Co-cultivation of Chaetoceros calcitrans and Arthrospira platensis

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Co-cultivation of *Chaetoceros calcitrans* and *Arthrospira platensis* growing on palm oil mill effluent under outdoor condition to produce fucoxanthin and c-phycocyanin

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ABSTRACT

Fucoxanthin from microalgae is promising since the productivity is higher compared to macroalgae. However, dynamic environmental and nutritional factors in large scale and outdoor condition still limiting the production. This research investigated co-cultivation of *Chaetoceros calcitrans* and *Arthrospira platensis* growing under outdoor condition and evaluated the pigment production when using palm oil mill effluent (POME) as medium growth. Results showed that co-cultivation strategy produced abundant biomass when growing up to 30 PSU salinity. The addition of POME to cultivation medium reduced fucoxanthin production, while C-phycocyanin (C-PC) production could be maintained. Mixed culture produced 27 mg/L C-PC and 5.4 mg/L fucoxanthin by using synthetic medium. The optimal condition for co-cultivation was 33% POME, 100 mg/L urea, and 17 PSU salinity to produce 1.7 mg/L fucoxanthin and 25 mg/L C-PC.

1. Introduction

Fucoxanthin is a kind of pigment from algae that is important as anti-obesity, antidiabetic and anti-inflammatory activities. It shows preventive effects on various types of cancers and tumor (Leong et al., 2022). The research on the health benefits of fucoxanthin in pharmaceutical and the applications in food and cosmetic industries has been reviewed previously (Lourenço-Lopes et al., 2021; Pajotet al., 2022; Silva et al., 2022). Due to the wide range of applications, the price of the purified fucoxanthin has been estimated at around 22.95 €/mg (Singh et al., 2022; Joel, 2016). Large-scale production of fucoxanthin currently comes mainly from macroalgae such as brown algae (Din et al., 2022). Microalgae could produce higher fucoxanthin content, grow faster and cultivation time is shorter than macroalgae (Mohamadnia et al., 2021) . Therefore, microalgae are promising as a source of fucoxanthin. However, due to the high cost of nutrition and the cultivation process, the production of fucoxanthin from microalgae seems difficult to compete with macroalgae. Therefore it is necessary to seek strategies on cultivating microalgae in outdoor and large scale condition to produce high biomass, and utilize low nutritional costs such as using wastewater lower the price (Saratale et al., 2022).

Previous researchers have demonstrated the use of palm oil mill effluent (POME) as a medium growth for microalgae to produce valuable compounds (Nur and Buma, 2019; Nur et al., 2022a,b). Microalgae do not compete with other agricultural products, do not require arable land for cultivation, can be grown using non-potable water (sewage, agricultural wastewater, seawater, etc.) and are

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highly productive compared to other agricultural products. Therefore, it is a promising renewable, sustainable and valuable source (Udayan et al., 2022; Abdelfattah et al., 2022). The utilization of wastewater for microalgae to produce high biomass and other valuable products appears promising because the production costs can be minimized (Sun et al., 2022). Previous research reported the potency of diatom growing on POME to produce fucoxanthin (Nur et al., 2019; M. M. A. Nur, 2021). Fernando et al. (2021) reported the utilization of POME as medium growth of *Haematococcus pluvialis* and *Chromochloris zofingiensis* to produce pigment astaxanthin. Previous study reported the potency of POME as medium growth of *A. Platensis* to produce polyhydroxybutyrate and pigment C-phycocyanin (Nur, 2022). However, all these findings were performed under controlled nutritional and environmental conditions which were optimized for the growth. Several challenges such as wastewater quality, dynamic changes in environmental conditions, and the effects of pollutant availability would affect biomass quality and quantity of microalgae when grown outdoors such as in open ponds and on a large scale production (Mattsson et al., 2021; Del Campo et al., 2007). Therefore, scaling microalgal cultures to wastewater using monocultures is difficult and laborious to maintain (Stirk et al., 2021; Lage et al., 2021).

It has been reported that co-cultivation (polyculture) of microalgae with bacteria, fungi, yeast or other algae can solve the problems (Alam et al., 2022; Liu et al., 2022). In co-culture conditions, microalgae can grow symbiotically and better adapt to dynamic environmental and nutrient conditions (Ray et al., 2022). Nutrients and environmental conditions are important for microalgal growth when cultures are carried out in a large scale (Nur et al., 2019). Previous research found that the co-cultivation approach using cyanobacteria—*Leptolyngbya tenuis* and green alga—*Chlorella ellipsoidea* enhanced biomass productivity, CO_2 fixation, lipid production, and cadmium bioremediation efficiency (Satpati and Pal, 2021a,b). It has been hypothesized that co-cultivation of diatom and cyanobacteria on POME and in outdoor conditions could generate high biomass and pigment production, while the nutritional cost could be decreased. In this study, we examined monoculture and mixed cultures of cyanobacteria-*Arthrospira platensis*, and diatom-*Chaetoceros calcitrans* under outdoor conditions using synthetic media and POME. Optimal nutritional and environmental conditions were also investigated using response surface methodology (RSM). *A. platensis* was chosen since the strain could grow on high POME fraction and produce high C-phycocyanin (C-PC) pigment.

