Enhancement of phycocyanin and carbohydrate production from Spirulina platensis growing on tofu wastewater by employing mixotrophic cultivation condition

by Muhamad Nur

Biocatalysis and Agricultural Biotechnology 47 (2023) 102600

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Enhancement of phycocyanin and carbohydrate production from *Spirulina platensis* growing on tofu wastewater by employing mixotrophic cultivation condition

Muhamad Maulana Azimatun Nur[a,b,*], Shelina Dewi Rahmawati[a], Indri Wulan Sari[a], Zubaidi Achmad[a], Tutik Muji Setyoningrum[a], Danang Jaya[a], Sri Wahyu Murni[a], Ira Nurhayati Djarot[b]

[a] *Chemical Engineering Department, Faculty of Industrial Engineering, UPN Veteran Yogyakarta, Jl. Padjajaran 104, Condongcatur, Sleman, Yogyakarta, Indonesia*
[b] *Research Center for Sustainable Production System and Life Cycle Assessment, National Research and Innovation Agency – BRIN Indonesia, Gedung Pusat Inovasi dan Bisnis Teknologi, No.720, Kawasan Puspiptek Serpong, Tangerang Selatan, Banten, Indonesia*

ARTICLE INFO

Handling Editor: Dr. Ching Hou

Keywords:
Spirulina platensis
Tofu wastewater
C-phycocyanin
Carbohydrate production
Mixotrophic

ABSTRACT

The high carbohydrate content of readily commercialized microalgae, *Spirulina platensis*, has gained more attention since it could be utilized as a source of bioethanol. However, the high cost of cultivation needs to be pressed to lower the bioethanol price. The purpose of this study was to study the potency of *Spirulina platensis* growing in tofu wastewater (TWW) as C-phycocyanin (C-PC) and carbohydrate feedstock under mixotrophic conditions. *S. platensis* was cultivated on different TWW fractions and different organic carbon sources. Box-behnken response surface methodology was employed to optimize organic carbon concentration, UV-C irradiation, and FeEDTA addition. Results showed that glucose addition on 15% TWW accelerates carbohydrate and C-PC productivity. Box-behnken RSM revealed that 0.67 g/L glucose, 5 min UV-C irradiation time, and 10 mg/L FeEDTA resulted in high carbohydrate (49.94 mg/L/d) and C-PC (26.93 mg/L/d) productivity. Interaction of glucose and UV-C irradiation significantly enhances carbohydrate and C-PC productivity.

1. Introduction

Microalgae have gained popularity in recent years as a potential sustainable biomass feedstock for the manufacture of a variety of high-value added goods, such as food, pharmaceuticals, cosmetics, biofuels, animal and aquaculture feed, natural colors, etc (Nur and Buma, 2019). Large-scale accumulation of an abundance of algal biomass is necessary for the industrial manufacturing of microalgae-based products. The high expense, however, greatly limits the production of microalgae on a wide scale. For instance, the cost of fixed assets, energy usage during the cultivation and harvesting process, and raw materials are significant for pilot or industrial scale manufacturing (Tawfik et al., 2022). Additionally, a lot of water and nutrients are required for the culture process. The use of chemical fertilizers and ordinary water as a production medium appears to be unsustainable, especially when producing low-cost products like biofuels, fertilizer, or biopolymer. The expense of large-scale microalgae production can be decreased by substituting digestate,

* Corresponding author. Chemical Engineering Department, Faculty of Industrial Engineering, UPN Veteran Yogyakarta, Jl. Padjajaran 104, Condongcatur, Sleman, Yogyakarta, Indonesia.
E-mail address: lanaazim@upnyk.ac.id (M.M.A. Nur).

https://doi.org/10.1016/j.bcab.2023.102600
Received 8 October 2022; Received in revised form 9 December 2022; Accepted 2 January 2023
Available online 4 January 2023

Table 1
Growth rate (GR), carbohydrate content (C), C-phycocyanin content (C-PC), biomass productivity (P_x), C-phycocyanin productivity (P_{-CPC}), and carbohydrate productivity (P_c) of *S. platensis* cultivation under different culture media. Average values are shown. SD was shown after ± symbol. Sharing letters indicate no significant value (P > 0.05).

Culture media	P_x (mg/L/d)		GR (/day)		C-PC (%)		P_{-CPC} (mg/L/day)		C (%)		P_c (mg/L/day)	
Zarrouk	86.43	±4.28^{a}	0.32	±0.01^{a}	13.21	±0.33^{a}	11.42	±0.42^{a}	6.91	±0.30^{a}	6.33	±0.99^{a}
5% TW	217.82	±7.09^{b}	0.26	±0.03^{b}	9.12	±0.12^{b}	19.39	±0.65^{b}	10.29	±0.15^{b}	23.96	±0.49^{b}
10% TW	196.03	±11.89^{b}	0.18	±0.01^{c}	8.94	±0.02^{b}	17.53	±0.24^{b}	11.56	±0.46^{b}	22.70	±2.10^{b}
15% TW	209.37	±8.54^{b}	0.21	±0.01^{c}	8.96	±0.43^{b}	16.67	±0.13^{b}	13.77	±0.95^{b}	28.77	±1.11^{c}

wastewater for standard media (Chong et al., 2022). Furthermore, an integration concept might be applied by exploiting whole biomass into several valuable products, such as extracting pigments, while the residue can be used as biofuel products (i.e. biogas, bioethanol) (McGinn et al., 2011).

Traditional tofu is a very nutritious and easily digestible food. It has long been a global phenomenon, especially in Asian nations (Ali et al., 2021). Traditional tofu is produced by grinding the soybeans, boiling, coagulating the protein, filtering, and preserving. For every ton of processed soybeans, it generates one ton of soybean soaking wastewater, five tons of soybean yellow pulp water and ten tons of clean wastewater (Wang and Serventi, 2019). The COD (Chemical Oxygen Demand) concentration of the tofu wastewater is typically high, and is regarded as a severe environmental problem (Putro and Hadiyanto, 2021). However, tof wastewater can be used as a good substrate in the fermentation sector since it contains rich of nutrients. Tofu whey wastewater (TWW) has been used as a medium to produce a variety of products as reviewed previously (Chua and Liu, 2019). It is also thought to be a viable medium for the growth of microalgae.

