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Influence of photodegradation on the removal of color and phenolic compounds from palm oil mill effluent by *Arthrospira platensis*

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Abstract

Palm oil mill effluent (POME) released from conventional treatment systems poses severe environmental problems due to its dark color, its high chemical oxygen demand (COD), and high content of phenolic compounds. However, the possible biodegradation of phenolic compounds and color by microalgae was not well explored. This research aimed to reveal optimal conditions for pollutant removal through biodegradation by the cyanobacterium *Arthrospira platensis*. This species was grown under a range of POME fractions and environmental conditions (irradiance, salinity, nutrients) during which growth, final biomass, color, COD, and phenolic compound levels were followed. POME fractions influenced *A. platensis* growth rate, final biomass, COD, and color removal. The optimization of phenolic compound removal by using central composite design (CCD) response surface methodology (RSM) showed that low light and high initial phenolic compounds promoted the activity of *A. platensis* to degrade phenolic compounds. The combination of high initial phenolic compounds and high light intensity increased the growth rate up to 0.45 days⁻¹ and final biomass up to 400 mg L⁻¹, while total phenolic compounds were almost completely (94%) removed. Finally, this study showed that phenolic compounds and color degradation from POME were dominated by the activity of photodegradation at high irradiance, while the activity of *A. platensis* dominated at low light intensity.

Keywords Biodegradation · POME · *Arthrospira platensis* · Phenolic compounds · Color

Introduction

Indonesia is the largest palm oil producer in the world. About 600–870 kg of palm oil mill effluent is generated when processing a tonne of fresh palm fruits. Direct discharge of untreated POME into aquatic environments may cause problems. When POME is released from the palm oil mills, its characteristics are the following: temperature ranges between 70 and 80 °C, pH is low (between 4 and 5), it is highly colloidal, it has high chemical and biological oxygen demand, and a high concentration of phenolic compounds which give it a particular deep reddish to

brown color (Chaijak et al. 2017). Regulatory standards for POME aim to reduce the wastewater discharges taking chemical oxygen demand (COD) and biological oxygen demand (BOD) as key parameters. However, after reaching regulatory standard parameters, the effluent still has a strong smell, high levels of phenolic compounds, and a dark color that could discomfort the local community and the environment (Igwe and Onyebado 2007; Poh et al. 2010). Physical treatments using membrane systems such as ultrafiltration and nanofiltration are able to remove the color completely (Ahmad et al. 2006; Amat et al. 2015). However, the investment cost for the membrane filtration system could be too high for a medium to small industry. Furthermore, in conventionally treated aerobic POME, the phenolic compounds can reach concentrations between 280 and 680 mg L⁻¹ and are dominated by gallic acid, followed by p-hydroxybenzoic acid, protocatechuic acid, caffeic acid, syringic acid, and vanillic acid (Chantho et al. 2016). Previous research has shown that the maximum toxicity concentrations for phenolic compounds range between 10 and 24 mg L⁻¹ for humans and between

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9 and 25 mg L⁻¹ for fish (Kulkarni and Kaware 2013). Discharging traditionally treated POME into rivers could thus pose a high environmental risk for the surrounding area. Therefore, additional treatments are needed to eliminate the phenols.

In general, physiochemical and biological methods could be applied to remove phenolic compounds from the wastewater (Mohammadi et al. 2015; Pradeep et al. 2015). Nowadays, wastewater treatment using microalgae is preferable compared to yeast and bacteria since the biomass produced has potentially higher economic value (Surkatti and Al-Zuhair 2018; Nur and Buma 2019). Several strains of microalgae have the capability to utilize phenols as energy sources as reviewed recently (Surkatti and Al-Zuhair 2018; Lindner and Pleissner 2019). Several species such as *Anabaena variabilis*, *Chlorella* sp., and *Scenedesmus obliquus* have shown good efficiency in removing phenolic compounds from wastewater by utilizing it as an organic carbon source in the presence of light (Naoki et al. 1979; Klekner and Kosaric 1992; Scragg 2006; Papazi et al. 2019). It has been reported that phenol degradation involves phenol monooxygenase which converts phenol into catechol, followed by further metabolization processes (Semple and Cain 1996). Lee et al. (2015) found that *Arthrospira maxima* could remove 400 mg L⁻¹ phenol through biodegradation rather than bioadsorption processes. Several factors such as the type and concentration of the phenolic compounds could influence the rate and efficiency of the removal (Surkatti and Al-Zuhair 2018; Priyadharshini and Bakthavatsalam 2016; Hirooka et al. 2003). Yet, earlier results did not consider phenol removal by photodegradation, although it had been shown that light could contribute to phenolic compound degradation (Lika and Papadakis 2009). In contrast, earlier research found that photodegradation activity also contributed to the antibiotic tetracycline in a domestic wastewater treatment system, using microalgae in the light (Norvill et al. 2017).

Previous research has demonstrated the removal of COD and color by *Chlorella sorokiniana* by varying POME fractions (Haruna et al. 2018). However, these results did not include the potential role of photodegradation. The presence of photodegradation and microalgae has been found to be important for the degradation of phenolic compounds (Norvill et al. 2017). Nevertheless, the growth of microalgae could also be affected by the light intensity. The relationship between photodegradation and microalgal activity was not well explored so far. Therefore, in the present study, factors affecting phenolic compound and POME color removal were considered by including photodegradation and microalgal activity by varying the initial phenol concentration in POME, irradiance, POME fractions, external nitrogen addition, and salinity. We hypothesized that at high irradiance, phenolic compound and color would be more degraded by photodegradation activity, while at low light irradiance,

A. platensis would relatively be more active degrading phenol and color. Gallic acid was varied and chosen as external phenolic compound since it is the most abundant phenolic compound in POME (Chantho et al. 2016). *Arthrospira platensis* was chosen since it can be easily cultured in outdoor and large-scale facilities. Furthermore, information on the ability of *A. platensis* to degrade phenolic compounds is lacking. At the same time, this species synthesizes high value-added compounds such as phycocyanin (Sukumaran et al. 2018; Nur et al. 2019a). The aim of this research was therefore to explore and optimize the utilization of *A. platensis* to remove color and phenolic compounds from POME by varying nutritional and environmental conditions. A range of control experiments (without microalgae) was done, to unravel the contribution of photodegradation to the removal processes under consideration.

Materials and methods

Wastewater preparation

Treated POME was obtained from a small factory in Sumatra, Indonesia, as used previously (Nur et al. 2019b). POME was stored at -20 °C to avoid degradation over time. Prior to experimental use, POME was thawed and filtered using GF/C glass fiber filter (Whatman) to remove suspended solids followed by autoclaving (121 °C, 15 min). Based on an earlier study, the pretreatment process (filtration and autoclave) reduced 22% and 20% of color and COD, respectively, compared to raw POME (Nur et al., 2019). Filtered and autoclaved POME contained 1425 mg L⁻¹ COD, 56 mg L⁻¹ total dissolved nitrogen, 6.93 mg L⁻¹ dissolved PO₄³⁻P, 240 mg L⁻¹ total phenolic compounds, and 3600 ptco color as estimated by spectrophotometry using appropriate assay kits LCK 318, LCK349, LCK138 (Hach Lange) (Nur et al. 2019b), and Folin-Ciocalteu reagent (Sigma-Aldrich, Netherlands).

