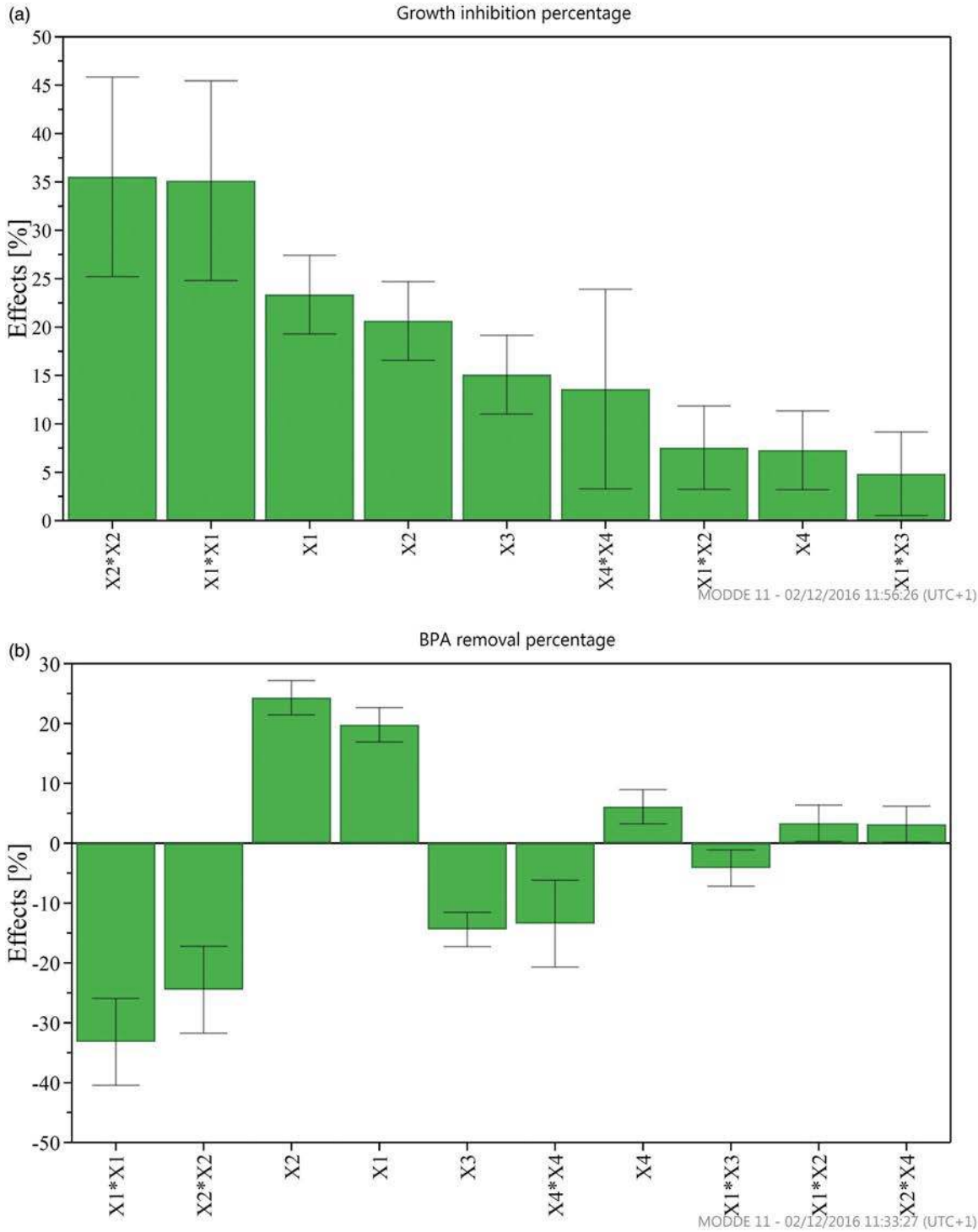

Figure 2. Effect plot for growth inhibition (a) and BPA removal percentage (b).