According to Christwardhana and Hadiyanto (2022), Botryococcus braunii could produce 2.4 g/L dry dry biomass and 0.87 g/L of lipid on 10% TWW fraction. Nugroho et al. (2014) reported that Chlorella vulgaris could remove 58.6% phosphorus, and 84% ammonia in aerobic treated TWW when growing in raceways open ponds. Hadiyanto et al. (2019) found that the optimal growth of Spirulina sp. (0.26/day) was found on 10% pre-treated TWW using ozone/UV, while 50% TWW resulted in lower growth rate (0.18/day). Previous findings reported that the addition of TWW in the medium growth of Botryococcus braunii shifted hydrocarbon from C34H58 to C32H54 and enhanced the growth (Yonezawa et al., 2012). In It is found that TWW could enhance the biomass productivity of Chlorella pyrenoidosa two times higher compared to BG-11 medium when grown in mixotrophic condition (Wang et al., 2018). Similar to this, mixotrophic cultivation of microalgae growing on palm oil mill effluent wastewater could enhance polyhydroxy butyrate production, which is associated with biopolymer accumulation (Nur et al., 2022).

Chong et al. (2021) proposed that by using wastewater and adding external organic carbon from food waste hydrolysate might accelerate the growth and polyhydroxybutyrate accumulation of microalgae. The addition of organic carbon sources enhanced the production of pigment and lipids in green algae and diatoms when growing on wastewater (Nur, 2021). Furthermore, the accumulation of carbohydrates could be influenced by physical factors (i.e. temperature, UV irradiation) and nutrients (i.e. nitrogen depletion, and the addition of metals). It is hypothesized that the cultivation of S. platensis on TWW under mixotrophic condition might increase the production of C-phycocyanin pigment and carbohydrates. S. platensis was chosen since the strain has already commercialized and easily to be cultured in large-scale production (Christwardana et al., 2013). The purpose of this study was to utilize raw TWW as the culture medium for S. platensis in mixotrophic condition to produce C-phycocyanin and carbohydrates by investigating TWW fractions and organic carbon sources. Box-behnken response surface methodology (RSM) was employed to unravel the optimal condition for physical and nutritional factors. This study is significant because it offers a less expensive alternative for growing microalgae, lowers the chance that TWW may pollute the environment, and produces valuable compounds from microalgal biomass.

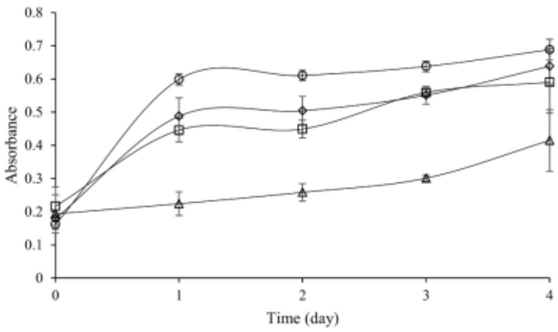

Fig. 1. Growth curve of *S. platensis* cultivated under different media. Zarrouk medium (Δ), Zarrouk + 5% tofu wastewater (□), Zarrouk + 10% tofu wastewater (◇), Zarrouk + 15% tofu wastewater (○). Average values are shown (n = 3).

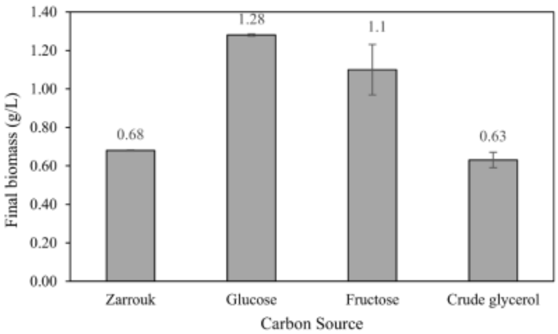

Fig. 2. Final biomass of *A. platensis* cultivated under different organic carbon source addition on Zarrouk medium + 15% TWW. Average values are shown (n = 3). Sharing letters indicate no significant value (P > 0.05).

Table 2
Growth rate (GR), carbohydrate content (C), C-phycocyanin content (C-PC), biomass productivity (P_x), C-phycocyanin productivity (P_{-CPC}), and carbohydrate productivity (P_c) of *S. platensis* cultivation on Zarrouk + 15% TWW medium under different organic carbon sources. Average values are shown. SD was shown after ± symbol. Sharing letters indicate no significant value (P > 0.05).

Carbon Source	P_x (mg/L/d)		GR (/day)		C-PC (%)		P C-PC (mg/L/day)		C (%)		PC (mg/L/day)	
Glucose	183.05	±30.04[a]	0.36	±0.01[a]	7.56	±0.13[a]	13.34	±0.45[a]	21.09	±1.50[a]	38.32	±5.41[a]
Fructose	190.04	±42.11[a]	0.49	±0.08[b]	7.65	±0.03[a]	14.54	±0.51[a]	15.07	±2.64[b]	28.43	±7.32[b]
Crude Glycerol	108.48	±14.20[b]	0.32	±0.03[c]	6.94	±0.42[b]	7.32	±0.42[b]	11.62	±1.12[c]	12.71	±3.33[c]

2. Materials and methods

2.1. Wastewater preparation

TWW was collected from a small tofu factory in Yogyakarta, Indonesia. This TWW was the filtrate from the process of protein coagulation followed by filtration. To eliminate the large-sized solid particles, TWW was filtered using GF/C filter paper. TWW was sterilized by adding 10 ppm NaOCl and settled overnight to remove the remaining chlorine. TWW was stored in a freezer to avoid denaturation. TWW was characterized according to previous research (Faisal et al., 2014; Nur et al., 2019). Tofu wastewater contained COD (7,800 mg/L), NH3-N (23.43 mg/L), and PO4-P (0.43 mg/L).

2.2. Inoculum culture

Spirulina platensis was obtained from BBPBAP culture collection, Jepara, Indonesia. The cells were inoculated in Zarrouk medium at 15 PSU salinity and incubated at 28°C in an orbital shaker at 120 rpm, exposed to 85 μmol photons m−2 s−1 of cool-white fluorescent light with 16/8-h light/dark cycle. The cells were used as an inoculum for subsequent tests after two weeks of cultivation.

2.3. Effect of TWW fractions

S. platensis was cultivated in a 500 mL polyethylene bottle under constant illumination at a light intensity of 100 μmol photons m−2 s−1 and 28°C. S. platensis (10% v/v) was inoculated in a mixture of TWW and Zarrouk medium at different fractions (0-15%). Salinity was adjusted by using commercial salt at 15 PSU, and pH was adjusted to 9.5 by using 1 M NaOH. Every three times a day, the bottles were manually shaken to mix the cultures and avoid settling. The growth was measured indirectly by using a spectrophotometer (Hach DR/2400) every day as stated previously (Nur et al., 2021). At the end of the exponential phase, samples (300 g mL) were taken for biomass, carbohydrate, and C-PC determination (see the analysis section below).

