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Co-production of polyhydroxybutyrate and C-phycocyanin from *Arthrospira platensis* growing on palm oil mill effluent by employing UV-C irradiation

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Abstract
Biopolymer made from bacteria is still not attractive compared to conventional commercial polymer because of the high cost of the substrate. The cyanobacterium *Arthrospira platensis* accumulates polyhydroxybutyrate (PHB) by using photosynthesis, making the production more promising compared to non-photosynthetic bacteria. However, this is still impractical for large-scale application. To increase PHB content and make it more feasible, the cultivation of *A. platensis* could be done on wastewater to reduce commercial fertilizer, whereas the valuable compound, C-phycocyanin (C-PC), can be exploited by modifying environmental factors. This study investigated the production of PHB and C-PC of *A. platensis* growing in batch mode on palm oil mill effluent (POME) as a medium for the cultivation by employing UV-C irradiation. Two experiments were conducted to investigate POME fractions (0–70% v/v) supplemented with modified Zarrouk medium and UV-C (20 W m^{-2}) irradiation time (0–30 min). Results showed that 5 min of irradiation time and 50% POME stimulated *A. platensis* to attain 7 mg L^{-1} day^{-1} of PHB and 16 mg L^{-1} day^{-1} of C-PC productivity.

Keywords *Arthrospira platensis* · Polyhydroxybutyrate · C-Phycocyanin · UV-C irradiation · Palm oil mill effluent

Introduction

Biopolymer made from bacteria is still not attractive compared to conventional commercial polymer because of the high cost of the substrate. Currently, the investigation on cyanobacteria as a source of polymer (bioplastic) has increased since they can grow photoautotrophically by utilizing carbon dioxide and light to produce biomass and accumulate a biopolymer, polyhydroxybutyrate (PHB) (Costa et al., 2019). However, because of the low productivity and high energy requirements in the downstream process, biopolymer made from cyanobacteria is still more difficult to produce than conventional commercial polymer (Carpine et al., 2020).

Several options have been proposed to make cyanobacterial biopolymer production more feasible, such as modifying the environmental and nutritional conditions, growing the cyanobacteria on wastewater to replace commercial fertilizer, and recovering value-added items from biomass, such as pigment and protein, before extracting the biopolymer (Amadu et al. 2020; Onen et al. 2020). According to Nur and Buma (2019), digested palm oil mill effluent (POME) generated from oil palm factories could be a potential medium source for cyanobacteria because it contains a high concentration of macro- and micronutrients required for growth. Each tonne of palm oil processing generates about 3 tonnes of POME which is hazardous for surrounding environment if not properly treated (Ahmad et al., 2003). Nur et al (b; c; 2019a) have reported that POME supplementation promotes the growth and fucoxanthin and sulfated exopolysaccharide in the marine diatom *Phaeodactylum tricornutum*. Fernando et al. (2021) reported that the addition of 7.5% raw POME to the cultivation medium for *Haematococcus pluvialis* resulted the highest astaxanthin accumulation and reduced the specific freshwater consumption up to 50%. Nur et al. (2019c) successfully utilized 50% POME in the culture medium of the cyanobacterium *Arthrospira platensis* to produce C-PC, thus reducing the cost of nutrients. The use of POME medium could lower the nutrient cost, reducing

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water consumption and potentially lowering the production cost of the biomass. However, the modification of nutritional and environmental conditions needs to be studied. Previous studies (Duangsri et al 2020; García et al., 2021) have found that cyanobacteria and algae accumulate high PHB content under stressed condition such as nitrogen depletion; however, POME is rich in micro- and macronutrients.