Experimental setup

Arthrospira platensis (SAG 21.99) was from the Culture Collection of Algae of the University of Göttingen (SAG, Germany). The culture was grown and maintained in Zarrouk medium (Zarrouk 1966), at a temperature of 27 °C and an irradiance of 150 μmol photons m⁻² s⁻¹ in a 16:8 h light/dark cycle. Three step-wise experiments were executed: the first experiment was aimed to study the effect of POME fraction (in ultrapure water, Milli-Q) on *A. platensis* growth rate, final biomass, POME color, phenolic compounds, and chemical oxygen demand (COD) removal, without adding external nutrients; the second experiment was done to study the most influencing factor (POME fraction, salinity, irradiance level, and nitrate concentration) as well as their possible

interactive effects on biomass productivity and total POME color removal by using a factorial design; the third experiment was aimed to examine the interactive effect of irradiance level and initial phenol concentration on growth, final biomass, and phenol removal by *A. platensis* using central composite design (CCD) response surface methodology (RSM). The cultures were acclimated to the experimental conditions for at least 1 week prior to experimentation.

Effect of POME

Arthrospira platensis was cultured in duplicate in 100 mL sterilized Erlenmeyer flasks with 75 mL working volume. The experimental temperature was set at 27 °C by placing the flasks in a U-shaped water bath, and cultures were illuminated by a steady light source (Osram Biolux L 36W/965) which was placed in an U design, in a 16:8 h light/dark cycle as described previously (Nur et al. 2019b).

Five percent (v/v) of culture inoculum, equal to 50 mg L⁻¹ dry weight, was added to five different dilutions of POME in ultrapure water (10–100% v/v), in triplicate. Since natural 100% POME has 4 PSU salinity, the final salinity for each POME dilution below 100% was set to 4 PSU by adding NaCl. Furthermore, the initial pH was set to 9.0 ± 0.2 by using 2 N HCl or 2 N NaOH since *A. platensis* grows well at high pH. Initial light intensity was set to a saturating level of 200 μmol photons m⁻² s⁻¹ for each POME condition, as measured in the center of the 100 mL sterilized Erlenmeyer culture flasks (75 mL working volume), using a spherical light sensor (Biospherical Instrument QSL2101, USA). The light penetrating into the flask was adjusted by using 1–4 layers of black neutral density screen since the turbidity of POME was different for each fraction. Every day, the optical density of the cells was measured at 750 nm to determine the growth rate. At the beginning of the incubations, the control (without *A. platensis*) was analyzed to determine the initial phenol, color, and COD concentration (see analysis section below). At the end of the incubation (6 days), final biomass in the culture was determined by centrifugation followed by spectrophotometric detection (see below), whereas COD concentration, color, and phenolic compounds were determined in the supernatant (see analysis section below). To follow photodegradation of POME over time, triplicate control experiments were done by incubating 10–100% POME without adding *A. platensis* in the same experimental setup.

Effects of nutritional and environmental conditions

Arthrospira platensis was cultured in 75 mL working volume in 100 mL sterilized Erlenmeyer flasks in a water bath as described above. Five percent (v/v) of *A. platensis* culture was used as inoculum to the medium as described above. Full factorial design with four variables (POME, salinity,

irradiance, nitrate) was performed to reveal the influencing factors and the possible interaction between these factors with respect to POME color removal by *A. platensis* (Table 1). The experiments were carried out at 27 °C, and the initial pH was adjusted to 9.0 ± 0.2 by using 2 N HCl or 2 N NaOH. Salinity was adjusted using ultrapure water (Milli-Q), NaCl, or natural filter sterilized sea water. At the beginning of the experiment, the control (without *A. platensis* inoculum) was analyzed to determine the initial color. At end of the exponential growth phase (7–10 days), the cultures were centrifuged, and the supernatant was kept to determine color. The biomass measurement was done at the beginning and the end of cultivation by using spectrophotometry. An additional control experiment was done by incubating POME without adding *A. platensis* in order to follow photodegradation of POME over time within the same experimental setup. Two replicates were done for the experiment.

Effect of light and initial phenolic compounds

To understand the interaction between light intensity and phenol addition on growth, final biomass, and phenol removal, different light intensities and concentrations of external gallic acid were applied following the CCD RSM approach. The addition of gallic acid was chosen because this phenolic substance dominates in POME (Chantho et al. 2016), while light intensity was chosen since it was the most influencing factor based on previous experiments (“Effects of nutritional and environmental conditions” section). A fixed POME fraction of 50% was used in this experimental series since, based on experiment 1, the final biomass was not significantly lower than 100% POME. Based on a preliminary experiment, color and phenolic compounds showed a strong correlation ($R^2 = 0.95$) (Supplementary Material 1). Therefore, different initial phenolic concentrations in POME combined with different light intensities were applied to understand the interaction of these factors. A total of 13 experimental runs were performed (Table 3). The ranges used for these runs were 8.6–290 mg L⁻¹ for gallic acid (x_1) and 80–220 μmol photons m⁻² s⁻¹ for initial light intensity (x_2). The empirical form of the second-order polynomial model (Eq. 1) can be described as:

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

where y is the predicted value; β_0 , β_i , β_{ii} , and β_{ij} are a constant, linear, quadratic, and the interaction coefficient, respectively; and x_i and x_j are independent variables of the model.

Cultivation was carried out in the same setup as the first experimental series. Fifty percent of POME (diluted with ultrapure Milli-Q) was mixed with gallic acid, stirred for 4 h at 1500 rpm at room temperature on a magnetic stirrer (Stuart SD162, England). The initial pH was adjusted to

Table 1 Factorial design experiment

Run	POME (%)	Salinity (PSU)	Light intensity ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	Nitrate (mg L^{-1})
1	50	15	50	0
2	50	15	50	100
3	50	30	50	0
4	50	30	50	100
5	100	15	50	0
6	100	15	50	100
7	100	30	50	0
8	100	30	50	100
9	50	15	200	0
10	50	15	200	100
11	50	30	200	0
12	50	30	200	100
13	100	15	200	0
14	100	15	200	100
15	100	30	200	0
16	100	30	200	100

9.0 ± 0.2 by using 2 N HCl or 2 N NaOH. Salinity was adjusted to 20 PSU as optimum level by using NaCl based on previous results (Nur et al. 2019a). External urea (800 mg L^{-1}) was added to the medium to avoid nitrogen limitation based on previous experiments (Nur et al. 2019a). Every day, the optical density of the culture was measured at 750 nm to determine the growth rate. At the end of the exponential phase (5 days), the cultures were harvested, and the filtrate was stored to determine phenol removal efficiency. Additional measurements were done to determine the correlation of initial total phenols and initial total color in POME (Supplementary 1). Two additional control experiments were executed: in the first control experiment, 50% POME was incubated without adding *A. platensis* (Table 3), in order to follow photodegradation of POME over time under the experimental conditions applied (initial and final sampling only). A second control experiment was done to understand the evolution of color degradation over time for a range of initial phenolic compounds, by performing daily sampling (Supplementary 2). Two replicates were done for the experiment.

Analytical methods

Growth rate and biomass measurement

Every day, 3 mL of sample was taken and centrifuged at 10,000 rpm for 15 min, after which the pellet was resuspended in 3 mL of ultrapure water (salinity 4 PSU). This

process was repeated twice to avoid color interferences between POME and *A. platensis* absorption. Growth rate was determined by plotting the relationship between time (days) versus cell absorbance of the natural logarithm of $\text{OD}_{750\text{nm}}$. The specified wavelength (750 nm) was chosen to avoid the interference of *A. platensis* pigments (Griffiths et al. 2011). Growth rate was determined by the slope of the linear regression analysis.

Dry biomass was measured indirectly based on optical density as described previously (Nur et al. 2019a). Since POME may strongly interfere with the *A. platensis* absorption measurements, samples (3 mL) were taken and centrifuged at 10,000 rpm for 15 min to separate the algal biomass from the POME medium. The pellet was washed twice using 0.75% NaCl and resuspended in 3 mL 4 PSU.