removal either by direct photolysis or oxidation through the increase in reactive species generated by the excessive amount of photons (Laoufi *et al.* 2008). BPA removal can be passive, by hydrophobic binding on microalgal cell walls (Endo *et al.* 2007) or to the extracellular polysaccharides, and active when accumulation and biotransformation/biodegradation occur within cells (Ben Ouada *et al.* 2018). In the present study, BPA removal did not show a monotonic relationship with temperature and light intensity; at high temperature and light intensity, the removal efficiency decreased. These results indicate that the BPA removal is not generated simply because of the chemical properties of BPA but also by biological effects related to the microalgae degradation processes (Zazouli *et al.* 2014). In part, some of these effects are related to the direct influence of temperature and light intensity on algal growth and photosynthesis. Indeed, as shown in Figure 3a, the PBA removal was maximal at temperature range 21–32 °C and light intensity range 30–82 µmol photons m^{-2} s^{-1}, which represent the optimal conditions for *Picocystis* growth. An increase in both factors

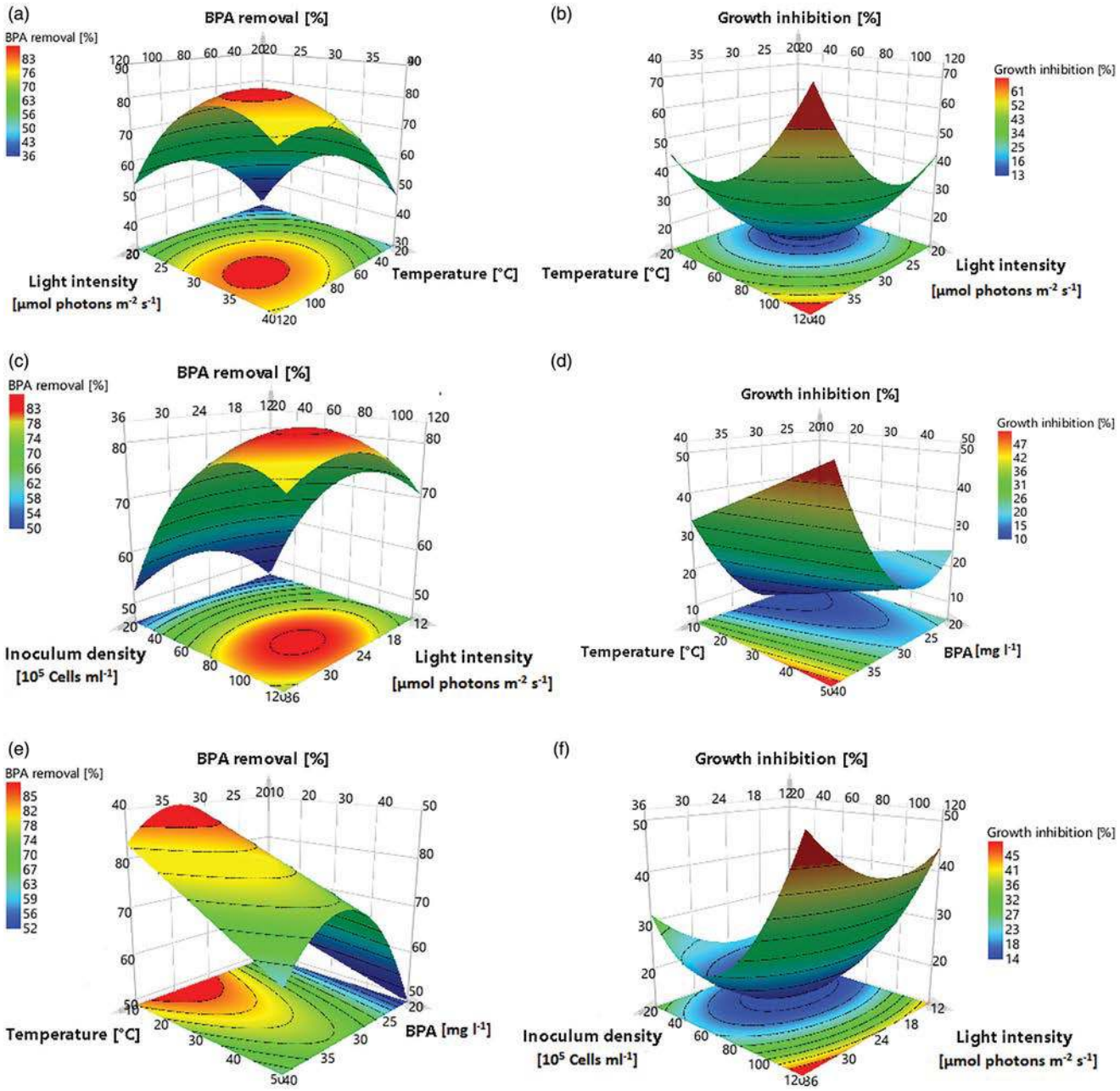

Figure 3. Response surfaces plot for the 24 central composite designs (a) X_1–X_2 (Y_2); (b) X_1–X_2 (Y_1); (c) X_2– X_4(Y_2); (d) X_1–X_3 (Y_1); (e) X_1–X_3(Y_2); (f) X_2–X_4 (Y_1).

beyond the optimal zone induced an increase in the growth inhibition (did not exceed 61%) and thus a decrease in the efficiency of microalgae to remove BPA.

Figure 3c shows the effect of initial BPA concentration and temperature on the growth inhibition percentage and the BPA removal efficiency. The optimal temperature for BPA removal was between 30 and 36 °C as earlier. However, a declining trend is noted with increasing BPA concentration in the experimental range; BPA removal took maximum value when BPA concentration was low (−1). This decrease may be correlated to the increase in growth inhibition percentage as seen in Figure 3d. The effect of BPA concentration on Y_1 and Y_2 was accentuated at high temperatures. This observation may be explained by the increase in BPA solubility and biodisponibility and consequently by its enhanced toxicity at high temperatures (Si *et al.* 2014).