Environmental and nutritional factors influence PHB production in cyanobacteria (Price et al., 2020). Previous research has investigated the possibility of using UV irradiation to induce PHB in *A. platensis* growing in a large-scale production (Kavitha et al., 2016). However, it has not been investigated whether UV-C irradiation of *A. platensis* growing on POME medium could affect PHB accumulation. It is hypothesized that by combining the addition of POME containing organic carbon with UV-C irradiation, the accumulation of PHB would be increased. Furthermore, based on previous studies, PHB accumulation has increased in some cyanobacterial strains as result of modification in nutritional conditions such as the addition of organic carbon sources, which results in mixotrophic cultivation (Correa and Teixeira, 2021). Nevertheless, the nutritional and environmental conditions such as low nitrogen availability, low salinity, and high UV-C irradiation could lower biomass and C-phycocyanin productivity (Ou et al., 2011; Nur et al., 2019c). Therefore, to obtain high PHB and C-phycocyanin production, conditions need to be optimized. The purpose of this study was to investigate into the potential of *A. platensis* growing on POME medium to produce PHB and C-phycocyanin by using UV-C irradiation.

Material and methods

POME preparation

POME was immediately collected after being discharged from a facultative aerobic treatment pond at a small factory in Sumatra, Indonesia. POME was filtered through GF/C (Whatman) filter paper to remove particles and the filtrate was then sterilized for 15 min at 121 °C and then stored at − 18 °C to prevent degradation. The POME had a COD concentration of 1230 mg L^{-1}, a total nitrogen concentration of 230 mg L^{-1}, and a dissolved phosphate concentration of 20 mg L^{-1}.

Experimental setup

Arthrospira platensis (SAG 21.99) stock cultures were grown on a modified Zarrouk medium (Zarrouk 1966) with the salinity adjusted to 15 PSU using commercial-grade salt (Refina, Indonesia), and contained 10 g L^{-1} $NaHCO_3$ and 1 g L^{-1} $NaNO_3$ and other micronutrients. The culture was acclimated for at least 1 week to the experimental conditions. The cyanobacteria were grown in a 12:12 light/dark cycle. If the culture reached the stationary phase, it was diluted with fresh modified Zarrouk medium.

Effect of POME on growth, PHB, and C-PC production

Arthrospira platensis cultures were grown in 50-mL culture flasks (Grenier Bio-one) with a working volume of 40 mL. The medium was consisted of modified Zarrouk medium and POME with different fractions (10, 30, 50, and 70% POME). The sample was inoculated with 2% *A. platensis* (initial optical density 0.6 at 680 nm). Commercial NaCl was used to adjust the salinity to 15 PSU and 0.1 N NaOH was used to adjust the pH to 9. The culture flasks were placed in a separate chamber equipped with a water bath and three fluorescent lamps (Philips, Indonesia) and the temperature was set to 30 °C with a light intensity of 100 µmol photons m^{-2} s^{-1} according to previous work (Nur et al., 2019c).

At least three times a day the culture was manually shaken to mix the culture medium. Growth was monitored spectrophotometrically. A 2 mL culture sample was centrifuged at 4000 x*g* for 10 min. The supernatant (2 mL) was discarded and replaced with 2 mL of Milli-Q (15 PSU) water before being shaken for 10 s in a homogenizer at 3000 rpm. The absorbance of the sample was measured at 680 nm. The cultivation was ended when it reached the end of the exponential phase. After cultivation, the biomass was collected and harvested by centrifugation at 2000 × *g* for 15 min to determine the C-PC and PBH concentration (see the "Analyses" section below). The experiment was done in two replicates ($n = 2$).

Effect of UV irradiance on growth, PHB, and C-PC production growing on 50% POME

Arthrospira platensis culture was grown on a medium consisting of 50% POME and 50% modified Zarrouk medium under the same conditions as above.

The culture bottle was irradiated with a 20 W m^{-2} UV-C lamp placed 5 cm above the cultures for 5, 10, 15, and 30 min at the start of the cultivation. The cultivation was then continued until the end of the exponential phase. Every day, the growth was monitored with a spectrophotometer (MBA 2000, Perkin Elmer). The experiment was done in two replicates ($n = 2$).