2. Material and methods

2.1. Experimental setup

Two commercially microalgae strains were used: *Arthrospira platensis*, and *Chaetoceros calciltrans*. The strains were purchased from BBPBAP Jepara Central Java, Indonesia. *Chaetoceros calcitrans* culture was maintained on Guillard medium (Guillard, 1975) at 25 PSU salinity. *Arthrospira platensis* was maintained on Zarrouk medium (Zarrouk, 1966) at 15 PSU salinity.

2.2. Pre-treatment of POME

POME was collected from a small factory in Sumatra, Indonesia. POME was obtained from final discharge of a traditional open pond treatment after being digested and aerated to lower organic compounds. POME contained 2100 mg/L chemical oxygen deman (COD), 220 mg/L total nitrogen, and 4 mg/L dissolved phosphate. POME was pretreated using GF/C filter paper (Whatman) to remove suspended solid and then autoclaved at 121 °C for 15 min (Nur et al., 2019).

2.3. Microalgae cultivation in synthetic medium

Cultivation was done in two stages: indoor and outdoor condition. For indoor cultures, microalgae were cultured in 1.5 L polyethylene bottles (1 L working volume) at 28 °C, pH was maintained at 8.5 with 0.5 N NaOH or HCl, and light intensity was maintained at 200 μmol photon m^{-2} s^{-1} in 16:8 day and night shift with fluorescent lighting (Philips, Indonesia). Salinity was optimally adjusted using commercial salt as previously described. The bottle was shaken by hand 3 times a day to mix the cultures. After reaching log phase, the microalgae were transferred to 200 ml (150 ml working volume) clear sterile polypropylene injection bags and the cultures were acclimated outdoors.

Microalgae acclimation is being conducted at Center for Biomass and Renewable Energy, UNDIP Tembalang, Semarang (location: 7°03′16.0″ S 110°26′23.5″E). A sterile infusion bag containing 10% v/v indoor microalgae and the medium was immersed in a 100 L water tank. The container was equipped with a shading mesh to reduce light penetration, reaching below 300 μmol photons m^{-2} s^{-1}. Light intensity was measured on the water surface in the tank. Salinity and pH were adjusted as previously described. The infusion bag was shaken by hand 3 times a day to mix the culture. Microalgae growth was monitored using a spectrophotometer (see analysis section below). Acclimatization was performed when the cultures reached the end of the exponential growth phase and were ready to be used as inoculum for further experiments. For co-cultivation, microalgae were grown by using 5% of total 10% v/v the acclimatized microalgae strains (0.4 optical density at 750 nm). Synthetic medium was used for co-cultivation condition contained 500 mg/L $NaHCO_3$, 100 mg/L $NaNO_3$, KH_2PO_4 10 mg/L, 1 mg/L Na_2SiO_3, and 1 mg/L trace elements from Guillard medium. Salinity were varied (15,20,30 PSU) to understand the effect on biomass and pigment production.

2.4. Effect of POME

Cultivation was carried out in outdoor condition by using 200 mL polypropylene bags as mentioned above (section 2.3). POME fractions were varied (30–60%) and diluted using double distilled water. The fraction was enriched with 1 mg/L Na_2SiO_3. Ten percent of acclimatized microalgae were used for inoculum (0.4 optical density at 750 nm). Light intensity was maintained below 300 μmol photons m^{-2} s^{-1} by using shading mesh. Initial pH was set to 8 by using 1 N NaOH or 1 HCl. The cultures were shaken manually to mix the media every day. The growth was evaluated by measuring the cells indirectly using a spectrophotometer at 750 nm.

To avoid the interference of POME, the cells were pretreated as mentioned previously (Nur et al., 2021). At the end of exponential phase, the cultures were harvested and the COD content in the effluent was evaluated.

2.5. Optimization of POME, salinity, and urea

Box-behnken response surface methodology (RSM) was used to design the experiment (Table 3). POME: 30–50% v/v (diluted using synthetic medium), urea: 100–400 mg/L, and salinity: 15–30 PSU were used to optimize pigment production. Salinity was adjusted by using a commercial salt, pH was controlled in 8.5 using 0.5M NaOH or 0.5 HCl, and POME was diluted using double distilled water. The sterile bags were placed in a water basin at outdoor condition as stated previously. The growth of microalgae were monitored daily by using spectrophotometer. Samples (5 mL) were taken and centrifuged at 2000 × *g* for 15 min to separate the algal biomass from POME due to the possible interference of the color from POME in the spectrophotometry measurements. At the end of exponential phase, the cultures were harvested for further analysis (see analysis section below).