2.4. Effect of organic carbon source

S. platensis was cultivated in a 500 mL polyethylene bottle under constant illumination at a light intensity of 100 μmol photons m−2 s−1 and 28°C. S. platensis (10% v.v) was inoculated on a mixture of TWW and Zarrouk medium at the best fraction from the first experiment (section 2.3), and 500 mg/L of different external organic sources (glucose, crude glycerol, or fructose) was added. Salinity was adjusted by using commercial salt at 15 PSU, and pH was adjusted to 9.5 by using 1 M NaOH. The cultures were shaken manually three times a day to avoid setting. The growth was monitored indirectly by using a spectrophotometer (Hach DR/2400). At the end of the exponential phase (4–7 days), the cultures were harvested for dry biomass, carbohydrate, and C-PC determination (see the analysis section below).

2.5. Optimization of UV-C and organic carbon

The best organic carbon source (section 2.4) was further investigated and optimized by varying the concentrations and combining with UV-C irradiation times, and iron (FeEDTA) addition. Box-behnken RSM was employed to design the experiment (Table 3). *S. platensis* cultures were grown in 500 mL polyethylene bottles by using Zarrouk + 15% TWW. The cultures (10% inoculum) were culti-

Table 3
Observed and predicted values of carbohydrate productivity and C-phycocyanin productivity of *Spirulina platensis* growing on 15% TWW at different glucose concentrations, UV-C irradiation times, and FeEDTA concentrations. Average observed values are shown (n = 3). SD are shown after ± symbol.

Run	Glucose (g/L)	UV-C (time)	FeEDTA (mg/L)	P C (mg/L/d)			P C-PC (mg/L/d)		
				Observed		Predicted	Observed		Predicted
1	1.00	10.00	30.00	19.00	±3.23	30.40	15.43	±1.43	19.01
2	0.50	15.00	20.00	15.00	±4.23	53.82	19.23	±3.45	15.09
3	0.50	10.00	30.00	18.80	±1.34	48.61	21.23	±4.32	18.52
4	1.00	10.00	10.00	15.43	±2.54	50.15	13.54	±2.12	15.70
5	0.75	5.00	30.00	24.32	±3.65	38.25	19.43	±1.43	24.40
6	0.75	15.00	10.00	18.42	±3.21	60.63	18.42	±4.53	18.34
7	0.75	15.00	30.00	19.43	±1.20	56.27	17.54	±2.34	19.60
8	0.50	5.00	20.00	27.54	±1.11	32.22	23.45	±3.42	27.73
9	0.75	5.00	10.00	25.43	±1.93	51.39	20.45	±2.34	25.25
10	0.75	10.00	20.00	20.12	±4.32	42.81	21.43	±2.34	20.38
11	1.00	5.00	20.00	18.43	±3.24	32.97	14.56	±1.15	18.33
12	1.00	15.00	20.00	19.45	±3.43	38.64	15.64	±3.21	19.25
13	0.75	10.00	20.00	20.54	±2.34	42.81	21.32	±3.64	20.38
14	0.50	10.00	10.00	21.45	±1.54	46.37	21.45	±4.62	21.43
15	0.75	10.00	20.00	20.50	±2.12	42.81	21.54	±2.78	20.38

vated under constant illumination at a light intensity of 100 μmol photons m−2 s−1 and 28°C. Salinity was adjusted to 15 PSU, and pH was adjusted to 9 by using 1 M NaOH. The cultures were shaken manually three times a day to avoid setting. At the end of the exponential phase (4-7 days), the cultures were harvested for dry biomass, carbohydrate, and C-PC determination.

2.6. Analysis

2.6.1. Growth rate and biomass determination

Growth rate was calculated from a linear regression of the natural logarithm of absorbance that was measured at 750 nm. A regression between the biomass dry weight and the optical density at 750 nm was used from a previous study (Nur et al., 2021) (Eq (1)).

$$y = 0.59(OD750) - 0.03 \quad \text{Eq. 1}$$

where y is biomass dry weight of *S. platensis* (g/L) and OD750 is optical density at 750 nm.

The volumetric biomass productivity was calculated based on the initial and final biomass, and the duration of the cultivation at the end of exponential phase (Eq. (2)),

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

where Px is the carbohydrate productivity (mg/L/day), X_t is the final biomass concentration (mg/L), X_0 is the initial biomass concentration (mg/L), and t is the total duration of the cultivation (days).

2.6.2. C-PC determination

C-PC extraction was performed according to the previous method (Hadiyanto and Suttrisnohadi, 2016). Three hundred milligrams of dry biomass was extracted by adding 3 mL of cold buffer phosphate (pH = 6.8) followed by two times freezing and thawing, and sonication for 2 min using an ultrasonic bath. The extract was separated from the residue by centrifugation (4500 rpm, 4 °C, 30 min). The remaining residue was stored for carbohydrate determination. The concentration of C-PC in the supernatant was determined using a spectrophotometer (Hach DR/2400), by measuring the optical density at 620 nm and 652 nm (Moraes et al., 2011). The concentration of C-PC was determined in Eq. 3

$$C - PC = \frac{OD_{620} - 0.474\,(OD_{652})}{5.34} \quad \text{Eq. 3}$$

where C-PC is phycocyanin concentration (mg/ml), OD_{620} is the optical density of the extract at 620 nm, and OD_{652} is the optical density of the extract at 652 nm.

Volumetric C-PC productivity (Eq. (4)) was determined from the biomass productivity and the specific C-PC content in the biomass (Nur et al., 2019).

$$P_{c-PC} = \frac{(X_t - X_0)\,.C_{C-PC}}{t} \quad \text{Eq. 4}$$

where $C_{C\text{-}PC}$ is C-PC content (%), $P_{C\text{-}PC}$ is C-PC productivity (mg/L/day), X_t is final biomass (mg/L), X_0 is initial biomass (mg/L), and t is the total duration of the cultivation (d).

