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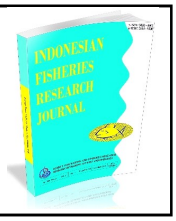

POTENTIAL OF *Spirulina* sp. FOR REMEDIATING POLLUTANTS IN AQUACULTURE WASTEWATER AND PRODUCING PHYCOCYANIN

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ABSTRACT

The sustainability of aquaculture wastewater treatment is challenging because it has a negative impact on the ecosystem if directly discharges to the environment. Aquaculture wastewater consists of high pollutants loading such as ammonia, phosphor, nitrate, and chemical oxygen demand. To offset the downsides of aquaculture effluent, effective solutions are required. *Spirulina* sp. is microalga that can convert nutrients in the wastewater and dissolve carbon into microbial biomass with value such as phycocyanin which can be employed as food, cosmetics, feed, and pharmacy feedstock. The effects of light intensity (6,000 - 10,000 lux) and urea addition (20-100 ppm) on biomass production, COD reduction, and phycocyanin yield were investigated in this study. For 10 days, *Spirulina* sp. was grown in a batch reactor at 25-27°C with a ratio of 30% inoculum and 70% wastewater under continuous aeration. *Spirulina* sp. produced the most biomass at 8,000 lux with the addition of 60 ppm of urea accounting for 0.71 ± 0.14 g/L ($P > 0.05$). Meanwhile, the maximum phycocyanin concentration was 4.21 ± 0.132% at 7,000 lux and 80 ppm urea ($P > 0.05$) with 96.51% of chemical oxygen demand reduction ($P < 0.05$). The outcomes of this study highlight the potential of aquaculture effluent to produce valuable microalgal biomass and phycocyanin, which can be used to generate lucrative products.

Keywords: Aquaculture wastewater; light intensity; phycocyanin; *Spirulina* sp.; urea

INTRODUCTION

Aquaculture is one of the world's fastest-growing food sectors, with significant development and intensification projected in practically all locations (Calderini et al., 2021). One of the major threats to the environmental sustainability of aquaculture is eutrophication of aquatic ecosystems induced by exposure to nutrient-rich (particulate and dissolved) in aquaculture wastewater (AW) (Andreotti et al., 2020). The nutrients in AW are primarily the result of unabsorbed feed in the ponds consisting of 3.87 mg/L phosphor, 5.69 mg/L nitrogen, 2,304 mg/L chemical oxygen demand (COD), and other contaminants (Fahrur et al., 2016; Gao et al., 2016; Guldhe et al., 2017). These compounds are usually discharged to the sea and are responsible for the environmental damage.

Some bioremediation approaches of AW have been conducted with various technologies. Microalgae cultivation is often suggested as the best cost-effective approach to be applied in such cases (Cardoso et al., 2022; Iber & Kasan, 2021; Dewi et al., 2024). They possess the ability of self-adaptive to low-quality medium such as wastewater that can remove up to 90% of wastewater nutrients and produce high added value biomass at low cost (Cardoso et al., 2022; Dewi et al., 2022; Malibari et al., 2018). *Spirulina* sp. is a popular microalga owing to its high content of protein (70%), lipids (3–9%), carbohydrates (15-30%), β-carotene (higher than carrots), and vitamins (B1, B2, and B12) (Cardoso et al., 2022; Pradana et al., 2020). Considering the quality composition of *Spirulina* sp., the biomass produced from cultivation have been considered as feedstock for a wide range of bio-based products (Putri et al., 2023; Nur & Dewi, 2024). *Spirulina* sp. contains a unique protein known as chromoprotein or phycobiliprotein. Phycobiliprotein, a pigment mostly composed of phycocyanin (PC), is

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a blue pigment that serves as a nitrogen reserve store and is well known for its antioxidant, anti-inflammatory, and anticarcinogenic properties (de la Jara et al., 2018; Nur & Buma, 2019; Soni et al., 2017; Wu et al., 2016). However, to produce a large amount of *Spirulina* sp., significant volumes of water are necessary for cultivation, and the exorbitant prices of synthetic fertilizers for mass cultivation of algae remain a major concern. Hence, new culture systems must be implemented to reduce costs and avoid negative environmental consequences by utilizing AW as medium for microalgae.

Several studies on microalgae cultivation in AW have been widely reported. Taqiyyah et al., (2023) mentioned that *Spirulina* sp. cultivated in 6 mg/L AW produced the best result of 0.78 g/L biomass, 50.44% protein, and 1.24 mg/L carotenoids. *Spirulina plantesis* yielded 0.22 g/L biomass and removed 94.8% nutrients while cultivated in the fish farming wastewater (Nogueira et al., 2018). Gao et al., (2016) found that the remediation rates of total nitrogen and total phosphorus from an aquaculture tank of *Penaeus vannamei* using *Chlorella vulgaris* were 86.1%. Ansari et al., (2017) used *S. obliquus* and succeeded removing 88.7% of *Nile tilapia* effluent. Batch mode *Mugil cephalus* effluent was successfully treated by *Tetraselmis suecica*, *Dunaliella tertiolecta* and *Isochrysis galbana* accounting for 94.4%, 95.4% and 91.9% nutrient removal efficiency (Andreotti et al., 2020). Malibari et al., (2018) mentioned that microalgae could grow well in AW and produced high lipid content, which accounts for 46.8%.