Analyses

Biomass and growth rate

The growth of *A. platensis* was monitored indirectly using a spectrophotometer at 680 nm. For biomass conversion, a

linear correlation between absorbance and dry biomass was used (Eq. 1). The correlation was created by serially diluting *A. platensis* cultured on modified Zarrouk medium with Milli-Q. The samples were collected using 1.2-µm pore size Whatman GF/C filters and oven-dried at 70 °C until constant weight (Nur et al., 2019c). Gravimetric analysis was used to determine the amount of the dry biomass.

The OD_{680}/dry weight relationship was as follows:

$$X = 0.683(OD) + 0.003; R^2 = 98\% \quad (1)$$

where X is biomass dry weight (g L^{-1}), and OD is optical density at 680 nm.

C-PC analysis

A culture sample (15 mL) was centrifuged at 2000 × *g* for 30 min. The pellet was oven-dried as stated previously and added 3 mL of phosphate buffer (pH = 7.2) was added and the sample then was sonicated in a sonicator water bath at 35 °C for 30 min. The sample was then centrifuged at 2000 × *g* for 15 min. The supernatant containing C-PC was monitored at 620 nm and 652 nm and the C-PC content was calculated (Bennett and Bogorad, 1973) (Eq. 2):

$$C - PC = \frac{OD_{620} - 0.474(OD_{652})}{5.34} \quad (2)$$

where C-PC is C-phycocyanin concentration in milligrams per liter, OD_{620} is optical density of the sample at 620 nm, and OD_{652} is optical density of the sample at 652 nm.

To calculate C-PC productivity, Eq. 3 was applied:

$$P_{C-PC} = \frac{C_1 - C_0}{t_1 - t_0} \quad (3)$$

where $P_{C\text{-}PC}$ is C-PC productivity (mg L^{-1} day^{-1}), C_1 is C-PC concentration (mg L^{-1}) at the end of cultivation t_1 (day), and C_0 is C-PC concentration (mg L^{-1}) at the beginning of cultivation t_0 (day).

PHB analysis

A 15-mL sample of *A. platensis* culture was centrifuged at 2000 × g for 30 min. Then, the wet pellet was immediately mixed with 5 mL of NaClO (10% v/v) and sonicated at 40 °C for 30 min. The sample was separated by centrifugation at 2000 × *g* for 15 min and the supernatant discarded. The pellet obtained was used to determine PHB.

To determine PHB content, the sample pellet was placed in a small glass tube, and 3 mL concentrated H_2SO_4 was added. The sample was heated in a water bath at 100 °C for 15 min to convert PHB to crotonic acid, and the OD was then measured at 245 nm. A correlation between crotonic acid and PHB concentration was used (Rajankar et al., 2018).

To calculate PHB productivity, Eq. 4 was applied:

$$P_{PHB} = \frac{P_1 - P_0}{t_1 - t_0} \quad (4)$$

where P_{PHB} is PHB productivity (mg L^{-1} day^{-1}), P_1 is the PHB concentration (mg L^{-1}) at the end of cultivation t_1 (day), and P_0 is the PHB concentration (mg L^{-1}) at the beginning of cultivation t_0 (day).

Statistical analysis

For statistical analysis, Minitab ver. 18 was used. Analysis of variance (ANOVA) with a P value of 0.05 was used to compare the differences between treatments. For pairwise comparisons, post hoc tests (Tukey HSD) were performed for pairwise comparisons. The experimental results were obtained for two replicates and are expressed as means and standard deviations (± SD).

Results

Effect of POME on growth rate, biomass, C-PC, and PHB

Arthrospira platensis was cultured on different POME fractions diluted with modified Zarrouk medium (Table 1). Growth rate was significantly enhanced when POME was added above 10% ($P < 0.05$). The highest growth rate was found with 30% POME diluted with modified Zarrouk medium. The lowest growth rate was found on modified Zarrouk medium (0% POME) ($P < 0.05$). This result indicated that the growth was influenced by the addition of POME. This result was confirmed by the biomass with the highest biomass (0.78 g L^{-1}) found with control medium with the addition of 50% POME (Table 1).