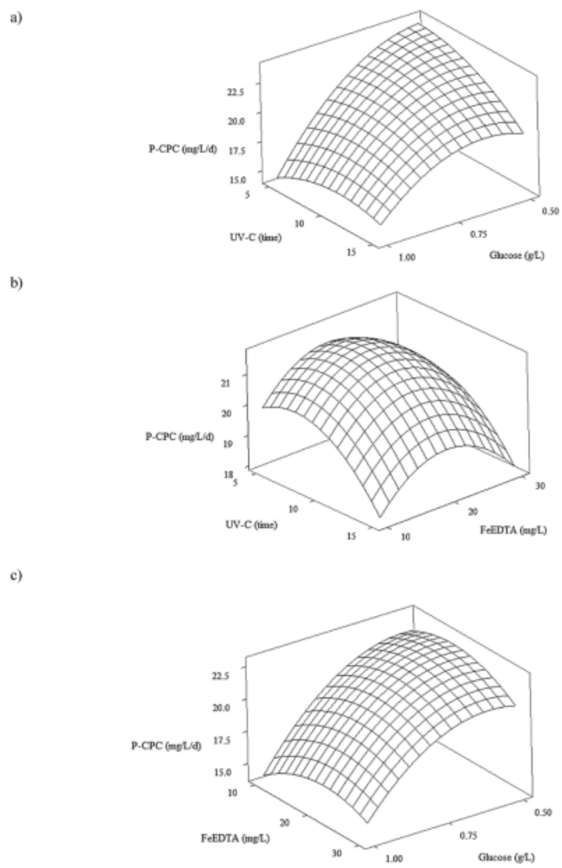

Fig. 3. Response surface of carbohydrate productivity from *Spirulina platensis* growing on 15% TWW under different UV-C irradiation time glucose concentration, and FeEDTA concentration at (a) fixed FeEDTA (20 mg/L), (b), glucose addition (0.75 g/L), and (c) UV-C irradiation time (20 min).

2.6.3. Total carbohydrate determination

Total carbohydrate content of *S. platensis* biomass was determined by using phenol sulphuric acid as stated by Dubois et al. (1956). The biomass residue after C-PC extraction (section 2.6.2) was used to determine the total carbohydrate. The biomass residue was oven dried at 80 °C for 3 h. The dry sample (200 mg) was placed in a vial bottle (5 mL). Carbohydrate extraction was carried out by adding 1 mL H_2SO_4 0.05 M in to the sample and performed at 60 °C for 1 h in a sonicator bath (60 Watt). The sample was then centrifuged at 5000 × *g* for 5 min at 28°C. The supernatant was collected to analyse total carbohydrates, using the total carbohydrate assay kit (Sigma, MAK104-1 KT) at 490 nm. D-Glucose was used as a standard curve.

The volumetric carbohydrate productivity (P_c) was calculated based on the initial and final biomass, total sugar content, and the duration of the cultivation at the end of exponential phase (Eq. (5)),

$$P_c = \frac{(X_t - X_0)\,.C}{t} \qquad \text{Eq. 5}$$

where *Pc* is the carbohydrate productivity (mg/L/day), X_t is the final biomass concentration (mg/L), X_0 is the initial biomass concentration (mg/L), C is the total sugar content per total biomass dry weight (w/w), and t is the total duration of the cultivation (days).

2.7. Statistical analysis

Minitab ver. 18 (USA) was used for statistical analysis. One-way analysis of variance (ANOVA) was used to determine the differences between treatments with P value of 0.01. Post hoc tests (Tukey HSD) were performed for pair-wise comparisons. The experimental results were obtained based on three replicates as expressed in the standard deviations (± SD).

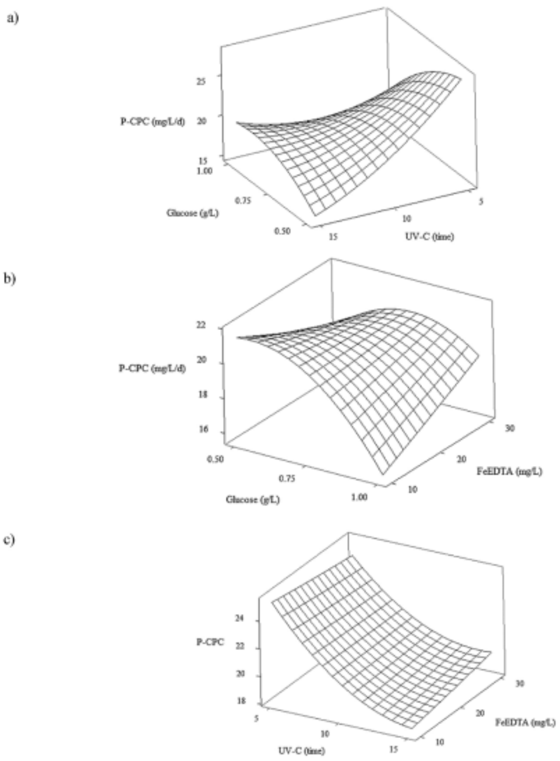

Fig. 4. Response surface of C-PC productivity from *Spirulina platensis* growing on 15% TWW under different UV-C irradiation time glucose concentration, and FeEDTA concentration at (a) fixed FeEDTA (20 mg/L), (b) UV-C irradiation time (20 min), and (c) glucose addition (0.75 g/L).

3. Results and discussion

3.1. *Effect of TWW addition*

The effect of TWW was evaluated by varying the ratio of TWW and Zarrouk medium in media cultivation. The highest growth rate (0.32/day) was recorded in S. platensis growing on Zarrouk medium (Table 1). All media supplemented with TWW fractions showed substantially lower growth rates, ranging between 0.26 and 0.21/day (Table 1, Fig. 1). Maximum growth was observed at 5% TWW: Above this fraction, growth rates decreased slightly. The growth for media supplemented with TWW resulted in a shorter exponential phase. While for Zarrouk, the growth seems to continue to grow above 4 days (Fig. 1). TWW contains macro and micronutrients that could enhance the growth of microalgae. Previous findings indicated that TWW contained proteins, oligopeptides, oligosaccharides, and some chemicals such as saponins, isoflavones, and phytic acid (Corzo-Martínez et al., 2016; Matemu et al., 2012). TWW contained carbohydrate that was mainly dominated by sucrose, fructose, glucose, and raffinose (Chua and Liu, 2019). TWW is also rich in nitrogen, phosphorous, and some metal elements, i.e. Fe, Cu, Zn, Mg, Mn, and Ca which is an excellent source for microalgae to grow (Su et al., 2011; Qiu et al., 2019). Previous research investigated the potency of pre-treated TWW by using ozone as medium growth of Spirulina sp and resulted in the enhancement of biomass production (Widayat and Hadiyanto, 2015; Hadiyanto, 2018). Another researcher found that the best medium composition for Chlorella sp. was 10% soybean wastewater supplemented with synthetic medium (Song et al., 2020). Qiu et al. (2019) found that Chlorella sp could utilize soybean wastewater at 10 times dilution. Wang et al. (2018) reported that the optimal growth of Chlorella pyrenoidosa was found in 60% of tofu wastewater. However, higher TWW contained high organic content that might inhibit growth. It is reported that high COD of palm oil mill effluent above 2000 mg/L inhibited the growth of Chlorella sp and Haematococcus pluvialis (Fernando et al., 2021). It is reported that soybean wastewater, including TWW, contains high organic carbon that consisted of soluble protein and carbohydrates that could be toxic to microalgae (Guo et al., 2018). A pretreatment process was suggested to lower organic carbon in the wastewater, thus protein and amino acids can be converted to ammonia, and carbohydrate content can be converted to simple organic carbons such as organic acids (Pan et al., 2021).