The combined effect of inoculum cell density and light intensity, keeping the other factors at basic levels, is shown in Figure 3e. It is noticed from the plots that BPA removal exhibited maximum value when inoculum cell density was between 23 and 30×10^5 cells ml^{-1} and light intensity between 82 and 105 µmol photons m^{-2} s^{-1}. The decrease in BPA removal with increasing initial inoculum cell density could be attributed to the increase in growth inhibition percentage (Figure 3f). Indeed, the higher inoculum density involves a decrease in nutrient availability per microalgae cells which might be the reason for the increase in the growth inhibition percentage (López-Elías *et al.* 2011). Photooxidation of BPA during the incubation procedure is likely to be negligible, considering that this mechanism is induced by UV radiation and solar light (Rosenfeldt and Linden 2004; Garg *et al.* 2019).

Table 5. Optimizer run list including factors, responses, iterations, and Log (D).

X_1	X_2	X_3	X_4	Y_1	Y_2	Iter.	log(D)
31.7248	80.6454	10.0004	24.9867	91.3493	11.9841014	153	−0.3129
31.6895	80.6096	10.0006	25.1502	91.3502	11.9864401	146	−0.313
31.5256	80.8468	10.0006	25.4215	91.3144	11.9424251	154	−0.311
31.7443	80.6183	10.0009	24.9963	91.3597	12.0099038	168	−0.3132
31.625	80.966	10.0009	25.0585	91.3416	11.9759636	135	−0.3124
31.7161	80.4308	10.0012	25.2248	91.3539	11.9971505	160	−0.3132
31.625	80.966	10.0009	25.0585	91.3416	11.9759636	135	−0.3124
31.625	80.966	10.0009	25.0585	91.3416	11.9759636	135	−0.3124

X_1: temperature (°C); X_2: light intensity (µmol photons m^{-2} s^{-1}); X_3: initial BPA concentration (mg l^{-1}); X_4: initial inoculums cell density (10^5 cells ml^{-1}); Iter.: number of iterations; D: overall desirability.