The addition of POME also enhanced PHB accumulation. The lowest PHB content was found on modified Zarrouk medium without POME ($P < 0.05$) (Table 1). Figure 1 shows the effect of POME on C-PC and PHB production. It was found that the highest C-PC and PHB productivity was on 50% POME with 32 mg L^{-1} day^{-1} C-PC productivity and 1.8 mg L^{-1} day^{-1} PHB productivity (Fig. 1). This finding was then used for the second experiment.

Effect of UV-C irradiation on A. platensis biomass growing on POME

UV-C irradiation was investigated to understand the effect on *A. platensis* growing on 50% POME. The *A. platensis*

culture was irradiated by using UV-C at the beginning of the cultivation. UV-C irradiation (20 W m^{-2}) significantly influenced growth rate, biomass, C-PC, and PHB content. The highest specific growth rate of 0.7 day^{-1} was found with 0 min UV-C irradiation (control). When UV-C irradiation was introduced, growth rate and biomass were reduced significantly ($P < 0.05$) (Table 2). The optimal condition to obtain co-production was in 5-min irradiation of UV-C which resulted 7 mg L^{-1} day^{-1} of PHB production and 16 mg L^{-1} day^{-1} C-PC production in 3-day cultivation (Fig. 2).

Table 1 Effect of POME fractions diluted with modified Zarrouk medium on growth rate, final biomass, C-phycocyanin (C-PC), and polyhydroxybutyrate (PHB) content of *A. platensis* growing in batch cultivation at 12:12 day to light at 100 µmol photons m^{-2} s^{-1}

POME (%)	C to N ratio	Growth rate (day^{-1})	Final biomass (g L^{-1})	C-PC (%)	PHB (%)
0	NA	0.37 ± 0.02a	0.41 ± 0.03a	12.32 ± 0.32a	0.55 ± 0.04a
10	0.27	0.36 ± 0.00a	0.54 ± 0.01b	11.15 ± 0.95a	0.68 ± 0.00b
30	0.75	0.66 ± 0.02b	0.67 ± 0.06 cd	11.45 ± 0.62a	0.72 ± 0.06b
50	1.17	0.52 ± 0.01b	0.78 ± 0.04c	12.15 ± 0.19a	0.70 ± 0.05b
70	1.53	0.58 ± 0.01b	0.65 ± 0.03bd	10.79 ± 0.84b	0.83 ± 0.03b

*Carbon to nitrogen (C to N) ratio is calculated based on theoretical total weight ratio of organic carbon and nitrogen in the medium. Total organic carbon from POME was calculated from $\frac{12}{32} \times$ COD. Standard deviations are shown after ± symbol. Values that do not share letters are significantly different ($P < 0.05$). NA means not applicable. Average values are shown ($n = 2$)

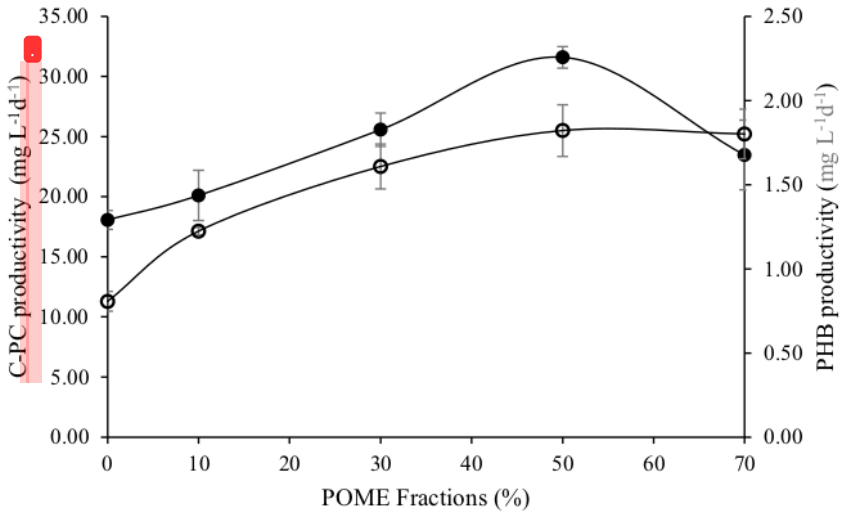

Fig. 1 Effect of POME fractions on polyhydroxybutyrate (PHB) productivity (open circles) and C-phycocyanin productivity (closed circles) at the end of exponential phase from batch cultivation of *A. platensis* grown in modified Zarrouk medium at 100 photons m^{-2} s^{-1} light intensity. Values are shown in average ($n = 2$). Bars indicate SD of the values