The highest biomass production was found on above 10% TWW ($P < 0.05$). While the lowest biomass was found in Zarrouk medium (0.6 g/L). The biomass productivity of TWW in all fractions did not differ significantly (Table 1) ($P > 0.05$). When TWW was supplemented with Zarrouk medium, the cultivation condition was shifted from autotrophic to mixotrophic condition (Wang et al., 2018). The presence of organic content in the medium could be utilized by S. platensis to produce higher biomass since the presence of energy sources from organic and inorganic carbon was abundant. Previous findings reported that the utilization of 10% TWW fractions resulted 2.5 g/L dry biomass of Botryococcus braunii (Christwardhana and Hadiyanto, 2022). Another finding reported that

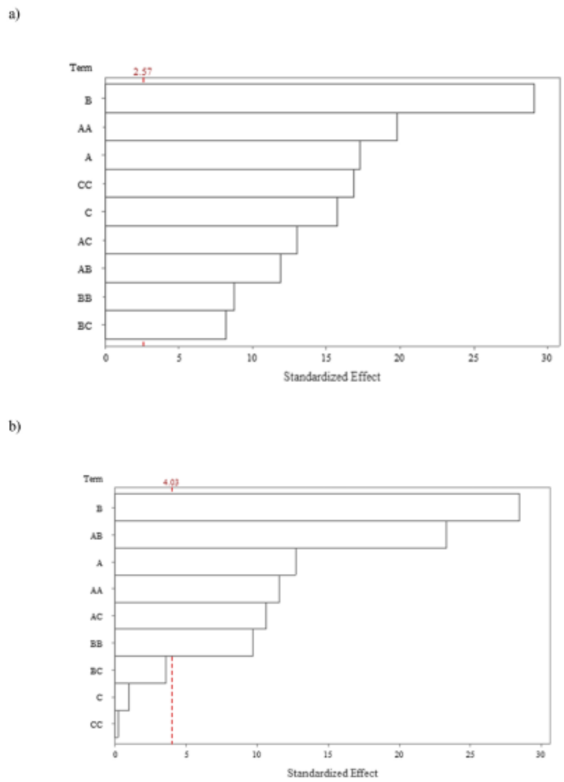

Fig. 5. Pareto chart of standardized effects a) carbohydrate productivity, and b) C-phycocyanin productivity. A is glucose concentration, B is UV-C irradiaton time, C is FeEDTA. Bars crossing vertical dashed line are significant factors ($P < 0.01$).

pure TWW could be utilized and resulted in higher biomass productivity compared to BG medium for Chlorella pyrenoidosa (Wang et al., 2018). This finding is supported by the decrease of C-PC content when TWW was supplemented to Zarrouk medium (from 13 to 8%) and the increasing of carbohydrate content from 6 to 13% (Table 1). This indicated that the growth of Spirulina platensis on Zarrouk media supplemented with TWW was in a mixotrophic condition. Previous research reported that C-PC content of Spirulina decreased when the culture was cultivated in mixotrophic condition by using wastewater or adding external organic carbon (Markou et al., 2021; Nematollahi et al., 2020). Pereira et al. (2019) reported that Spirulina platensis accumulated higher carbohydrate content when the growth was in mixotrophic condition.

3.2. Effect of external carbon source

The addition of external carbon sources to the media supplemented with TWW significantly enhanced final biomass and growth rate in all kinds of organic carbon sources compared to control (Zarrouk + 15% TWW only) (Fig. 2, Table 1). The addition of fructose resulted in the highest growth rate (0.49/day). The highest final biomass was found on glucose addition (1.2 g/L), followed by fructose (1.1 g/L). The addition of crude glycerol to the medium reduced the final biomass (0.6 g/L). Previous researchers showed that the strategy of adding external organic carbon to the wastewater medium increased the biomass productivity of microalgae since the growth was in mixotrophic condition (Pang et al., 2019; Lowrey et al., 2015). Previous researchers investigated that the supplementation of glycerol to palm oil mill effluent enhanced biomass production to 1.68 g/L and accumulate 15% lipid of Chlorella sorokiniana (Cheah et al., 2018). Chu et al. (2022) reported the enhancement biomass of Isochrysis when growing on aquaculture wastewater supplemented with glycerol. Cheah et al. (2018) reported that the growth of C. sorokiniana was better on POME when the cultivation was added with external organic carbon (glucose, and glycerol). It is found that the biomass and lipid productivity of green algae and diatom was enhanced when glycerol was added to wastewater (Nur, 2021).

For carbohydrate productivity, the highest value was recorded on the media supplemented with glucose (23 mg/L/d) compared to fructose and crude glycerol. This indicated that the higher growth rate of *S. platensis* resulted in lower carbohydrate accumulation. Microalgae may accumulate high carbohydrates or lipids when the growth was inhibited such as unfavorable nutritional and environmental conditions (Olguín et al., 2022). The presence of glucose or fructose as excess energy might be stored as a form of carbohydrate (Villanova et al., 2017). It is reported that the addition of glucose, glycerol, or acetate significantly enhanced carbohydrate accumulation in green algae and diatom (Smith et al., 2020). In this research, the addition of crude glycerol to the medium seems not effectively enhanced carbohydrate productivity. It seems that the presence of impurities such as methanol, soap, free fatty acids (FFA), and other chemical elements might be toxic for *S. platensis* thus disturbing cell growth and intracellular carbohydrate production (Kumar et al., 2019). Even though the side effect might be dependent on the concentration and the strain tolerance.

The addition of external organic carbon significantly reduced C-PC content. The lowest C-PC content was found on the medium supplemented with crude glycerol (Table 2). The addition of glucose or fructose resulted in around 13.5 mg/L/d. This result was significantly lower compared to the medium without external carbon supplementation. The addition of TWW in Zarrouk medium reduced C-PC content from 13 to 7%. In Zarrouk medium, S. platensis utilizes only light and inorganic carbon as the substrate as an energy source, and therefore, a higher C-PC level (13%) was found. S. platensis produced more pigments to capture light energy in photosynthesis reactions (Borowiak and Krzywonos, 2022). In the presence of TWW, S. platensis utilize both light, inorganic and organic carbon source for growth, thus reducing photosynthetic activity. It is reported that the phycocyanin content of Gardieria sulfaria decreased when organic carbon was presented in the medium (Abiusi et al., 2021). Previous findings also recorded that pigment content (chlorophyll-a, carotenoids, and phycocyanin) in *S. platensis* were lower in mixotrophic compared to autotrophic condition (Li et al., 2018).