2.6. Analyses

2.6.1. Growth rate and biomass determination

Monitoring of the cultures were done indirectly by using a spectrophotometer at 750 nm to evaluate the growth profile. Growth rate was calculated by the slope of the linear regression analysis.

A linear correlation between dry biomass and absorbance was used for co-cultivation was shown in Eq (1).

$$X_C = 0.441\ (OD_C) - 0.0126;\ R^2 = 99.75\%\ . \qquad \text{Eq. 1}$$

where X_C is biomass dry weight (g/L) of the mixture from co-cultivation, and OD_C is optical density at 750 nm from the mixture of co-cultivation *Arthrospira platensis*, and *Chaetoceros calcitrans*.

For *Arthrospira platensis*, a linear correlation between dry biomass and absorbance was shown in Eq. (2).

$$X_A = 0.569\ (OD_A) - 0.0019\ R^2 = 99.70\%\ . \qquad \text{Eq. 2}$$

where X_s is biomass dry weight (g/L) for *A. platensis*, and OD_N is optical density at 750 nm for *A. platensis*.

For *C. calcitrans*, a linear correlation between dry biomass and absorbance was shown in Eq. (3).

$$X_B = 0.563\ (OD_B) - 0.0179\ R^2 = 98.33\%\ . \qquad \text{Eq. 3}$$

where X_B is biomass dry weight (g/L) for *C. calcitrans*, and OD_B is optical density at 750 nm for *C. calcitrans*.

To prepare dry biomass, the samples were collected by using Whatmant GF/C filter paper, washed using NH_3HCO_3 and oven-dried at 95 °C until constan weight reached (Nur et al., 2019). Gravimetric analysis was used to determine the dry biomass.

Biomass productivity was calculated from the initial and final biomass dry weight, and the time of the cultivation (Eq. (4)),

$$P_x = \frac{(X_t - X_0)}{t} \qquad \text{Eq. 4}$$

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

2.6.2. Pigment analysis

2.6.2.1. C-PC extraction was done. C-PC extraction was done as according previous method (Nur et al., 2019). Dry biomass was weighed (100 mg) and extracted by adding 10 mL cold buffer phosphate (0.1 M, pH = 6.8) followed by sonication at 60 °C for 15 min. After extraction, the extract was separated from the residue by centrifugation (4500 rpm, 4 °C, 30 min). The concentration of C-PC in the extract was determined by using Hach DR2400 spectrophotometer, by measuring the optical density as described previously (Moraes et al., 2011). The concentration of C-PC was determined in Eq. (5).

$$C - PC = \frac{OD_{620} - 0.474\left(OD_{652}\right)}{5.34} \qquad \text{Eq. 5}$$

where C-PC is phycocyanin concentration (mg/ml) in the extract, 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. The C-PC concentration was.

Fucoxanthin extraction was conducted using spectroscopic method as described previously with little modification (Khoo et al., 2022). Dry biomass (100 mg) was added to 10 ml of ethanol, and then sonicated for 15 min at 60°. Residue was separated from the supernatant by centrifugation at 4000 × *g* for 15 min. The concentration of fucoxanthin in the extract supernatant was determined by using Hach DR2400 spectrophotometer, by measuring the optical density. The concentration of fucoxanthin was determined in Eq. (6).

$$\text{Fuco} = \frac{(OD_{445}) - 0.1425}{0.0703} \qquad \text{Eq.6}$$

where Fuco is the concentration of fucoxanthin in the extract (ug/ml), OD_{445} is the absorbance of the extract in ethanol at 445 nm.

2.6.3. COD analysis

COD determination was performed by Method 8000 (HACH Company, USA). Samples (100 mL) were taken and centrifuged at 10,000 rpm for 15 min to remove microalgae cells. Two milliliter of the sample (diluted with ultrapure water if necessary) was carefully pipetted to a Hach digestion Vial (HACH 21258-25) (Hach, USA). This dilution was also used to lower the interference of the salinity during the COD analysis. The tube was gently inverted and then hydrolyzed to a temperature block at 150 °C for 120 min. After cooling to room temperature, the COD value in the tube was read based on Hach DR 2400 spectrophotometer program.

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

$$R - COD = \frac{COD_i - COD_f}{COD_i}$$

where R-COD removal is COD removal efficieny (%), CODi is initial COD of the sample medium before cultivation, and COD*f* is COD of the effluent at the end of cultivation (mg/L).