Table 2 Effect of UV-C (20 W m^{-2}) irradiation time on growth rate, final biomass, C-phycocyanin (C-PC), and polyhydroxybutyrate (PHB) content of *A. platensis* growing in batch cultivation at 12:12 day to light at100 µmol photons m^{-2} s^{-1}

Time (min)	Growth rate (day^{-1})	Final biomass (g L^{-1})	PHB (%)	C-PC (%)
0	0.54 ± 0.01a	0.68 ± 0.00a	0.80 ± 0.072a	12.70 ± 0.19a
5	0.52 ± 0.035b	0.59 ± 0.06b	3.71 ± 0.12b	8.08 ± 0.05b
10	0.50 ± 0.064b	0.56 ± 0.00b	5.10 ± 0.19c	7.58 ± 0.34b
15	0.43 ± 0.045b	0.45 ± 0.00b	6.79 ± 0.26d	6.56 ± 0.9b
30	0.37 ± 0.033b	0.34 ± 0.04b	8.45 ± 0.12e	5.09 ± 0.57c

Average values are shown ($n = 2$). Standard deviations are shown after ± symbol. Values that do not share letters are significantly different ($P < 0.05$)

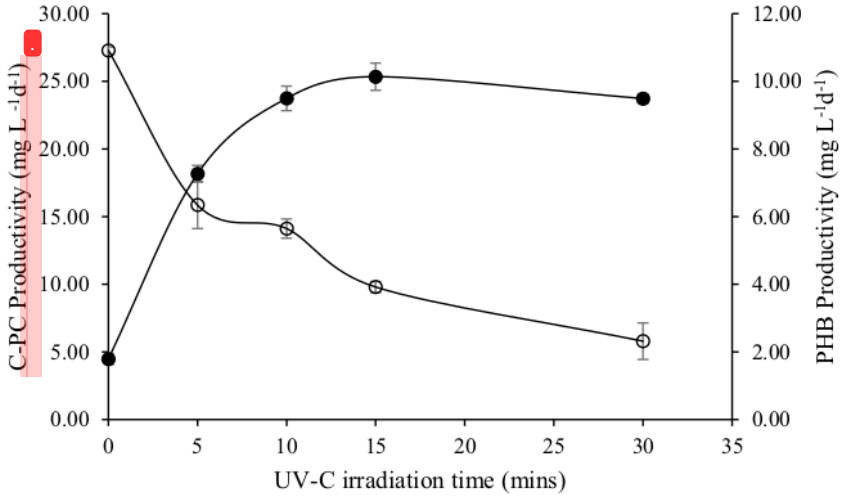

Fig. 2 Effect of UV-C irradiation time on polyhydroxybutyrate (PHB) productivity (closed circles) and C-phycocyanin productivity (open circles) at the end of exponential phase from batch cultivation of *A. platensis* growing in 50% POME and modified Zarrouk medium using 100 photons m^{-2} s^{-1} light intensity. Values are shown in average ($n = 2$). Bars indicate SD of the values

Discussion

Effect of POME

POME is rich in micronutrients and organic carbon that could enhance the growth of microalgae. When POME was added to modified Zarrouk medium, the cultivation changed from autotrophic to mixotrophic condition. Previous research found that the addition of acetate to modified Zarrouk medium enhanced the growth and biomass of *A. platensis* (Maheswari and Ahilandeswari 2011). In mixotrophic condition, where an organic carbon source is available, growth and biomass production are enhanced compared to autotrophic condition (Nematollahi et al., 2020).