3.3. Optimization of glucose, UV-C and FeEDTA

The addition of glucose to the cultivation medium resulted in the highest carbohydrate and C-PC productivity compared to other organic carbon sources. However, the high organic carbon cost would not be economically feasible for large-scale production. Further investigation is needed to optimize the concentration by using Box-behnken RSM. Other factors (UV-C irradiation and FeEDTA) that may induce carbohydrate and C-PC productivity were also studied (Table 3). The interaction of high UV-C irradiation time and low glucose concentration or high FeEDTA concentration and low glucose concentration significantly enhanced carbohydrate productivity (Fig. 3). The interaction of high UV-C irradiation time and low FeEDTA concentration also induced carbohydrate productivity. FeEDTA addition did not significantly influence C-PC productivity. Low glucose addition at high FeEDTA concentration lower C-PC productivity, while UV-C irradiation at low level and high glucose addition significantly lower C-PC productivity (Fig. 4). Overall, low glucose addition could promote C-PC productivity at low FeEDTA concentration or low UV-C irradiation time. The interaction of UV-C and FeEDTA did not significantly influence C-PC productivity (Fig. 5). It is shown that the optimal condition to reach the highest carbohydrate (49.94 mg/L/d) and C-PC productivity (26.93 mg/L/d) were 0.67 g/L glucose, 5 min UV-C irradiation time, and 10 mg/L FeEDTA (Figs. 3 and 4).

External organic carbon addition is important to enhance the biomass productivity of microalgae. However, pigment content that is important to capture light would be reduced since the condition requires less light as a source of energy (Abreu et al., 2022; Nur, 2021; Narayan et al., 2005). In the excess of energy, microalgae accumulate energy in the form of lipids or carbohydrates (Chambonniere et al., 2022; Baicha et al., 2016). Previous findings reported that the addition of 0.88 g/L glucose coupled with yellow LED light could induce phycocyanin production (Nosratimovafagh et al., 2022). Cordeiro et al. (2021) found that the interaction of magnetic fields and the addition of external carbon to Zarrouk medium could accelerate carbohydrate and pigment production. In this experiment, the addition of glucose coupled with UV-C and FeEDTA could accelerate carbohydrate 8-fold and C-PC productivity 2.5-fold compared to Zarrouk medium only. Zaparoli et al. (2020) stated that UV-C irradiation coupled with iron enhanced carbohydrate synthesis up to 55.85 mg/L/d of Spirulina platensis. Costa et al. (2021) reported that UV-C irradiation is a good strategy to alter the chemical composition of microalgae (i.e. accumulate lipids or carbohydrates). Iron also plays important role in carbohydrate and pigment production. Previous researchers found that the addition of iron to the cultivation medium accelerated carbohydrate production in Dunaliella and Alexadrium tamarense (He et al., 2010; Rizwan et al., 2017). By combining UV-C and iron, the concentration of glucose was reduced from 1 to around 0.5 g/L to obtain the highest carbohydrate and C-PC productivity. It seems that the presence of external organic carbon in the cultivation medium has changed the carbon to nitrogen ratio. High carbon-to-nitrogen ratio is needed in the cultivation when using wastewater as a growth medium to accelerate the growth and remove more nutrients (Gao et al., 2019). In this experiment, the interaction of glucose and UV-C irradiation has a positive effect on carbohydrate accumulation. UV-C irradiation attacks DNA and RNA, which leads to cell damage (Sydney et al., 2018). Furthermore, the presence of iron can also act as an inducer, thus, disrupting the photosynthetic reaction when the iron concentration is accelerated. Thus, C-PC productivity was lower. Previous findings reported that microalgae accumulate higher carbohydrates or lipids as storing energy when the growth is in a stressed condition (Debnath et al., 2021; Poh et al., 2020). By adding iron at a low concentration, the production of C-PC could be maintained at the desired concentration.

4. Conclusion

TWW supplemented to Zarrouk medium has significantly enhanced carbohydrate and C-PC productivity. Glucose addition resulted higher carbohydrate and C-PC productivity compared to fructose and crude glycerol. Optimization by using Box-behnken RSM revealed that the optimal condition was found on 0.67 g/L glucose, 5 min UV-C irradiation time, and 10 mg/L FeEDTA to reach carbohydrate (49.94 mg/L/d) and C-PC (26.93 mg/L/d). The interaction of glucose and UV-C irradiation significantly influenced carbohydrate and C-PC productivity. We purpose that mixotrophic cultivation on TWW coupled with physical and nutritional factors could enhance valuable compounds from *S. platensis*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

References

Abreu, A.P., Morais, R.C., Teixeira, J.A., Nunes, J., 2022. A comparison between microalgal autotrophic growth and metabolite accumulation with heterotrophic, mixotrophic and photoheterotrophic cultivation modes. Renew. Sustain. Energy Rev. 159, 112247.

Ali, F., Tian, K., Wang, Z.X., 2021. Modern techniques efficacy on tofu processing: a review. Trends Food Sci. Technol. 116, 766–785.

Baicha, Z., Salar-García, M.J., Ortiz-Martínez, V.M., Hernández-Fernández, F.J., De los Ríos, A.P., Labjar, N., et al., 2016. A critical review on microalgae as an alternative source for bioenergy production: a promising low cost substrate for microbial fuel cells. Fuel Process. Technol. 154, 104–116.

Borowiak, D., Krzywonos, M., 2022. Bioenergy, biofuels, lipids and pigments—research trends in the use of microalgae grown in photobioreactors. Energies 15 (15), 5357.

Chambonniere, P., Ramírez-Romero, A., Dimitriades-Lemaire, A., Sassi, J.F., Delrue, F., 2022. Photosynthetic carbon uptake correlates with cell protein content during lipid accumulation in the microalga Chlorella vulgaris NIES 227. Fermentation 8 (11), 614.

Cheah, W.Y., Show, P.L., Juan, J.C., Chang, J.S., Ling, T.C., 2018. Enhancing biomass and lipid productions of microalgae in palm oil mill effluent using carbon and nutrient supplementation. Energy Convers. Manag. 164, 188–197.

Chong, J.W.R., Khoo, K.S., Yew, G.Y., Leong, W.H., Lim, J.W., Lam, M.K., et al., 2021. Advances in production of bioplastics by microalgae using food waste hydrolysate and wastewater: a review. Bioresour. Technol. 342, 125947.