2.7. Statistical analysis

Minitab ver. 18 (demo version) was employed for statistical analysis and evaluation for Box-behnken RSM design. Differences between treatments and the interaction between factors were analyzed with analysis of variance (ANOVA). Significant ($P < 0.05$) differences among treatments were further analyzed by pairwise comparisons using Tukey (Post Hoc Test). The experimental results were recorded based on three replicates ($n = 3$) except for fatty acid profiles, and expressed as the means and standard deviations (±SD).

3. Results

Table 1 shows biomass productivity, and pigment production of monoculture and mixed culture cultivated at different salinities. The addition of salinity above 20 PSU lower biomass productivity of *C. calcitrans* and *A. platensis* significantly ($P < 0.05$). Interestingly, biomass productivity of co-cultivation was not lower significantly when growing on high salinity. Pigment content was also influenced by salinity. Fucoxanthin content was found low (0.89%) at 30 PSU salinity. C-PC content was low (9%) when 30 PSU salinity was used. The highest fucoxanthin production was found on *C. Calcitrans* when growing at 20 PSU salinity, while C-PC production was optimal at 15–20 PSU salinity. At above 30 PSU salinity, fucoxanthin and C-PC production were significantly lower both in monoculture and mixed cultures ($P < 0.05$).

POME fractions significantly influenced growth rate and final biomass of the co-culture. The increasing of POME fractions both significantly lowering growth rate and final biomass. The highest COD removal efficiency was found on 30% of POME fraction (initial COD 640 mg/L) which resulted 60% efficiency, and resulted 0.4/day growth rate, and 440 mg/L final biomass (Table 2).

Box-behnken RSM was used to optimize the effect of POME, urea, and salinity on the pigment production. It revealed that the addition POME fractions significantly lowered biomass production of mixed cultures (Fig. 1). The addition of urea from 100 to 200 mg/L also lower biomass production. Optimal salinity was found at 16–20 PSU (Table 3, S1).

For C-PC production, salinity is the most significant factor influencing the pigment, followed by the interaction of salinity and POME fractions. Urea addition has no effect on C-PC production. Higher salinity addition decreased C-PC production (Fig. 2). The addition of POME up to 40% enhanced C-PC production. An uncoded model describing C-PC production and the factors was shown in Eq. 7

Table 1
Effect of salinity on final biomass, and pigment production of *Chaetoceros calcitrans*, *Arthrospira platensis*, and co-cultivation. Average values are shown ($n = 3$). SD are shown after ± symbol. Values that do not share letter are significantly different ($P < 0.05$).

Species	Salinity (PSU)	Biomass productivity (mg/L/d)		Fuco content (%)		C-PC (%)		Fuco production (mg/L)		C-PC production (mg/L)	
Chaetoceros calcitrans	15	67.62	$\pm 14.39^a$	2.07	$\pm 0.33^a$	ND	ND	6.94	$\pm 1.02^b$	ND	ND
	20	69.43	$\pm 9.23^a$	2.33	$\pm 0.08^a$	ND	ND	8.14	$\pm 0.32^a$	ND	ND
	30	47.24	$\pm 3.23^a$	0.89	$\pm 0.32^c$	ND	ND	2.13	$\pm 0.54^c$	ND	ND
Arthrospira sp	15	55.43	$\pm 8.42^a$	ND	ND	14.61	$\pm 2.90^a$	ND	ND	40.82	$\pm 8.10^a$
	20	46.45	$\pm 4.63^a$	ND	ND	15.51	$\pm 2.21^a$	ND	ND	36.05	$\pm 5.14^a$
	30	38.42	$\pm 8.40^a$	ND	ND	9.09	$\pm 1.44^b$	ND	ND	17.58	$\pm 2.79^c$
Co-cultivation	15	72.43	$\pm 15.61^a$	1.23	$\pm 0.01^b$	8.54	$\pm 0.23^b$	4.74	$\pm 0.45^b$	30.92	$\pm 0.83^b$
	20	75.32	$\pm 10.82^a$	1.42	$\pm 0.03^a$	7.33	$\pm 0.65^b$	5.73	$\pm 0.13^b$	27.51	$\pm 2.44^b$
	30	42.40	$\pm 11.27^a$	0.67	$\pm 0.02^b$	5.95	$\pm 0.94^c$	1.43	$\pm 0.05^c$	12.64	$\pm 2.00^c$

Table 2
Effect of POME fractions on growth rate, final biomass and COD removal efficiency from co-cultivation of *Chaetoceros calcitrans*, *Arthrospira platensis*. Average values are shown (n = 3). SD are shown after ± symbol. Values that do not share letter are significantly different (P < 0.05).