In addition, this finding was supported by reduced C-PC content from modified Zarrouk medium compared to medium containing POME above 70%. In the control medium, *A. platensis* utilizes only light as an energy source, and therefore, a higher C-PC level (13%) was found since *A. platensis* produced more pigments to capture light energy in photosynthesis reaction. C-PC as a secondary pigment was more active in photoautotrophic activity compared to mixotrophic condition. In the mixotrophic condition, microalgae utilize both light and organic carbon source for the growth, thus reducing the activity of pigments to capture light. It has previously been reported that the phycocyanin content of *Gardieria sulfaria* decreased by up to 60% when grown mixotrophically (Abiusi et al, 2021). Li et al. (2018) also recorded that chlorophyll-*a*, carotenoids, and phycocyanin content in *A. platensis* were lower in mixotrophic compared to autotrophic condition.

When the growth was changed to mixotrophic condition, *A. platensis* accumulated a higher PHB content. Correa and Teixeira (2021) found that the accumulation of PHB by *A. platensis* was increased by adding glycerol as an organic carbon source. This is due to the excess energy from light intensity and organic carbon source (such as glucose) which was stored as a form of biopolymer (Costa et al., 2019). PHB content recorded in the present study (Table 1) was influenced by POME at above 70% addition. By adding POME to modified Zarrouk medium, a different C to N ratio was generated, since POME contains high organic carbon source (i.e., acetic acid, butyric acid, propionic acid, and formic acid) (Jasni et al., 2020). The higher POME fraction results in a higher C to N ratio, indicating that nitrogen in the media was more limited and it has been found that most *Arthrospira* species produce high PHB content (10–19.2%w/w dry weight) under nitrogen-depleted conditions supplied with carbon sources (Duangsri et al. 2020).

Photoperiod and light intensity are important factor for C-PC productivity in cyanobacteria. Previously, it was found that the highest C-PC yield was recorded with a 12:12 day to night photoperiod compared to 16:8 and 24:0 photoperiods (Lin et al. 2022). Nur et al (2019c) found that a 16:8 (day to night) photoperiod for *A. platensis* growing on 50% POME supplemented with external nitrate and 200 photons m^{-2} s^{-1} resulted in 14 mg L^{-1} C-PC. In the present study, C-PC production reached up to 90 mg L^{-1} on 50% POME supplemented with modified Zarrouk medium and a 12:12 photoperiod using 100 photons m^{-2} s^{-1}.

Effect of UV-C irradiation

UV-C irradiation has several benefits for microalgae to produce valuable compounds. Costa et al. (2021) have reviewed the potency of UV-C irradiation to enhance secondary pigments, carbohydrate, lipid, and polyhydroxybutyrate. In the present study, the culture irradiated with UV-C (20 W m^{-2})

Table 3 Comparison of polyhydroxybutyrate productivity (P_{PHB}) and C-phycocyanin productivity ($P_{C\text{-}PC}$) (mg L^{-1} day^{-1}) of *Arthrospira* species growing in batch cultivation at various nutritional and environmental conditions

Strain	Media	TN	Cultivation system	Trophic mode	Optimal condition	Cultivation (day)	P_{PHB}	$P_{C\text{-}PC}$	References
A. platensis RRGK	Zarrouk	2.5 g L^{-1} $NaNO_3$	Open raceways pond	Autotrophic	UV-B irradiation (10 min, 3 W m^{-2})	30	4.00	NA	Kavitha et al. (2016)
A. platensis LEB18	Biopolymer extraction waste + Zarrouk medium	5 mg L^{-1} N-NO_3	Tubular reactor	Mixotrophic	25% biopolymer waste	20	2.00	NA	da Silva et al. (2018)
A. platensis	Modified Zarrouk	0.25 g L^{-1} $NaNO_3$	Transparent bottles of polyethylene terephthalate	Autotrophic	Reduced medium	25	0.16	2.4	dos Santos et al. (2019)
A. platensis	Modified Zarrouk	1.25 g L^{-1} $NaNO_3$	Germination chamber	Mixotrophic	Pure glycerol addition	7	11.06	42.25	Correa and Teixeira (2021)
A. platensis	Modified Zarrouk	1 g L^{-1} $NaNO_3$	Erlenmeyer flask	Autotrophic	-	3	0.75	21.93	This study
A. platensis	Modified Zarrouk + 50% POME	1 g L^{-1} $NaNO_3$	Erlenmeyer flask	Mixotrophic	50% POME	3	1.8	32	This study
A. platensis	Modified Zarrouk + 50% POME	1 g L^{-1} $NaNO_3$	Erlenmeyer flask	Mixotrophic	UV-C irradiation (5 min, 20 W m^{-2})	3	7.26	15.84	This study