Chong, C.C., Cheng, Y.W., Ishak, S., Lam, M.K., Lim, J.W., Tan, I.S., et al., 2022. Anaerobic digestate as a low-cost nutrient source for sustainable microalgae cultivation: a way forward through waste valorization approach. Sci. Total Environ. 803, 150070.

Christwardana, M., Hadiyanto, H., 2022. Cultivating microalgae Botryococcus braunii in tofu whey medium. J. Bioresour. Environ. Sci. 1 (1), 12–19.

Christwardana, M., Nur, M.M.A., Hadiyanto, H., 2013. *Spirulina platensis*: potensinya sebagai bahan pangan fungsional. J. Apl. Tek. Pang. 2 (1).

Chu, R., Ma, J., Zhou, C., Liu, D., Wang, G., Ruan, R., et al., 2022. Improved growth of bait microalgae Isochrysis and aquacultural wastewater treatment with mixotrophic culture. Bioproc. Biosyst. Eng. 45 (3), 589–597.

Chua, J.Y., Liu, S.Q., 2019. Soy whey: more than just wastewater from tofu and soy protein isolate industry. Trends Food Sci. Technol. 91, 24–32.

Cordeiro, A.P., Nogueira, A.O.D.M., Salgado, H.Z., Kokuszi, L.T., Costa, J.A., de Lima, V.R., Santos, L.O., 2021. Simultaneous application of mixotrophic culture and magnetic fields as a strategy to improve Spirulina sp. LEB 18 phycocyanin synthesis. Curr. Microbiol. 78 (12), 4014–4022.

Corzo-Martínez, M., García-Campos, G., Montilla, A., Moreno, F.J., 2016. Tofu whey permeate is an efficient source to enzymatically produce prebiotic fructooligosaccharides and novel fructosylated α-Galactosides. J. Agric. Food Chem. 64, 4346–4352.

Costa, J.A.V., Moreira, J.B., Izaguirres, G.G., Teixeira, L.M., de Morais, M.G., 2021. Microalgae-Based UV Protection Compounds. Bioprospecting of Microorganism-Based Industrial Molecules, pp. 201–224.

Debnath, C., Bandyopadhyay, T.K., Bhunia, B., Mishra, U., Narayanasamy, S., Muthuraj, M., 2021. Microalgae: sustainable resource of carbohydrates in third-generation biofuel production. Renew. Sustain. Energy Rev. 150, 111464.

Faisal, M., Mulana, F., Alam, P.N., Daimon, H., 2014. Wastewater characteristics from tofu processing facilities in Banda Aceh. In: Proceedings of the Annual International Conference, vol. 4. Syiah Kuala University-Life Sciences & Engineering Chapter, pp. 118–121.

Fernando, J.S.R., Premaratne, M., Dinalankara, D.M.S.D., Perera, G.L.N.J., Ariyadasa, T.U., 2021. Cultivation of microalgae in palm oil mill effluent (POME) for astaxanthin production and simultaneous phycoremediation. J. Environ. Chem. Eng. 9 (4), 105375.

Gao, F., Yang, H.L., Li, C., Peng, Y.Y., Lu, M.M., Jin, W.H., et al., 2019. Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae Chlorella sp. Bioresour. Technol. 282, 118–124.

Guo, K., Shang, Y., Gao, B., Xu, X., Lu, S., Qi, Q., 2018. Study on the treatment of soybean protein wastewater by a pilot-scale IC-A/O coupling reactor. Chem. Eng. J. 343, 189–197.

Hadiyanto, H., 2018. Ozone application for tofu waste water treatment and its utilisation for growth medium of microalgae Spirulina sp. In: E3S Web of Conferences, vol. 31. EDP Sciences.

Hadiyanto, H., Suttrisnorhadi, S., 2016. Response surface optimization of ultrasound assisted extraction (UAE) of phycocyanin from microalgae *Spirulina platensis*. Emir. J. Food Agric. 227–234.

Hadiyanto, H., Digda, A., Adetya, N.P., Evanty, M., 2019. Pretreatment of herbal, tofu and fertilizer waste using UV/ozon technique and its utilization for *Spirulina* sp cultivation. In: *IOP Conference Series: Earth and Environmental Sci.* 248. IOP Publishing.

He, H., Chen, F., Li, H., Xiang, W., Li, Y., Jiang, Y., 2010. Effect of iron on growth, biochemical composition and paralytic shellfish poisoning toxins production of Alexandrium tamarense. Harmful Algae 9 (1), 98–104.

Kumar, L.R., Yellapu, S.K., Tyagi, R.D., Zhang, X., 2019. A review on variation in crude glycerol composition, bio-valorization of crude and purified glycerol as carbon source for lipid production. Bioresour. Technol. 293.

Lowrey, J., Brooks, M.S., McGinn, P.J., 2015. Heterotrophic and mixotrophic cultivation of microalgae for biodiesel production in agricultural wastewaters and associated challenges—a critical review. J. Appl. Phycol. 27 (4), 1485–1498.

Markou, G., Diamantis, A., Arapoglou, D., Mitrogiannis, D., González-Fernández, C., Unc, A., 2021. Growing Spirulina (Arthrospira platensis) in seawater supplemented with digestate: trade-offs between increased salinity, nutrient and light availability. Biochem. Eng. J. 165, 107815.

Matemu, A.O., Katayama, S., Kayahara, H., Murasawa, H., Nakamura, S., 2012. Improving surface functional properties of tofu whey-derived peptides by chemical modification with fatty acids. J. Food Sci. 77, 333–339.

McGinn, P.J., Dickinson, K.E., Bhatti, S., Frigon, J.C., Guiot, S.R., O'Leary, S.J., 2011. Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. Photosynth. Res. 109 (1), 231–247.

Moraes, C.C., Sala, L., Cerveira, G.P., Kalil, S.J., 2011. C-phycocyanin extraction from Spirulina platensis wet biomass. Braz. J. Chem. Eng. 28, 45–49.

Narayan, M.S., Manoj, G.P., Vatchravelu, K., Bhagyalakshmi, N., Mahadevaswamy, M., 2005. Utilization of glycerol as carbon source on the growth, pigment and lipid production in Spirulina platensis. Int. J. Food Sci. Nutr. 56 (7), 521–528.

Nematollahi, M.A., Laird, D.W., Hughes, L.J., Raeisossadati, M., Moheimani, N.R., 2020. Effect of organic carbon source and nutrient depletion on the simultaneous production of a high value bioplastic and a specialty pigment by Arthrospira platensis. Algal Res. 47, 101844.

Nosratimovafagh, A., Fereidouni, A.E., Krujatz, F., 2022. Modeling and optimizing the effect of light color, sodium chloride and glucose concentration on biomass production and the quality of arthrospira platensis using response surface methodology (RSM). Life 12 (3), 371.