POME fractions (%)	Initial COD (mg/L)		Growth rate (/day)		Final biomass (mg/L)		COD removal (%)	
30	640	± 24	0.33	±0.03[a]	443	±23[a]	60	±23[a]
40	810	± 43	0.24	±0.05[b]	345	±41[b]	42	±19[b]
50	1040	± 11	0.19	±0.07[c]	243	±23[c]	34	±12[c]
60	1260	± 23	0.15	±0.06[d]	103	±11[d]	24	±11[d]

Table 3
Observed and predicted value fucoxanthin and C-phycocyanin production from co-cultivation growing on different POME fractions, salinity, and urea addition. Average values of observed lipid are shown (n = 2). SD are shown after ± symbol.

Salinity (PSU)	Urea (mg/L)	POME (%)	Fucoxanthin (mg/L)			C-PC (mg/L)		
			Observed		Predicted	Observed		Predicted
30	100	40	0.64	± 0.10	0.62	11.24	±1.80	10.42
22.5	250	40	1.91	± 0.12	2.85	15.62	±2.85	15.62
30	250	50	0.77	± 0.10	0.84	9.59	±0.84	9.98
22.5	250	40	1.91	± 0.12	2.85	15.62	±2.85	15.62
15	100	40	1.34	± 0.20	1.30	25.78	±2.78	26.24
22.5	400	50	1.89	± 0.05	1.87	10.00	±0.35	10.06
22.5	100	30	1.84	± 0.01	1.85	12.95	±1.40	12.88
30	400	40	0.92	± 0.03	0.95	13.95	±0.41	13.49
15	400	40	1.16	± 0.05	1.18	25.50	±0.50	24.52
22.5	400	30	1.95	± 0.01	1.90	13.39	±0.94	14.76
22.5	250	40	1.91	± 0.12	2.85	15.62	±2.85	15.62
15	250	50	1.00	± 0.08	0.99	15.14	±0.49	16.06
30	250	30	0.60	± 0.15	0.61	7.03	±0.50	6.11
15	250	30	1.27	± 0.15	1.29	27.28	±1.28	26.89
22.5	100	50	1.66	± 0.10	1.71	1.45	±11.98	10.61

$$P\text{-}cpc = 54.8 - 5.411\,X_1 - 0.0095\,X_2 + 1.806\,X_3 + 0.0509\,X_1^2 + 0.000008\,X_2^2 - 0.03727\,X_3^2 + 0.001063\,X_1.X_2 + 0.0490\,X_1.X_3 - 0.000404\,X_2.X_3 \quad \text{Eq.7}$$

where P-cpc is C-PC production, X_1 is salinity, X_2 is urea, and X_3 is POME fraction.

For fucoxanthin production, the highest production was found when 17–20 PSU salinity was used. Salinity is the most influencing factor for fucoxanthin production (Fig. 3). The interaction of salinity and urea also influenced fucoxanthin production. High fucoxanthin production was found when of urea or POME was added at salinity 20 PSU. An uncoded model for fucoxanthin production was shown in Eq. (8).

$$Fuco = -4.81 + 0.6109\,X_1 - 0.00300\,X_2 + 0.0299\,X_3 - 0.016123\,X_1^2 + 0.000001\,X_2^2 - 0.000900\,X_3^2 + 0.000101\,X_1.X_2 + 0.001470\,X_1.X_3 + 0.000019\,X_2.X_3 \quad \text{Eq.8}$$

where Fuco is fucoxanthin production, X_1 is salinity, X_2 is urea, and X_3 is POME fraction.

The interaction of salinity and urea was also significantly influenced the production. When high salinity was used, the addition of urea from 100 to 400 mg/L ehnaced fucoxanthin production from 0.5 to 1 mg/L. The optimal condition to produce the highest pigment was found on 33% POME, 100 mg/L urea, and 17 PSU salinity which resulted 1.6 mg/L fucoxanthin and 25 mg/L C-PC.

4. Discussion

The production of fucoxanthin from monoculture of *C. calcitrans* was low when high salinity was used (30 PSU). Conversely, when co-cultivation was applied, the fucoxanthin production was not differ significantly for high salinity. This implies that co-cultivation plays significant role during the growth. *C. Calcitrans* could tolerate higher salinity when *A. Platensis* was presented. Previous research mentioned that co-cultivation strategy was better compared to monoculture when the environmental and nutritional conditions were not controlled (Mattsson et al., 2021). It seems that exopolysaccharide (EPS) secreted from *A. platensis* might be utilized for *C. calcitrans* to protect the cells from different osmotic pressure. *A. platensis* is a kind of cyanobacteria that produce high sulfated exopolysaccharide under specific conditions (Rajasekar et al., 2019; Vergnes et al., 2019). *A. platensis* could accumulate higher EPS when salinity was above optimal condition (M.M.A. Nur, 2021). Previous finding revealed that co-cultivation of cyanobacteria and green algae resulted higher biomass and lipid production (Satpati and Pal, 2021a,b).