Biomass productivity was calculated as described previously (Nur et al. 2019a) following Eq. 2:

$$P_x = \frac{X_t - X_0}{t} \quad (2)$$

where P_x is biomass productivity $\text{mg L}^{-1} \text{ day}^{-1}$, X_t is final biomass (mg L^{-1}), X_0 is initial biomass (mg L^{-1}), and t is total duration of cultivation (day).

Determination of COD

For COD analysis, samples (3 mL) were taken and centrifuged at 10,000 rpm for 15 min to remove *A. platensis* cells. Two milliliter of the sample (diluted with ultrapure water if necessary) was carefully pipetted to a tube containing reagent LCK314 (Hach, Netherland). This dilution was also used to lower salinity due to the interference of salt during the COD analysis. The tube was gently inverted and then hydrolyzed to a temperature block (Hach LT 200) at 148°C for 120 min. After cooling to room temperature, the COD value in the tube was read based on spectrophotometry (Hach, DR3900).

COD removal efficiency was calculated using Eq. 3:

$$\text{TCOD} (\%) = \frac{\text{COD}_0 - \text{COD}_t}{\text{COD}_0} \times 100 \quad (3)$$

where TCOD is total COD removal (%), COD_0 is COD of the sample at day 0 of cultivation (mg L^{-1}), and COD_t is COD of the sample at the end of cultivation (mg L^{-1}). The calculation of COD removal by *A. platensis* (COD_s) and the control (COD_p) were also derived from Eq. 3.

COD removal by *A. platensis* was corrected by subtracting total COD removal (TCOD) with removal of the COD from control as stated in Eq. 4:

$$\text{COD}_s = \text{TCOD} - \text{COD}_p \quad (4)$$

where COD_s is COD removal efficiency as a result of activity

from *A. platensis* (%) and COD_p is COD removal efficiency from the control expressing photodegradation activity (%).

COD removal in absolute value was calculated from Eq. 5:

$$ACOD = COD_0 - COD_t \tag{5}$$

where ACOD is absolute value of COD removed by total activity ($mg\ L^{-1}$), COD_0 is COD at the first day of cultivation ($mg\ L^{-1}$), and COD_t is COD at the end of cultivation ($mg\ L^{-1}$). Equation 5 was also applied for control as expressed of photodegradation activity and *A. platensis* activity.

Absolute value of COD removed by *A. platensis* was corrected by subtracting the absolute total value of COD from control expressing photodegradation following Eq. 6:

$$ACOD_s = ACOD - ACOD_p \tag{6}$$

where $ACOD_s$ is the concentration of COD removed by *A. platensis* activity ($mg\ L^{-1}$), $ACOD$ is the concentration COD removed by total activity ($mg\ L^{-1}$), and $ACOD_p$ is the concentration of COD from control expressing photodegradation activity ($mg\ L^{-1}$).

Determination of color removal

Color measurements were done following Hach (2014) which is suitable for water, wastewater, and seawater samples. Samples (2 mL) were taken and centrifuged at 10,000 rpm for 15 min to remove *A. platensis* cells. The supernatant was then diluted in a ratio 1:3 by using ultrapure water and measured using a spectrophotometer (Hach Lange DR 3900) at 455 nm. A platinum cobalt standard solution (PtCo) was used to prepare a standard curve of measurements at 455 nm (spectrophotometer, program 120). Measurements were recorded in PtCon units.

Color removal efficiency was calculated using (Eq. 7):

$$TC = \frac{C_0 - C_t}{C_0} \times 100 \tag{7}$$

where TC is total color removal (%), C_0 is the color of the sample at day 0 of cultivation, and C_t is the color of the sample at the end of cultivation. The calculation of color removal by *A. platensis* (TC_s) and the control, representing photodegradation only (TC_p), was also applied from Eq. 7.

Color removal by *A. platensis* was corrected by subtracting total removal with removal of the color from photodegradation activity following Eq. 8:

$$TC_s = TC - TC_p \tag{8}$$

where TC_s is the color removal efficiency as a result of activity from *A. platensis* (%) and TC_p is the color removal efficiency from the control as a result of photodegradation activity (%).

Color removal in absolute values was calculated from Eq. 9:

$$ATC = TC_0 - TC_t \tag{9}$$

where ATC is the absolute value of color removed by total activity (ptco), TC_0 is the color at the first day of cultivation (ptco), and TC_t is the color at the end of cultivation ($mg\ L^{-1}$). Equation 9 was also applied for control expressing photodegradation activity and *A. platensis* activity.

The absolute value of color removed by *A. platensis* was corrected by subtracting the absolute total value of color from control expressing photodegradation, following Eq. 10:

$$ATC_s = ATC - ATC_p \tag{10}$$

where ATC_s is the concentration of color removed by *A. platensis* activity (ptco), ATC is the concentration color removed by total activity (ptco), and ATC_p is the concentration of color removed from control expressing photodegradation activity (ptco).

Determination of phenolic compounds

Total phenolic compounds were determined by applying minor modifications of the Folin-Ciocalteu method as described previously (Ergül et al. 2011; Chantho et al. 2016). First, samples were centrifuged at 10,000 rpm for 15 min to eliminate *A. platensis*. Then, 200 μ L of the sample (diluted with ultrapure water if necessary) was placed in a 15-mL dark conical tube and mixed with 1 mL Folin-Ciocalteu phenol reagent (Sigma-Aldrich, Netherlands) (4-fold dilution with ultrapure water). After 5 min, 1 mL of a saturated sodium carbonate solution ($200\ g\ L^{-1}$) was added, vortexed at 2500 rpm for 10 s, and left for 30 min at room temperature. The absorbance was measured spectrophotometrically (Hach, 5200, Netherland) at 725 nm. A calibration curve of gallic acid was used as a standard, with 5 set points in the range 0–850 $mg\ L^{-1}$.

Phenol removal efficiency (TP) was calculated using Eq. 11:

$$TP = \frac{P_0 - P_t}{P_0} \times 100 \tag{11}$$

where TP is total phenol removal (%), P_0 is phenol of the sample at day 0 of cultivation ($mg\ L^{-1}$), and P_t is the concentration of phenol in the sample at the end of cultivation ($mg\ L^{-1}$).

Phenol removal by *A. platensis* was corrected by subtracting total phenol removal with phenol removal from the control expressing photodegradation activity as stated in Eq. 12:

$$TP_s = TP - TP_p \tag{12}$$

where TP_s is phenol removal efficiency as a result of activity from *A. platensis* (%) and TP_p is phenol removal efficiency from the control as a result of photodegradation (%).

The absolute value of total phenolic compounds removed was calculated by Eq. 13:

$$AbTP = TP_0 - TP_t \quad (13)$$

where $AbTP$ is the absolute value of phenolic compounds removed by total activity (mg L^{-1}), TP_0 is total phenolic compounds at the first day of cultivation (mg L^{-1}), and TP_t is total phenolic compounds at the end of cultivation (mg L^{-1}). Equation 9 was also applied for photodegradation activity and *A. platensis* activity.

Concentration of phenol removed by *A. platensis* was corrected by subtracting the absolute total value of phenolic compounds by the absolute concentration of phenol removed from the control expressing photodegradation activity following Eq. 14:

$$ATPs = AbTP - ATPp \quad (14)$$

where $ATPs$ is the absolute concentration of phenolic compounds removed by *A. platensis* activity (mg L^{-1}), $AbTP$ is the concentration of phenolic compounds removed by total activity (mg L^{-1}), and $ATPp$ is the concentration of phenolic compounds removed from the control expressing photodegradation activity (mg L^{-1}).

Data analysis

Experimental design and statistical analyses were done using ANOVA Factorial Design and Response Surface Methodology, run in Minitab 18. ANOVA analysis was done with $\alpha = 0.05$ for attribution of sources of variation. Optimization experiments were generated as per CCD model. Pareto charts and contour plots were used to understand the most influencing factors and the correlations between parameters.