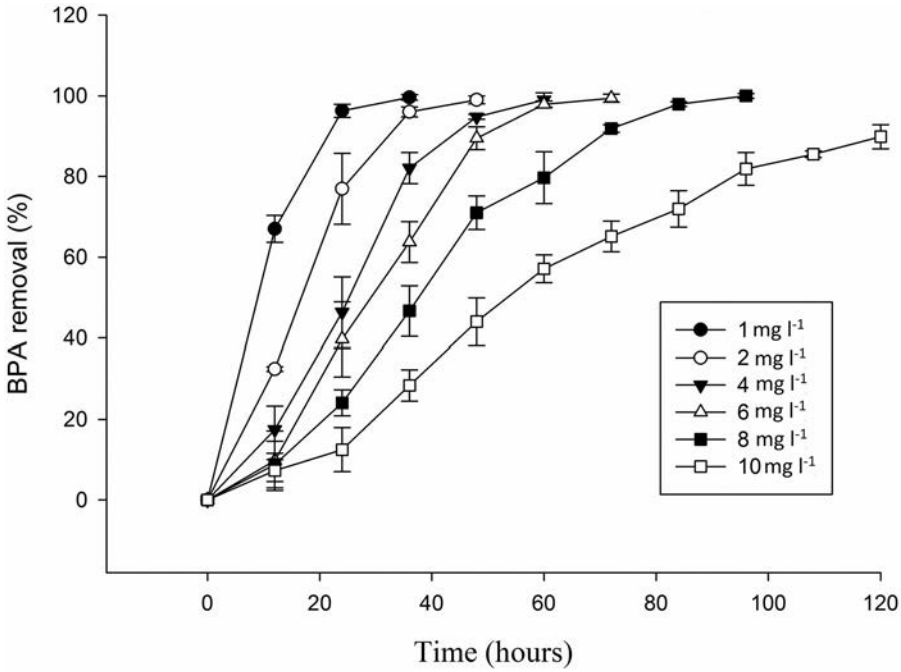

Figure 4. Variation of BPA removal percentage as a function of time at different initial BPA concentrations. Data presented as mean ± standard deviation.

Optimization

The optimal conditions for the BPA removal and microalgae growth according to the models were determined using the optimizer function of MODDE 11.0 software. The optimizer uses a Nelder Mead simplex method with the fitted response functions to optimize an overall desirability (D) function combining the individual desirability of each response (Nelder and Mead 1965; Kuhn 2009). Optimal conditions were characterized by maximizing BPA removal yields and keeping the growth inhibition percentage lower than 15%. The best overall solution generated by the D function predicted a maximum BPA removal of 91.36% with a growth inhibition of 12% when temperature, light, inoculum cell density, and BPA concentration were 30.7 °C; 80.6 µmol photons m^{-2} s^{-1}; 25×10^5 cells ml^{-1} and 10 mg l^{-1}, respectively (Table 5).

In order to ensure the feasibility and the validity of the model, an experiment was performed using the latter optimal settings and showed that BPA removal percentage after 5 days of exposure was estimated to 89.85% ± 2.98 (Figure 4) with a growth inhibition percentage lower than 15%. This result reveals good agreement with the value predicted from the model.

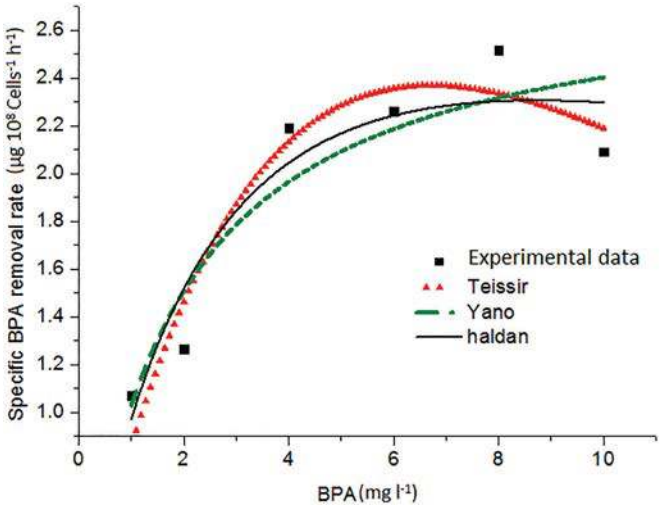

Figure 5. Comparison of various models with experimental values for BPA removal by *Picocystis* sp.