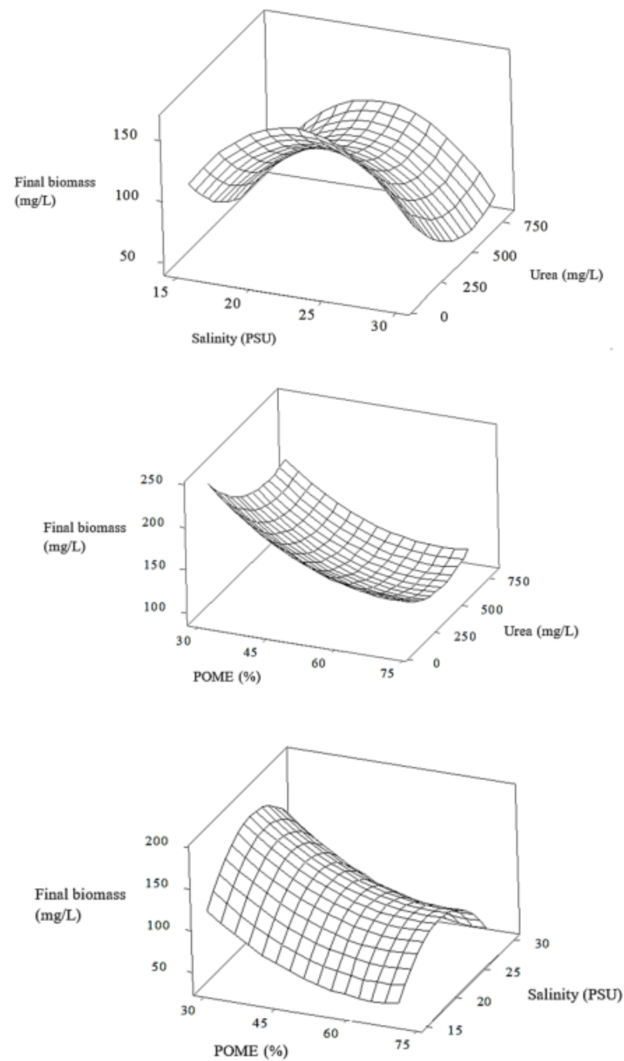

Fig. 1. Effect of POME fractions, urea addition, and salinity of co-cultivation on final biomass.

When using POME as medium growth, biomass production was declining. Co-cultivation of microalgae could utilize low substrate from POME with initial COD 640 mg/L (Table 2). This result revealed that 60% of COD might be utilized by microalgae in mixotrophic cultivation condition. Previous research mentioned that organic carbon such as acetic acid contained in POME is a promising carbon supply for microalgae to grow (Nur et al., 2021). The higher COD level in POME lowered the growth rate and final biomass production. It seems that phenolic compounds in POME could inhibit the growth due to the toxicity effect (Papazi et al., 2019; Low et al., 2021).

Based on the pigment composition, fucoxanthin production was detected around 1–1.6 mg/L, which was almost 4-fold times compared to synthetic medium (Table 3). For C-PC production, it was slightly decreased from 30 mg/L to 25 mg/L when POME was introduced in synthethic medium. This indicated that *C. calcitrans* could not grow well on POME, while POME fraction has positive effect for *A. platensis*. In this experiment, POME has no effect on fucoxanthin production (Fig. 3). Previous research reported that *Chaetoceros* produced lower biomass when ammonium was introduced as nitrogen source to the medium rather than nitrate and urea addition (Karthikeyan et al., 2013). This indicated that *C. calcitrans* could not tolerate to high ammonium content which presented in POME. High organic carbon contained in POME might also influence *C. calcitrans* to grow. However this finding needs to be investigated in the future. Interestingly, the addition of POME from 30 to 50% into medium did not significantly decrease or increase fucoxanthin production. It seems that the interaction between *C. calcitrans* and *A. platensis* could also played important role in the cultivation. EPS production from *A. platensis* might protect *C. calcitrans* to grow on POME. The interaction of salinity and urea influenced fucoxanthin production significantly. Fucoxanthin was only increased when urea was introduced at high salinity. This implies that at