Results

Effect of POME fraction

Arthrospira platensis was grown well on all POME fractions, including 100% POME. Between 10 and 100% POME final biomass showed an almost 1.5-fold significant increase, whereas POME fractions above 30% POME showed no significant differences ($P > 0.05$) (Fig. 1). The lowest final biomass was found on 10% POME (135 mg L^{-1}) ($P < 0.05$), whereas at 100% POME, a final biomass of 190 mg L^{-1} was recorded. For growth rate, POME fractions above 30% showed significantly increased growth rates compared with

the lower fractions ($P < 0.05$). No significant differences in growth rate were found between 50 and 100% POME ($P > 0.05$). The final biomass concentration was increased 2.5 to 3.8-fold for 10 to 100% POME, respectively, compared to the initial biomass concentration at day zero.

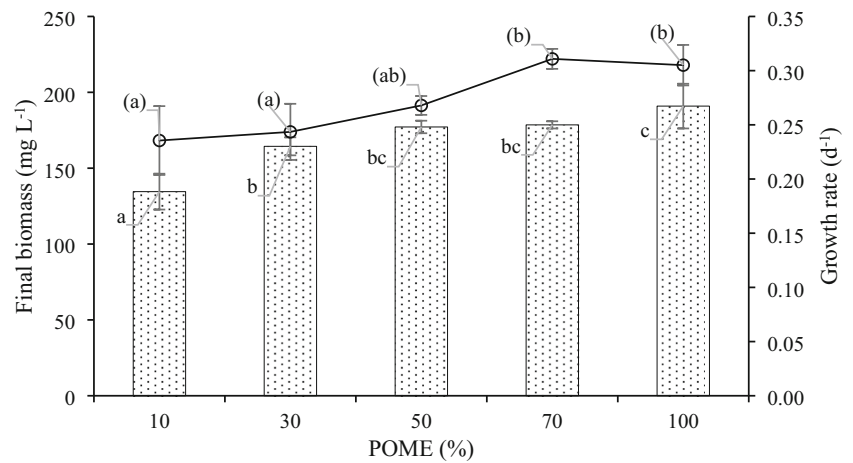
Increasing the POME fraction from 10 to 20% significantly enhanced COD removal in absolute value by photodegradation (ACODp), from 28 to 72 mg L^{-1} . Above 30% POME, no significant increase in ACODp removal was recorded (125 to 245 mg L^{-1} , Table 2). Higher POME fractions also significantly influenced COD removal by *A. platensis* (ACODs). The highest ACODs was recorded at 100% POME which removed 317 mg L^{-1} ($P < 0.05$), and the activity of *A. platensis*, ACODs, was significantly higher compared to photodegradation activity ACODp ($P < 0.05$). The interaction of POME fractions and total activity significantly influenced COD removal in absolute value. By combining photodegradation and *A. platensis* activity (total activity), the highest absolute value for COD removal was found at 100% POME ($P < 0.05$) (Table 2).

Absolute phenolic compound removal (ATPp) and color removal (ATCp) were significantly influenced by POME fraction when considering photodegradation activity. Above 50%, POME fractions significantly influenced the removal of ATPp and ATCp. For *A. platensis* activity, the removal of phenolic compounds (ATPs) and color (ATCs) were not significantly influenced by POME fractions ($P < 0.05$). At 100% POME, color was removed up to 2239, 365, and 2605 ptco by photodegradation, *A. platensis* activity, and total activity, respectively. The highest removal of phenolic compounds and color were found at 100% POME considering total activity (Table 2). The interaction of total activity and POME fractions significantly influenced the removal of ATC and AbTP ($P < 0.05$). The lowest activity was found for *A. platensis* activity both for phenolic compounds (ATPs) and color removal (ATCs) ($P < 0.05$).

For COD removal (in percentage value) by photodegradation (CODp), the value was not significantly affected by POME fraction ($P > 0.05$). POME fraction significantly affected COD removal efficiencies by *A. platensis* activity (CODs) ($P < 0.05$): increasing POME fractions from 10 to 50% significantly enhanced COD removal efficiencies with almost 3-fold (Fig. 2). For POME fractions above 50%, CODs removal was not significantly different. When considering total COD removal (TCOD), the lowest removal was found at 10% POME.

POME fractions influenced the removal efficiency of phenolic compound, both through photodegradation (TPp) and through the activity of *A. platensis* (TPs). TPp was found to be significantly higher at 100% POME compared to 50% POME and lower ($P < 0.05$). However, no significant difference was found between 70 and 100% POME ($P > 0.05$). Low phenolic compound removal by *A. platensis* activity (TPs)

Fig. 1 Final biomass (calculated dry weight in mg L^{-1}) and growth rate of *A. platensis* cultivated on different POME fractions. Average values of triplicate cultures are shown. Error bars indicate the SD of the mean. Bar is final biomass; line is growth rate. Values that do not share letters are significantly different ($P < 0.05$)



was found for 10% and 100% POME, while high TPs removal was found around 30–50% POME (Fig. 3). For total phenolic compound removal (TP), increasing POME fraction from 10 to 50% significantly increased removal efficiency ($P < 0.05$). Above 50% POME fraction, the efficiencies were not significantly different from each other ($P > 0.05$).

Increasing POME fractions from 10 to 100% also influenced color removal efficiencies by photodegradation (TCp) as well as total activity (TC) ($P < 0.05$) (Fig. 4). However, the POME fraction did not significantly influence color removal based on *A. platensis* activity (TCs). The highest color removal efficiency was found at 100% POME both for TCp and TC ($P < 0.05$).

Effect of environmental and nutritional conditions on biomass productivity and total POME color removal

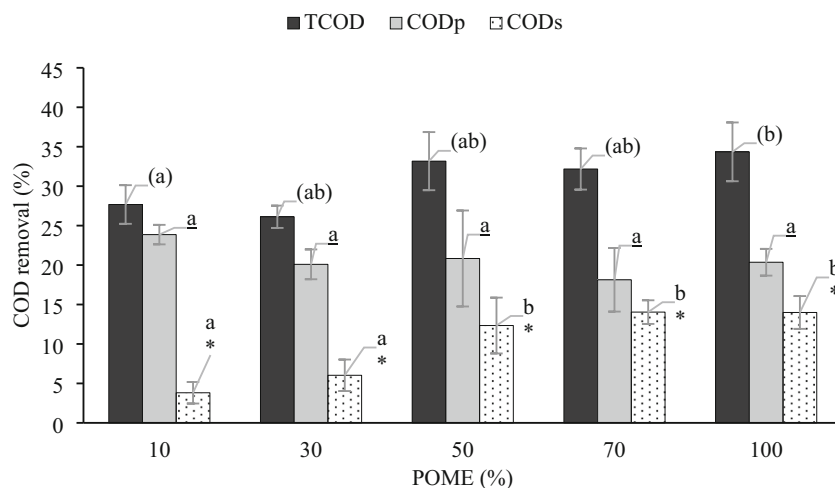
Environmental and nutritional conditions were studied to understand the interactive factors with respect to color removal and biomass produced by *A. platensis*. The most influencing factor for biomass productivity was found to be light intensity alone (Fig. 5a). Increasing light intensities, from 50 to 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, significantly enhanced *A. platensis* biomass productivity ($P < 0.05$) (Fig. 5a). Increasing salinity, from 15 to 30 PSU, significantly decreased the final biomass ($P < 0.05$) (Fig. 5a). The presence of nitrate at high POME also significantly enhanced biomass

Table 2 COD removal, phenolic removal, and color removal in absolute values at different POME fractions (%) and activities (photodegradation activity, *A. platensis* activity, and total activity)