Kinetics study

At the optimized temperature light intensity and inoculums density, BPA concentrations were varied to study the effect of initial BPA concentration on the removal kinetics. As the

Table 6. Value of kinetic parameters obtained from substrate inhibition models of BPA removal by *Picocystis* sp.

Model	Equation	R^2	R_m (μg 10^{-8}cell^{-1} h^{-1})	K_s (mg l^{-1})	K_i (mg l^{-1})	K_1 (mg l^{-1})	K_2 (mg l^{-1})
Yano and Koga	$R_s = R_m \frac{S}{Ks+S+\frac{S^2}{K_1}+\frac{S^3}{K_2^2}}$	0.84	2.8215	1.73		3.74E + 17	−3.72E + 12
Teissier	$R_s = R_m\left[\exp\left(\frac{S}{K_i}\right) - \exp\left(\frac{-S}{K_s}\right)\right.$	0.92	5.846	3.966	1.27E + 01		
Haldane	$R_s = \frac{R_m \times S}{K_s+S+\frac{S^2}{K_i}}$	0.89	3.851	2.9157	26.073		

optimal BPA concentration was at the −1 level, the concentration range selected was 1–10 mg l^{-1}. Kinetics of BPA removal efficiency at different initial concentrations are shown in Figure 4. It was clear that the time taken by *Picocystis* to remove BPA depends on its initial concentration. For instance, 2 mg l^{-1} BPA took about 36 h to be completely removed, whereas only 89% of 10 mg l^{-1} were removed after 120 h. Moreover, the total removal duration may be divided into two phases: the initial lag phase and active removal phase. The extent of the two phases, in turn, depended on initial BPA concentration.

The specific BPA removal rate (μg 10^{-8} cells^{-1} h^{-1}) was calculated and plotted against BPA concentration as shown in Figure 5. As seen in Figure 5, the rate of BPA removal increased with initial concentration up to 8 mg l^{-1} and decreased at 10 mg l^{-1}. These results indicated typical substrate inhibition kinetics, thus the batch kinetic of BPA removal by *Picocystis* was fitted to three inhibition models namely Yano and Koga, Teissier and Haldane models (Andrews 1968; Yano and Koga 1969; Edwards 1970). The best fit model was selected based on the highest correlation coefficient (R^2). The fitting function builder tools in Origin 8.5 software was used to fit the models to the experimental data (Figure 5). The kinetics parameters of each model are presented in Table 6. From the results, it was found that the Teissier model fits the data well for BPA removal by *Picocystis* sp. ($R^2 = 0.92$).

Conclusions

BPA removal performance by an extremophilic microalgae strain isolated from Tunisian household sewage appears promising. Maximum degradation of 91.36% was obtained at the optimum conditions (30.7 °C; 80.6 μmol photons m^{-2} s^{-1}; 25 × 10^5 cells ml^{-1} and 10 mg l^{-1} BPA).

The process parameters namely light intensity, temperature, inoculum cell density, and initial BPA concentration were varied and optimized using RSM. To the best of the authors' knowledge, this work is the first that applied statistical design for BPA removal using microalgae species. The results evidenced that the optimization tool can be effectively used to maximize the bio-removal process, allowing valuable tradeoff between organism sensitivity and bioremediation capacity, taking into account the general need to consider the tolerance of the microorganism to chemical toxicity when addressing issues of microbial bioremediation.

The effect of initial BPA concentration on the removal kinetics was studied at the optimized conditions. Various substrate inhibition models like the Haldane model, Yano and Koga, and Teissier models were used to fit the experimental data. Tessier's model was found best suited to describe BPA removal efficiency according to culture conditions and inoculum density and could be implemented as an operational tool to estimate PBA removal by microalgae.

However, applying the microalgae bioremediation tool on a large scale needs more investigations, mainly related to operational costs estimation, including those relative to the harvesting techniques of microalga biomass collected from the treatment process.

Disclosure statement

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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