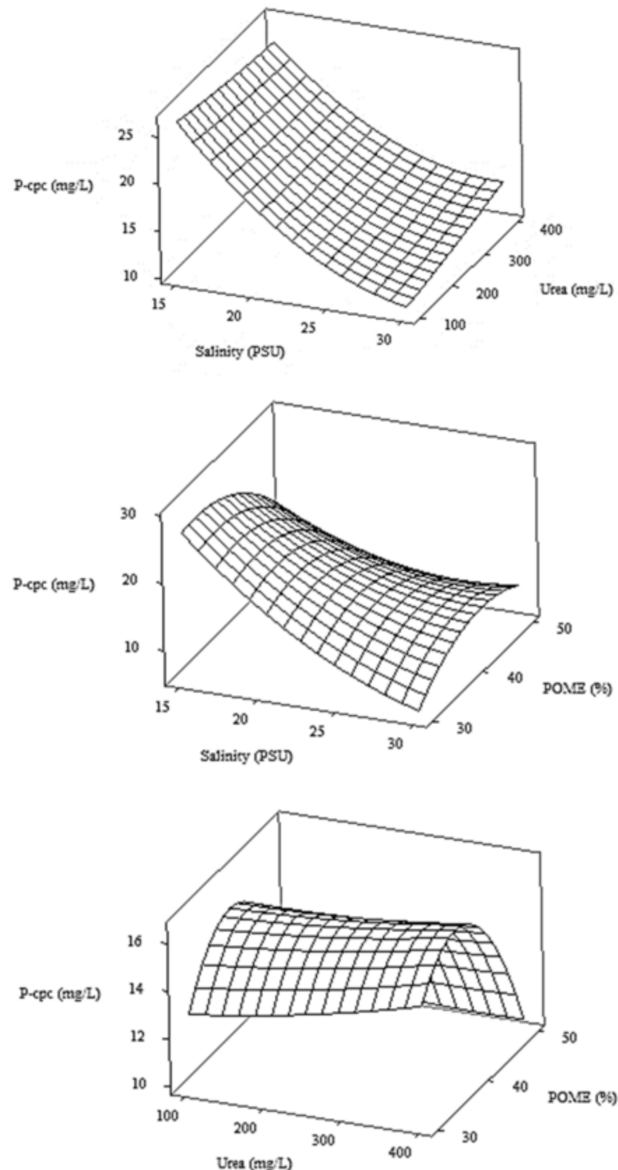

Fig. 2. Effect of POME fractions, urea addition, and salinity of co-cultivation on C-PC production.

high salinity and high urea, high EPS was secreted by *A. platensis* and the substance might be utilized for *C. calcitrans* to protect the cells from the toxic substance in POME.

Co-cultivation of *C. calcictrans* and *A. platensis* exhibit the final biomass production. This result was in line with previous studies (Table 4). It is found that co-cultivation strategies enhanced final biomass of mixed cultures growing on synthetic medium such as *Chlorella vulgaris* and *Scenedesmus dimorphus*, *Ettlia* sp. and *Chlorella* sp., *Leptolyngbya tenuis* and *Chlorella ellipsoidea,* compared to monoculture (Tang et al., 2022; Rashid et al., 2019; Satpati and Pal., 2021). Another researcher reported that co-cultivation strategy is suitable to remove nutrients in wastewater such as fishery wastewatter while the biomass and the composition (carbohydrate and lipid) were enhanced compared to monoculture (Wang et al., 2021). Previous finding also demonstrated the enhancement of valuable products such as lipid, pigments, and hydrogen by using co-cultivation strategy (Nur et al., 2022a,b; Wutthithien and Incharoensakdi, 2022; Maglie et al., 2021). Mixed culture might promote cell density and avoid light saturation, thus increasing the accumulating of pigment. In other hand, mixed culture can also accelerated nutrient deficiency, thus increasing the accumulation of lipid, and degrade pigment production. In this research, the final biomass production was enhanced due to the interaction between microalgae when growing in co-cultivation. The addition of POME to the medium accelerate the mixotrophic cultivation condition, thus lowering pig-