POME	Activity	COD removal (mg L^{-1})		Color removal (ptco)		Phenolic compounds removal (mg L^{-1})	
10	Photodegradation	28.63 ^a	± 1.48	105.67 ^a	± 20.31	11.46 ^a	± 3.03
30	Photodegradation	72.33 ^b	± 6.81	389.67 ^b	± 22.59	33.65 ^b	± 1.17
50	Photodegradation	147.00 ^c	± 19.29	737.67 ^c	± 53.16	69.32 ^c	± 7.95
70	Photodegradation	217.67 ^d	± 6.03	1377.33 ^d	± 35.57	108.21 ^d	± 7.20
100	Photodegradation	317.00 ^e	± 41.07	2239.33 ^e	± 54.50	171.25 ^e	± 1.75
10	<i>A. platensis</i>	17.03 ^(a)	± 2.70	37.33 ^(a)	± 9.24	0.00 ^(a)	± 0.00
30	<i>A. platensis</i>	55.67 ^(a)	± 8.14	163.67 ^(b)	± 8.96	10.74 ^(a)	± 2.23
50	<i>A. platensis</i>	125.00 ^(b)	± 36.50	225.67 ^(b)	± 54.37	21.17 ^(a)	± 16.22
70	<i>A. platensis</i>	152.33 ^(b)	± 33.86	166.00 ^(b)	± 28.84	12.76 ^(a)	± 10.48
100	<i>A. platensis</i>	246.33 ^(c)	± 20.50	365.67 ^(c)	± 56.75	4.28 ^(a)	± 6.29
10	Total	45.67 ^A	± 4.04	143.00 ^A	± 18.52	5.48 ^A	± 1.09
30	Total	128.00 ^A	± 6.93	553.33 ^B	± 15.28	44.39 ^B	± 1.09
50	Total	272.00 ^B	± 30.20	963.33 ^C	± 75.06	90.49 ^C	± 8.27
70	Total	370.00 ^C	± 30.00	1543.33 ^D	± 56.86	120.97 ^D	± 3.30
100	Total	563.33 ^D	± 61.10	2605.00 ^E	± 10.00	175.03 ^E	± 5.15

Average values are shown ($n = 3$). Value after ± symbol indicates standard deviation. Values that do not share the same letters indicate significant difference ($P < 0.05$)

Fig. 2 Total COD removal (TCOD), COD removal by photodegradation (CODp), and by *A. platensis* activity (CODs), as a function of POME fraction. Average values of triplicate incubations are shown. Error bars indicate the SD of the mean. TCOD is shown as black bar, CODp is shown as gray bar, and CODs is shown as white bar. Values that do not share letters are significantly different ($P < 0.05$)



productivity when the irradiance level was high (Fig. 5a). However, based on Pareto chart, the addition of nitrate alone, POME alone, and salinity alone did not enhance biomass productivity ($P > 0.05$).

For total color removal (TC), the most influencing factor was found to be irradiance only (Fig. 5b), while the other factors were not significantly influential. When considering the activity of *A. platensis* alone (TCs), none of the factors significantly influenced the color removal (Fig. 5c). Based on these results, light intensity was optimized to explore phenolic compound removal as the activity of *A. platensis* normalized to photodegradation (third experimental series).

Effect of light intensity and initial phenol concentration on phenol and color removal efficiency

Since light intensity was found to be the most influencing factor for color removal (second experimental series, Fig. 5b), the third experimental series was done to reveal the effect of photodegradation on phenolic compound removal during the cultivation of *A. platensis*. We plotted the effect of light

intensity and initial phenol concentration on phenol removal using POME only (without *A. platensis*, TPp). Phenolic compound removal was significantly influenced by light intensity and initial phenol concentration (Fig. 6a). Low initial phenol concentration and increasing light intensity (from 50 to 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) resulted in increasing phenolic compound removal from 30 to 70%. At a higher initial phenol concentration, the phenol removal increased from 60 up to 80%. Both the increasing initial phenol concentration and light intensity resulted in enhanced phenol removal efficiency.

For total phenol removal by *A. platensis* (TPs) which included the activity of *A. platensis* alone, increasing light intensity decreased phenolic compound removal efficiency (Fig. 6b). At low light (LL) ($< 100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), phenol degradation reached up to 30–40% for all initial phenol concentrations. When the light level exceeded 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, TPs was significantly lower, at around 10–15%.

For total phenol removal (TP), the highest phenol removal ($> 90\%$) was recorded when a medium irradiance level (ML) ($100\text{--}150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high initial phenol concentration were employed. The lowest phenol removal ($<$

Fig. 3 Total phenol removal (TP), phenol removal by photodegradation (TPp), and by *A. platensis* activity (TPs). Average values of triplicate cultures are shown. Error bars indicate the SD of the mean. TP is shown as black bar, TPp is shown as gray bar, and TPs is shown as white bar. Values that do not share letters are significantly different ($P < 0.05$)

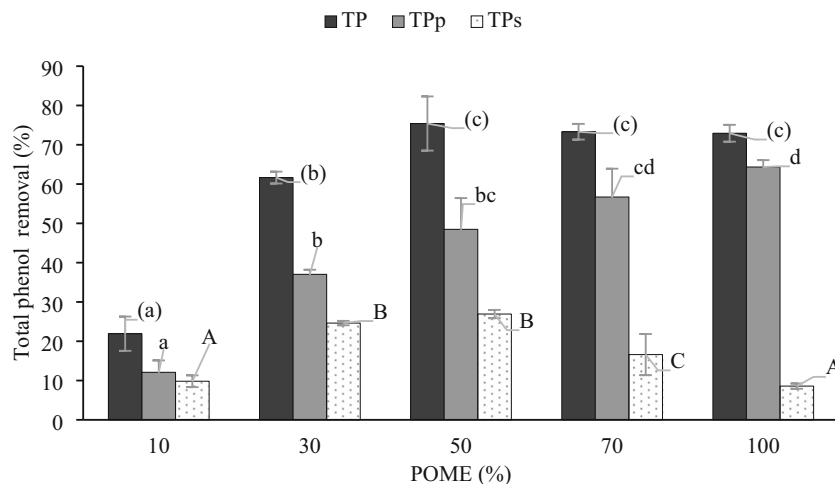
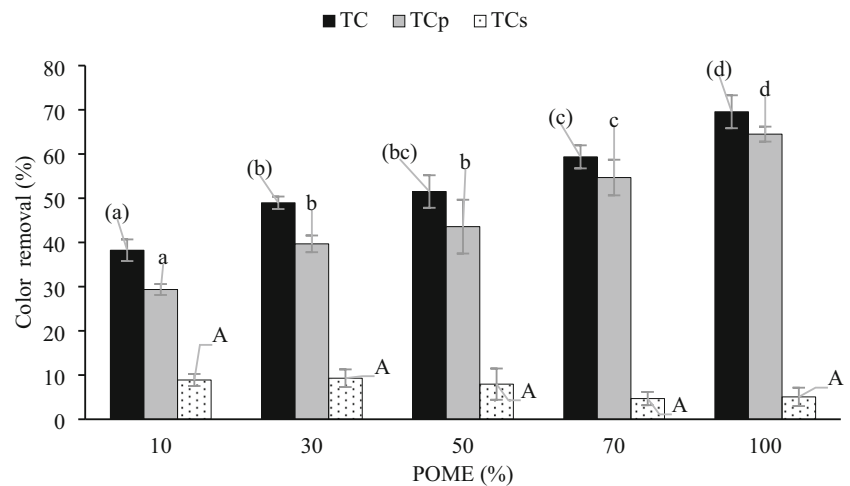


Fig. 4 Total color removal (TC), color removal by photodegradation (TCp), and by *A. platensis* (TCs) activity. Average values of triplicate cultures are shown. Error bars indicate the SD of the mean. TC is shown as black bar, TCp is shown as gray bar, and TCs is shown as white bar. Values that do not share letters are significantly different ($P < 0.05$)



75%) was recorded at low light and low initial phenol concentration (Fig. 6c).