PHB and C-PC productivity are calculated based on the concentration divided by the cultivation time. NA means not applicable. TN means initial nitrogen concentration in the medium

has a higher PHB content and lower growth rate compared to the control (without UV-C irradiation). This indicated that growth was interrupted by UV-C irradiation. The UV-C irradiation resulted in PHB accumulation, up to tenfold times higher compared to the control without UV-C irradiation. However, the CP-C content was up to fourfold times lower compared to the control without UV-C irradiation. Kavitha et al. (2016) also reported that UV-B irradiation (3 W m^{-2} for 10 min) resulted in high PHB accumulation. Similarly, total lipids of *Nannochloropsis* sp. and *Chlorella* sp. were significantly influenced by UV-C irradiation at or above 250 mJ cm^{-2} (Sharma et al. 2015, 2014). It has also been reported that UV-C irradiation resulted in higher carotenoid accumulation in microalgae because of induced DNA damage, and promotion of mutations in cells (Ikehata and Ono, 2011; Ahmed et al., 2015; Yi et al., 2015). UV-C irradiation can also reduce photosynthesis due to the degradation of the pigments in cyanobacteria (i.e., phycocyanin, phycoerythrin) (Li et al. 2020) as well as influencing lipid production in cyanobacteria where the lipid production is associated to PHB accumulation (Monshupanee et al., 2014; Choi et al., 2017). UV-C may induce DNA and protein damage in cyanobacteria by direct photochemical reaction or by indirect oxidative damage from reactive oxygen species (ROS) (Rinalducci et al. 2006).

Comparison of different treatments

In the present study, the utilization of 50% POME diluted with modified Zarrouk medium and using 5 min 20 W m^{-2} UV-C irradiation resulted in 7 mg L^{-1} day PHB and 16 mg L^{-1} day^{-1} C-PC productivity. This is slightly higher compared to a previous study which reported that the cultivation of *A. platensis* with added pure glycerol resulted in 1% PHB and 4% C-PC (around 2.5 mg L^{-1} day^{-1} and 10 mg L^{-1} day^{-1} of PHB and C-PC productivity) (Correa and Teixeira; 2021). Other studies have reported 2 to 3 mg L^{-1} day^{-1} PHB productivity in different cultivation treatments such as by using mixotrophic cultivation, UV-B irradiation, and nutrient deprivation. Overall, the modification of nutritional and environmental conditions enhanced PHB productivity (Table 3).

In the present study the cost of modified Zarrouk medium could be reduced by adding 50% POME to the culture medium, thus affecting the production cost of PHB and C-PC. By modifying cultivation conditions, PHB productivity could be enhanced, while on the other hand, the productivity of C-PC could be maintained.

Conclusion

This work reported the utilization of POME as medium for the growth of *A. platensis* to obtain PHB and C-PC for the first time by employing UV-C irradiation. The addition of POME to modified Zarrouk medium enhanced PHB and C-PC accumulation, particularly PHB accumulation. The highest PHB and C-PC production was found with 50% POME and 5 min UV-C irradiation. By using this method, co-production can be achieved and the wastewater could be utilized as a growth medium.

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Data availability The data generated during and/or analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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