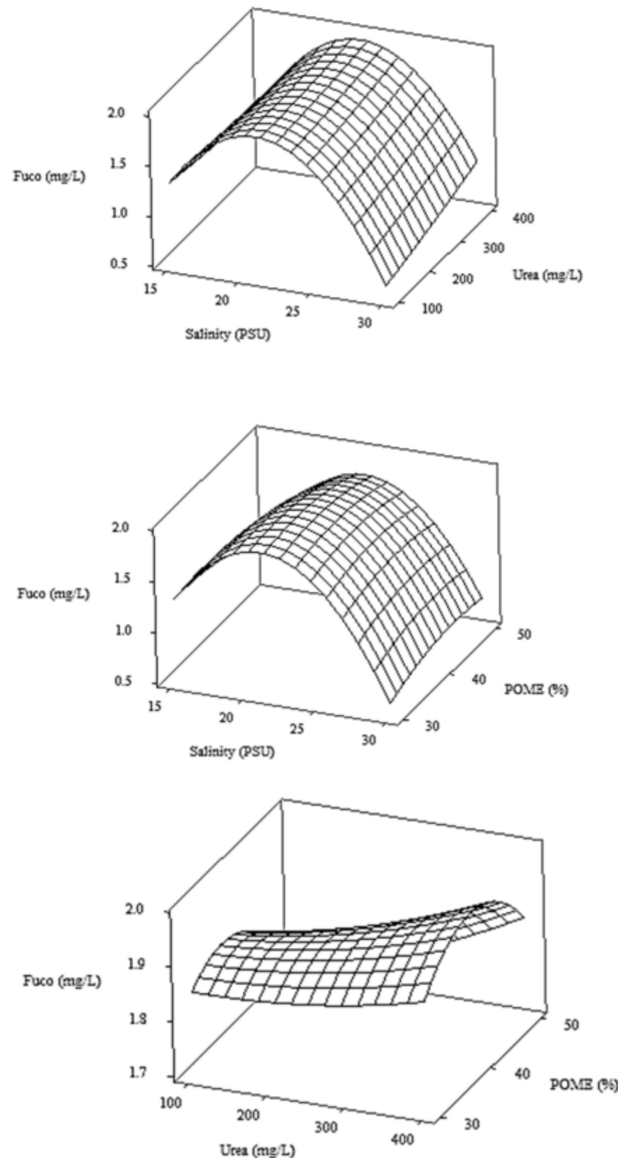

Fig. 3. Effect of POME fractions, urea addition, and salinity of co-cultivation on fucoxanthin production.

ment content (Nur, 2022). Nevertheless, the productivity of the pigment could be maintained at reasonable value when growing at 33% POME and supplemented with urea, resulting the production became more sustainable.

5. Conclusion

This research revealed that co-cultivation strategy enhanced biomass production of mixed culture *C. calcitrans* and *A. platensis*. Salinity significantly influenced biomass and pigment production. At high salinity, biomass production could be maintained when using co-cultivation condition. POME has positive effect on C-PC production, while fucoxanthin production was influenced by the interaction of salinity and urea. Synthetic medium was promising to lower nutritional cost when the cultivation was performed under outdoor condition. Mixed culture produced 27 mg/L C-PC and 5.4 mg/L fucoxanthin by using synthetic medium. Optimal condition for mixed culture was 33% POME, 17 PSU salinity, and 100 mg/L urea to produce 25 mg/L C-PC, and 1.6 mg/L fucoxanthin.

Table 4
Co-cultivation of microalgae and the valuable product.

Microalgae co-cultivation	Medium	Valuable product(s)	Reference
Tisochrysis lutea and *Nannochloropsis oculata*	f/2	Total carotenoid, chlorophyll-a, chlorophyll-c	Maglie et al. (2021)
Thalassiosira pseudonana and *Isochrysis galbana*	fishery wastewater	Carbohydrate, lipid content,	Wang et al. (2021)
Chlorella vulgaris and *Scenedesmus dimorphus*	landfill leachate	Final biomass and lipid	Tang et al. (2022)
Tisochrysis lutea and *Microchloropsis salina*	Modified f/2	Final biomass and DHA	Thurn et al. (2022)
Ettlia sp. and *Chlorella* sp. HS-2	BG-11	Final biomass	Rashid et al. (2019)
Diatom and green algae	POME	Fucoxanthin and lipid	Nur (2021)
Chlorella sp. U4341 and *Monoraphidium* sp.	Modified BBM	Increasing lipid productivity	Zhao et al. (2014)
Leptolyngbya tenuis and *Chlorella ellipsoidea*	BG-11 + cadmium nitrate	Biomass and lipid	Satpati and Pal (2021)
Cyanobacteria *Fischerella muscicola* TISTR 8215 and *Chlorella* sp.	BG-11	Hydrogen	Wutthithien and Incharoensakdi (2022)
Chaetoceros muelleri and *Amphora* sp.	f/2	Fucoxanthin	Ishika et al. (2019)
Dunaliella sp., *Spirulina* sp., *Nannochloropsis* sp., and *Chaetoceros calcitrans*.	POME	Lipid	Nur et al. (2022)
Chaetoceros calcitrans and *Arthrospira platensis*	Synthetic medium	27 mg/L C-PC and 5.4 mg/L fucoxanthin	This study
Chaetoceros calcitrans and *Arthrospira platensis*	33% POME	25 mg/L C-PC, and 1.6 mg/L fucoxanthin.	This study

Author statement

MMAN Performed literature review, conceptualization, editing, and writing manuscript. IND designed the experiment. NAS, ASP and H reviewed the manuscript.

Declaration of competing interest

Corresponding authors declare no conflict of interest.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2023.102611.

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