For the absolute concentration of total phenolic compounds removed by *A. platensis* activity (CTPs), increasing light intensity decreased phenolic compound removal, but these differences were not significant ($P = 0.052$). However, when initial total phenolic compounds were increased at lower light intensity, the concentration of phenolic compounds removed by *A. platensis* was found to be higher (Table 3). The interaction of light intensity and initial total phenolic compounds significantly influenced CTPs ($P < 0.05$). Based on RSM calculation, the highest concentration of total phenolic compounds removed by *A. platensis* was found at $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 411 mg L^{-1} of initial phenolic compounds which resulted in 136 mg L^{-1} CTPs.

Effect of light intensity and initial phenol concentration on growth rate and final biomass

The highest final *A. platensis* biomass ($> 400 \text{ mg L}^{-1}$) was recorded at high irradiance (HL) ($> 150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and high initial phenol concentration. The interaction of light intensity and initial phenol concentration significantly influenced the final biomass. When the light was set to HL, the final biomass was significantly influenced by initial phenol concentration (Fig. 7a). However, at LL, the increase of initial phenol concentration did not significantly influence final biomass production.

The highest growth rate ($> 0.45 \text{ days}^{-1}$) was also recorded at HL and high initial phenol concentration. At HL, the increase of initial phenol concentration significantly boosted the growth rate, while at LL, the increase of initial phenol concentration decreased the growth rate. Growth rate was significantly influenced by the interaction of light intensity and initial phenol concentration (Fig. 7b).

Discussion

In the present work, the color of POME was strongly correlated to phenolic compound concentration. The degradation of phenolic compounds was significantly influenced by the activity of photodegradation which also contributed to the degradation of color. It is possible that the presence of hydroxyl radicals (OH^\cdot), which are generated from irradiated fulvic acid-like substances in POME (Kongnoo et al. 2012), could degrade the phenolic compounds. Faust and Hoigne (1987) found that the combination of fulvic acid and sunlight could enhance the degradation of 2,4,6-trimethylphenol in natural waters, while Jacobs et al. (2012) found that the degradation of phenolic compound occurred through reaction with the hydroxyl radical (OH^\cdot) generated by irradiated fulvic acids. Possibly, at POME fractions above 50%, photodegradation became more pronounced, due to higher fulvic acid-like concentrations, resulting in higher absolute phenolic compound removal (ATPp). However, at very low POME values, it seems that color could not be degraded by the combined action of fulvic acid and light (see Supplementary 2). Furthermore, the presence of *A. platensis* activity could not significantly enhance the removal. This result is in agreement with a previous study which reported that the color of POME not only consisted of phenolic compounds but also of fulvic acid, humic acid, tannin, and lignin which could not be degraded by simple physical-chemical methods (Kongnoo et al. 2012). Furthermore, previous studies reported that simple phenolic compounds which contained lower hydroxyl group bound to the aromatic ring such as gallic acid were more easily degraded by hydroxyl radicals compared to more complex phenolic compounds such as caffeic acid and protocatechuic, which also contribute to color (Chantho et al. 2016; Sroka and Cisowski 2003).

Compared to photodegradation, the activity of *A. platensis* to remove phenolic compounds (ATPs) and color (ATCs) on

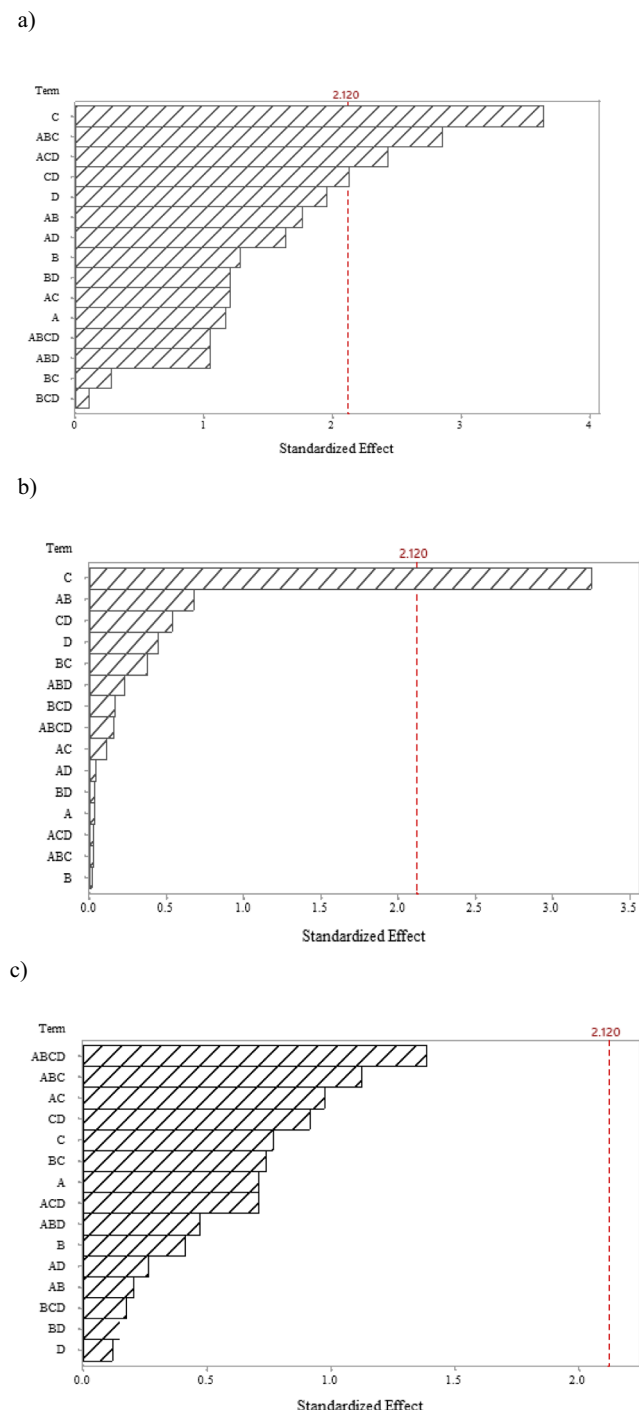


Fig. 5 Pareto chart showing the effects of the parameters on (a) biomass productivity, (b) total color removal (TC), and (c) color removal by *A. platensis* (TCs). A is POME, B is salinity, C is light intensity, and D is nitrate addition. The vertical line indicates the significance of the effects at 95% confidence level

POME fractions was lower. In experiment 1, with high irradiance levels (Table 2), *A. platensis* only contributed around 10–25% (Figs. 3 and 4). This indicated that *A. platensis* activity to remove phenolic compounds on POME was depended on the activity of photodegradation. At high light intensity,

hydroxyl radical can be more produced by fulvic acid and resulted higher photodegradation activity. This result was supported by experiment 3 which showed that the activity of *A. platensis* to remove phenolic compounds in absolute value was lower compared to photodegradation at high irradiance, and vice versa (Table 3).