Nugroho, W.A., Hermanto, M.B., Lutfi, M., Fakhri, M., 2014. Phosphorus removal of tofu processing wastewater in recirculated raceway pond bioreactor by *Chlorella vulgaris*. Nat. Environ. Pollut. Technol. 13 (4), 859.

Nur, M.M.A., 2021. Co-production of fucoxanthin and lipid from Indonesian diatom and green algae growing on palm oil mill effluent under mixotrophic condition. Biocatal. Agric. Biotechnol. 38, 102228.

Nur, M.M.A., Buma, A.G., 2019. Opportunities and challenges of microalgal cultivation on wastewater, with special focus on palm oil mill effluent and the production of high value compounds. Waste Biomass Valor 10 (8), 2079–2097.

Nur, M.M.A., Swaminathan, M.K., Boelen, P., Buma, A.G.J., 2019. Sulfated exopolysaccharide production and nutrient removal by the marine diatom *Phaeodactylum tricornutum* growing on palm oil mill effluent. J. Appl. Phycol. 31 (4), 2335–2348.

Nur, M.M., Garcia, G.M., Boelen, P., Buma, A.G., 2021. Influence of photodegradation on the removal of color and phenolic compounds from palm oil mill effluent by *Arthrospira platensis*. J. Appl. Phycol. 33 (2), 901–915.

Nur, M.M.A., Yuliestyan, A., Irfandy, F., Setyoningrum, T.M., 2022. Nutritional factors influence polyhydroxybutyrate in microalgae growing on palm oil mill effluent. J. Appl. Phycol. 34 (1), 127–133.

Olguín, E.J., Sánchez-Galván, G., Arias-Olguín, I.I., Melo, F.J., González-Portela, R.E., Cruz, L., et al., 2022. Microalgae-based biorefineries: challenges and future trends

to produce carbohydrate enriched biomass, high-added value products and bioactive compounds. Biology 11 (8), 1146.
Pan, M., Zhu, X., Pan, G., Angelidak, I., 2021. Integrated valorization system for simultaneous high strength organic wastewater treatment and astaxanthin production from Haematococcus pluvialis. Bioresour. Technol. 326, 124761.
Pang, N., Gu, X., Chen, S., Kirchhoff, H., Lei, H., Roje, S., 2019. Exploiting mixotrophy for improving productivities of biomass and co-products of microalgae. Renew. Sustain. Energy Rev. 112, 450–460.
Poh, Z.L., Kadir, W.N.A., Lam, M.K., Uemura, Y., Suparmaniam, U., Lim, J.W., et al., 2020. The effect of stress environment towards lipid accumulation in microalgae after harvesting. Renew. Energy 154, 1083–1091.
Putro, P.G.L., Hadiyanto, H., 2021. Water quality parameters of tofu wastewater: a review. In: IOP Conference Series: Materials Sci. Eng, vol. 1156. IOP Publishing. 1.
Qiu, Y., Zu, Y., Song, C., Xie, M., Qi, Y., Kansh, Y., et al., 2019. Soybean processing wastewater purification via *Chlorella* L166 and L38 with potential value-added ingredients production. Bioresour. Technol. Rep. 7.
Rizwan, M., Mujtaba, G., Lee, K., 2017. Effects of iron sources on the growth and lipid/carbohydrate production of marine microalga Dunaliella tertiolecta. Biotechnol. Bioproc. Eng. 22 (1), 68–75.
Smith, J.P., Hughes, A.D., McEvoy, L., Day, J.G., 2020. Tailoring of the biochemical profiles of microalgae by employing mixotrophic cultivation. Bioresour. Technol. Rep. 9.
Song, C., Han, X., Qiu, Y., Liu, Z., Li, S., Kitamura, Y., 2020. Microalgae carbon fixation integrated with organic matters recycling from soybean wastewater: effect of pH on the performance of hybrid system. Chemosphere 248, 126094.
Su, H., Zhang, Y., Zhang, C., Zhou, X., Li, J., 2011. Cultivation of *Chlorella pyrenoidosa* in soybean processing wastewater Bioresour. Technol. 102, 9884–9890.
Sydney, T., Marshall-Thompson, J.A., Kapoore, R.V., Vaidyanathan, S., Pandhal, J., Fairclough, J.P.A., 2018. The effect of high-intensity ultraviolet light to elicit microalgal cell lysis and enhance lipid extraction. Metabolites 8 (4), 65.
Tawfik, A., Niaz, H., Qadeer, K., Qyyum, M.A., Liu, J.J., Lee, M., 2022. Valorization of algal cells for biomass and bioenergy production from wastewater: sustainable strategies, challenges, and techno-economic limitations. Renew. Sustain. Energy Rev. 157, 112024.
Wang, Y., Serventi, L., 2019. Sustainability of dairy and soy processing: a review on wastewater recycling. J. Clean. Prod. 237.
Wang, S.K., Wang, X., Miao, J., Tian, Y.T., 2018. Tofu whey wastewater is a promising basal medium for microalgae culture. Bioresour. Technol. 253, 79–84.
Widayat, W., Hadiyanto, H., 2015. Pemanfaatan limbah cair industri tahu untuk produksi biomassa mikroalga nannochloropsis sp sebagai bahan baku biodiesel. Reaktor 15 (4), 253–260.
Yonezawa, N., Matsuura, H., Shiho, M., Kaya, K., Watanabe, M.M., 2012. Effects of soybean curd wastewater on the growth and hydrocarbon production of *Botryococcus braunii* strain BOT-22. Bioresour. Technol. 109, 304–307.
Zaparoli, M., Ziemniczak, F.G., Mantovani, L., Costa, J.A.V., Colla, L.M., 2020. Cellular stress conditions as a strategy to increase carbohydrate productivity in Spirulina platensis. BioEnergy Research 13 (4), 1221–1234.

Enhancement of phycocyanin and carbohydrate production from Spirulina platensis growing on tofu wastewater by employing mixotrophic cultivation condition

ORIGINALITY REPORT

10%	10%	6%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Muhamad Maulana Azimatun Nur, Ira Nurhayati Djarot, Nugroho Adi Sasongko, Agusta Samodra Putra, Hadiyanto. "Co-cultivation of Chaetoceros calcitrans and Arthrospira platensis growing on palm oil mill effluent under outdoor condition to produce fucoxanthin and c-phycocyanin", Biocatalysis and Agricultural Biotechnology, 2023 Publication	5%
2	www.researchgate.net Internet Source	5%

Exclude quotes On
Exclude bibliography On
Exclude matches < 5%