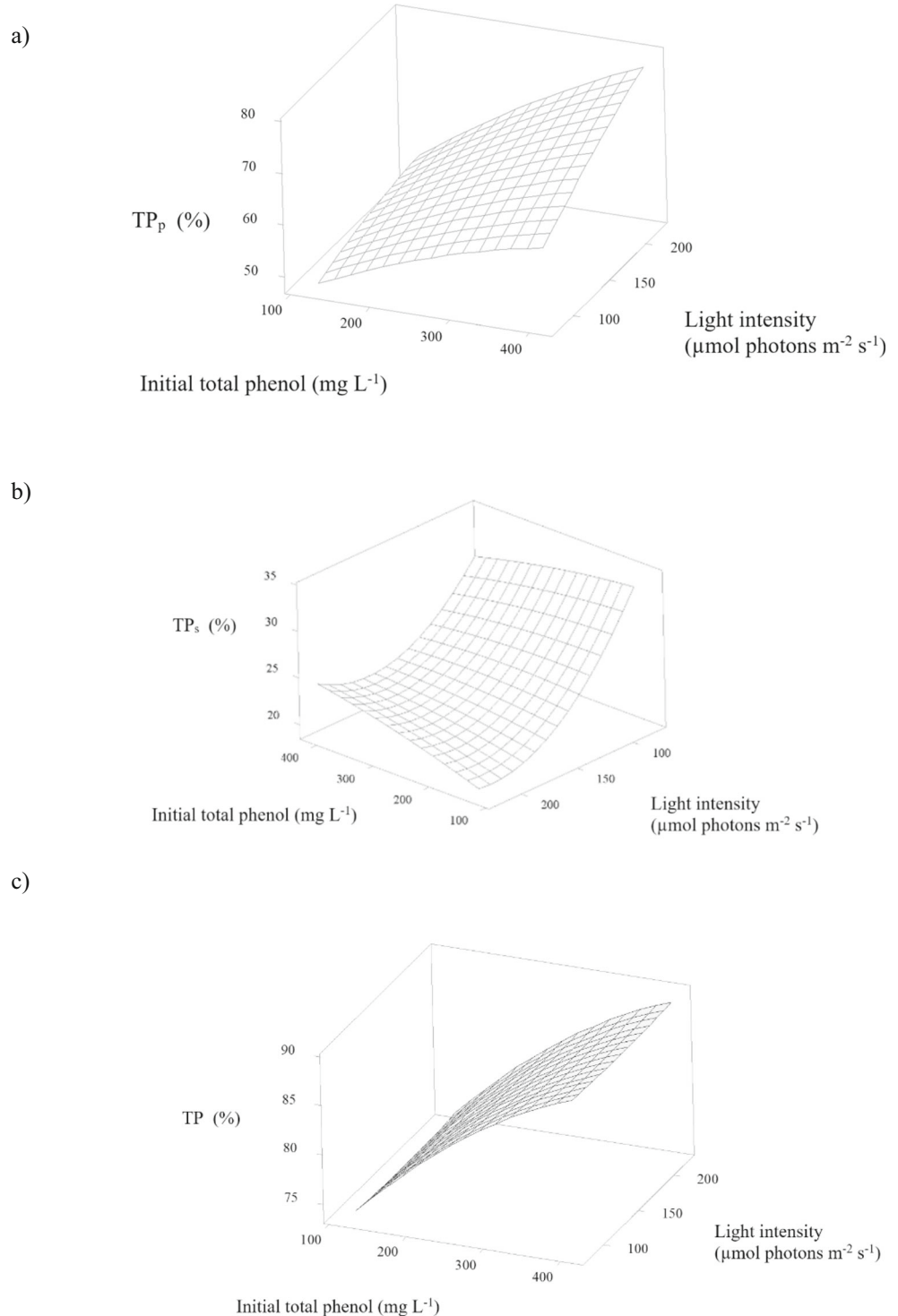
In this study, a combination of fulvic acid present in POME and light intensity also influenced absolute COD by photodegradation (ACOD_p) (Fig. 2). At 100% POME, 246.33 mg L⁻¹ of COD was removed by photodegradation. However, at 10% POME, only 28.63 mg L⁻¹ was removed. In this work, the presence of phenolic compounds seems to contribute to COD. At 100% POME, the decrease in phenolic compounds was found to be up to 65%, while COD only decreased up to 20% through photodegradation activity. The decrease of phenolic and color were not consistent with COD removal (Figs. 2, 3, and 4). This indicates that COD in POME consists of complex organic material such as acids, phenols, and tannins. Photodegradation activity was not enough to lower COD content to a very low level, while the presence of *A. platensis* activity could be promising to lower COD content when their cell concentration is high.

Previous research showed that total organic carbon in POME, which is associated with COD, was degraded in the presence of the hydroxyl radical and metals such as Fe²⁺ (Gamaraalage et al. 2019). It seems that trace metals contained in POME, such as iron, zinc, and copper, could also contribute to COD degradation after reacting with the hydroxyl radical (Safarzadeh-Amiri et al. 1996; Hurtado et al. 2016; Ding et al. 2016). Increasing the POME fraction might therefore result in higher iron and hydroxyl levels, thereby enhancing ACOD_p through photodegradation. Furthermore, the value of ACOD_p was significantly higher compared to COD degradation by *A. platensis* activity (ACOD_s). This indicates that the physical processes derived from photodegradation more quickly degraded COD compared to biological process. Previous work reported that digested POME contains approximately 180 mg L⁻¹ of iron (Shavandi et al. 2012).

In the presence of *A. platensis*, ACOD_s and total biomass was higher at increasing POME fractions. It is possible that higher POME fractions result in higher organic carbon levels which can be utilized by *A. platensis* for growth, resulting in higher biomass and higher breakdown of ACOD. This is supported by an earlier study, which demonstrated that organic carbon in treated POME such as acetic acid could be utilized as organic carbon source by *A. platensis* in mixotrophic conditions (Zainal et al. 2012). Poh et al. (2010) reported that digested POME contains acetic acid at concentrations between 1150 and 3500 mg L⁻¹.

With respect to the environmental factors, light intensity significantly enhanced the removal of total color (TC). Previous study found that at higher light intensities, phenol oxidation was more enhanced (Blaková et al. 1998), thus

Fig. 6 Response surface plot (3D) of (a) total phenolic compound removal by photodegradation (TP_p), (b) total phenolic compound removal by *A. platensis* (TP_s), and (c) total phenolic compound removal (TP), as a function of initial light intensity and initial total phenolic compounds



resulting higher color removal. While for color removal by *A. platensis*, the activity was not significantly affected by the selected environmental factors (Fig. 6c), even though the biomass productivity of *A. platensis* was significantly affected by light intensity and the interaction of light intensity and nitrate, the interaction of POME fractions and salinity, and the

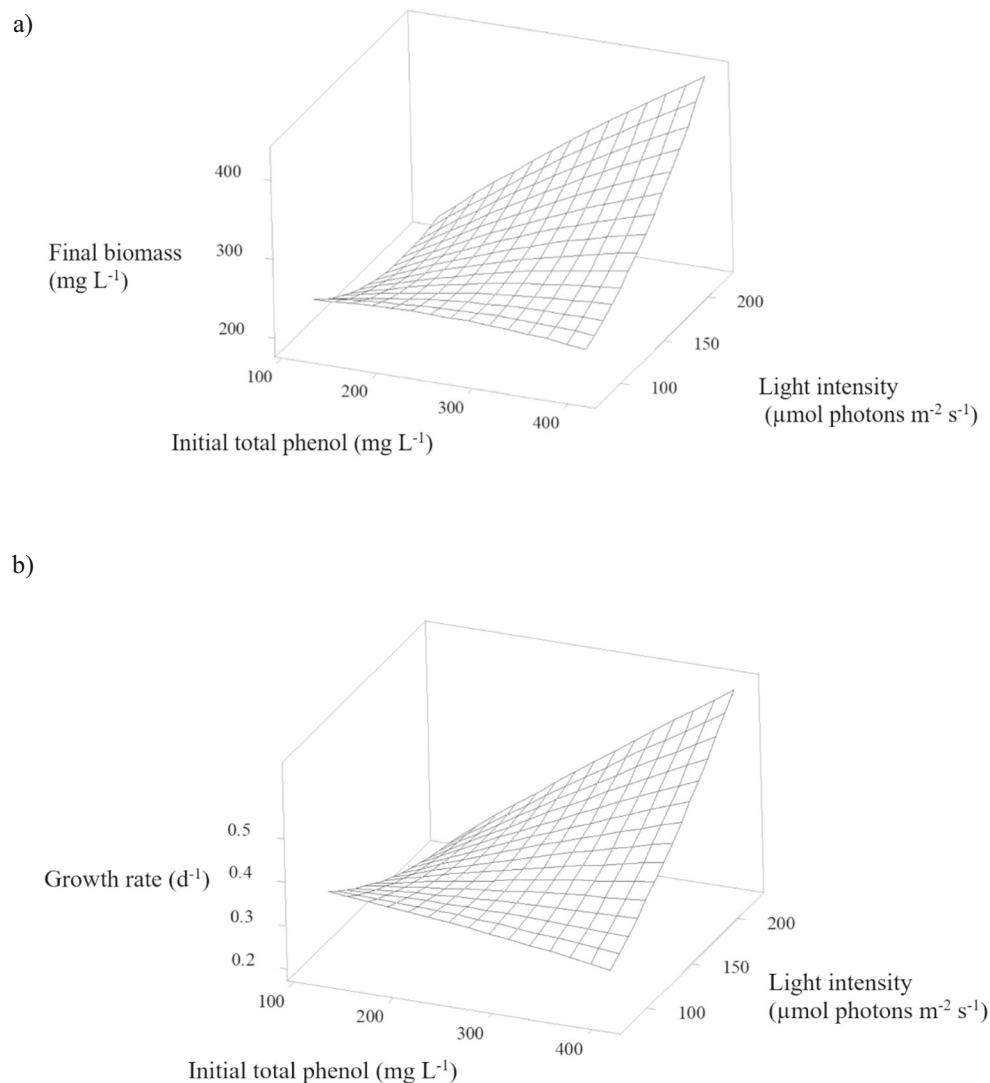
interaction of POME fractions and nitrate addition (Fig. 5a). It seems that a very high microalgal biomass is required to eliminate all color of POME. Baldev et al. (2013) reported that the removal of a synthetic dye was influenced by the initial inoculum concentration of *Coelastrella* sp. At the higher initial inoculum concentration, a higher number of the cells were

Table 3 Experimental design of CCD RSM and the responses of phenolic removal in absolute values

External GA addition (mg L ⁻¹)	Initial total phenolic (mg L ⁻¹)	Initial light intensity (μmol photons m ⁻² s ⁻¹)	AbTP (mg L ⁻¹)	ATPp (mg L ⁻¹)	ATPs (mg L ⁻¹)
8.58	128.58	150.00	96.68 ± 3.93	81.03 ± 0.49	15.65 ± 4.43
50.00	170.00	100.00	129.36 ± 1.97	71.99 ± 0.49	57.38 ± 1.48
50.00	170.00	200.00	138.75 ± 2.46	117.19 ± 0.49	21.56 ± 2.95
150.00*	270.00*	150.00*	231.60 ± 0.98	207.26 ± 0.98	24.34 ± 1.97
150.00	270.00	220.71	232.30 ± 0.00	209.00 ± 3.44	23.30 ± 3.44
150.00	270.00	79.29	235.78 ± 5.90	182.22 ± 36.39	53.55 ± 30.49
250.00	370.00	100.00	324.46 ± 0.49	207.96 ± 1.97	116.50 ± 2.46
250.00	370.00	200.00	339.06 ± 7.38	311.59 ± 3.93	27.47 ± 3.44
291.42	411.42	150.00	374.18 ± 11.80	338.71 ± 6.89	35.47 ± 4.92

Average values of the response are shown from duplicate experiments ($n=2$). SD is shown after the \pm symbol. AbTP is the concentration of phenolic compounds removed by total activity (mg L⁻¹), ATPp is concentration of phenolic compounds removed by photodegradation activity (mg L⁻¹), and ATPs is concentration of phenolic compounds removed by *A. platensis* activity (mg L⁻¹). * is the middle value in CCD RSM design

Fig. 7 Response surface plot (3D) of (a) final biomass expressed as dry weight (mg L⁻¹) and (b) growth rate as a function of initial light intensity and initial total phenolic compounds



exposed to the medium, thereby enhancing dye removal. Furthermore, Stephen and Ayalur (2017) found that *Chlorella pyrenoidosa* at an initial inoculum of around 3–4 g L⁻¹ could remove phenol up to 95% when supplemented with nutrients.

CCD RSM revealed that increasing initial phenolic compounds and light intensity significantly enhanced the phenol removal efficiency by photodegradation. It seems that gallic acid as the external phenolic compound was easily degraded by photodegradation. The higher gallic acid concentrations were applied, and the higher removal efficiency was obtained. On the long run, however, the remaining phenolic compounds are probably more difficult to degrade, and the remaining phenolic compounds could not be removed (see Supplementary 2).

Previous work demonstrated that microalgae could degrade phenolic compounds in several ways: by utilizing it as an organic carbon source or by oxidizing it due to the binding of dissolved oxygen produced by microalgae (Lika and Papadakis 2009). In our study, at low light intensity, photodegradation activity was low. It is possible that the hydroxyl radical produced from fulvic acid in POME was low at low light, thereby causing lower phenolic degradation rates. On the other hand, the degradation of phenolic compounds by *A. platensis* was high at low light intensity. This might imply that the phenolic compounds were assimilated by *A. platensis*, which would be in agreement with previous work demonstrating phenolic compound uptake by the cyanobacterium *Arthrospira (Spirulina) maxima* as organic carbon source (Lee et al. 2015). However, when the irradiance level was high, photodegradation became more pronounced compared with *A. platensis* activity (Table 3, Fig. 6b), even though *A. platensis* still contributed to phenolic compound removal at around 10–20%. This indicated that phenol removal was not directly linked to biomass growth at high light intensity. When photodegradation activity became more active, the presence of phenolic compounds as organic carbon source became limiting for the growth of *A. platensis*, even though the final biomass concentration was high. A possible explanation for this is that the degradation of phenol at high light intensity resulted in lower molecular weight acids which could be easily utilized by *A. platensis* (Vlyssides et al. 2011; Golmakani et al. 2012; Gamaralalage et al. 2019).

Nevertheless, the increase in final biomass seems inhibited the photodegradation activity. It was found that increasing irradiance from 150 to 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 400 mg L⁻¹ initial phenolic compound could not promote T_{pp} which is almost stagnant at 80% (Fig. 6a), while biomass concentration increased from 300 to 400 mg L⁻¹. By

combining high light intensity and high initial phenolic compound level, the final biomass and growth rate also increased.

Conclusion

This study reveals the capability of photodegradation activity to degrade COD, color, and phenolic compounds in POME wastewater in the presence of *A. platensis*. The initial fraction of POME that was present influenced growth rate, final biomass, COD removal absolute value, and absolute color removal by *A. platensis*. Based on a factorial design approach, it was shown that salinity, nitrogen addition, and initial POME concentration did not influence total color removal. High initial phenolic compound and high light intensity could increase the growth rate up to 0.45 days⁻¹ and final biomass up to 400 g L⁻¹, while on the other hand, total phenolic compounds were removed almost completely (94%). Photodegradation activity significantly contributed to color and phenolic compound removal. Phenolic compounds that are present in POME could be removed by *A. platensis* when cultivated on high POME fractions at low irradiance conditions. High phenolic compound removal can be achieved by combining *A. platensis* activity and photodegradation, while both activities seem not effective in lowering COD content. Overall, this study shows that phenolic compounds and color degradation from POME are dominated by the activity of photodegradation at high irradiance, while the activity of *A. platensis* dominates at low light intensity.

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