In addition, Chaiklahan et al., (2010) mentioned that cultivating *Spirulina* sp. in swine wastewater treated with urea addition could reduce the cost by up to 4.4 times and increased pigments productivity. Benvenuti et al., (2016) demonstrated that enriching commercial medium with nitrogen enhanced the biomass productivity of *Nannochloropsis* sp. Likewise, Nur et al., (2019) reported that PC content in *Spirulina* sp. was discovered to be irradiance and nitrate dependent while cultivated in POME, with PC tending to grow at higher nitrogen concentrations and light intensities. However, to the best of our knowledge, this culture mode has not been tried for *Spirulina* sp. cultivated on AW medium to produce biomass and PC. Thus, the purpose of this study was to examine the effects of different urea concentrations and light intensities on *Spirulina* sp. grown in AW by monitoring the production of biomass and PC as well as the efficiency of COD removal.

MATERIALS AND METHODS

Aquaculture Wastewater Preparation

Aquaculture wastewater (AW) was received from shrimp farm ponds in Yogyakarta, Indonesia. The particulate matter in AW was removed using GF/C Whatman filters (Whatman, 110 mm) with an initial chemical oxygen demand of 4,229 mg/L, 0.3 ppt salinity, and 7.43 pH. Following that, AW was sterilized for 30 minutes at 121°C to remove the likely influence of bacteria from AW on the cultivation and consider only algae that influenced the process.

Strain Preparation

Spirulina sp. cultures were used for this study due of their high protein content. The isolated algal strain was acquired from the Institute for Brackish Water Aquaculture Fisheries culture collection in Jepara, Central Java, Indonesia. The strain was phototropically cultured in Zarrouk fertilizer with 30 ppt filtered and sterilized saline water at 25-27°C under 6,000-7,000 irradiations. For 24 hours, a compressor was utilized to continuously aerate the culture. After a week of growth, the cells were used as an inoculum for further use.

Effect of Urea and Light Intensities on *Spirulina* sp. Cultivation

Spirulina sp. culture was mixed with AW in a 70/30 ratio (AW/inoculum) with a total volume of 2 L. Following that, urea was given to the medium at varied concentrations, namely 0, 20, 40, 60, 80, and 100 (mg/L). Light intensities of 6,000, 7,000, 8,000, 9,000, and 10,000 (lux) were attained using a 25 watt fluorescent lamp. The cultivation lasted 7 days at a temperature of 25-27°C, salinity of 30 ppt, and pH of 7-8 with constant aeration.

Specific Growth Rate and Biomass Measurement

Every day, the optical density of *Spirulina* sp. was measured with an Optima SP-300 spectrophotometer at 750 nm to determine the growth rate. The maximum specific growth rate (μ_{max}) was measured during exponential phase in accordance with Eq. (1).

$$\mu_{max} = \ln \frac{OD_t / OD_0}{t_t - t_0} \quad (1)$$

where OD_t and OD_0 denote the optical density on day t and 0; t_t and t_0 denote the cultivation day on day t and 0 accordingly.

While the biomass of microalgae was determined by calibrating the optical density value with a standard curve through linear equation as shown in Eq. (2).

$$x=4.9628(OD_{750})+2.109 \ (R^2=0.9904) \quad (2)$$

where x denotes biomass (g/L) and OD_{750} denotes the optical density at 750 nm for 7 days.

Phycocyanin Determination

The prior approach for concentration of phycocyanin (C-PC) extraction was used based on Hadiyanto and Suttrisnohadi' experiment (Hadiyanto & Suttrisnorhadi, 2016). Three hundred milligrams of dry biomass were extracted by adding three millilitres of cold buffer phosphate (pH = 6.8), freezing, and thawing twice, and sonication for ten minutes in an ultrasonic bath at 52.5°C. Centrifugation (4500 rpm, 4°C, 30 min) was used to separate the extract from the residue. Using an Optima SP-300 spectrophotometer, the optical density at 620 nm was measured to quantify the content of C-PC in the supernatant (Moraes et al., 2011). Eq. (3) was used to calculate the concentration of C-PC.

$$\%C\text{-}PC=\ln\left(\frac{OD_{620} \times 10 \times 100)}{7.3 \times \text{sample mass}}\right) \quad (3)$$

where C-PC is the phycocyanin content (%), OD_{620} is the extract's optical density at 620 nm.

Chemical Oxygen Demand Determination

The chemical oxygen demand (COD) analysis was carried out using Dewi's method (Dewi et al., 2022). To begin, standardize 0.01 N $KMnO_4$ solution in the Erlenmeyer flask by adding 0.01 N $H_2C_2O_4$, 4 N H_2SO_4, and a trace of phenolphthalein. The mixture was then placed beneath a calibrated burette containing the titrant and heated to 70-80°C for a predetermined amount of time. Afterwards, a small amount of titrant was then added to the analyte until the colour changed indicated the end point of titration. Eventually, the titrant volume was recorded (b mL). Then, 10 mL of sample was pipetted into the flask and mixed with 0.01 N $H_2C_2O_4$, 4 N H_2SO_4, b ml 0.01 N $KMnO_4$ and phenolphthalein. The mixture was then titrated with 0.01 N $KMnO_4$ until the colour changed (a mL). In summary, COD was calculated using Eq. (4).

$$COD=[(a+b) \times N_k \ KMnO_4 \ strd- V_o \times N_o \ H_2C_2O_4] \times 8000 \quad (4)$$

where a is titrant volume for samples (mL), b is titrant volume for standardization (mL), N_K is normality of $KMnO_4$, V_o is $H_2C_2O_4$ volume, N_o is $H_2C_2O_4$ normality.

Statistical Analysis

To evaluate the influence of the components being investigated on microalgae biomass, specific growth rate, PC content, and COD concentration, data were analysed using One-Way ANOVA. A Tukey test was used to identify which concentration had a significant effect ($P < 0.05$). IBM SPSS version 26 was used to perform one-way ANOVA analysis. The experimental results are provided in standard deviations (SD) based on three replicates.

RESULTS AND DISCUSSION

Results

Urea is deemed as a viable nitrogen source for *Spirulina* sp. cultivation on AW medium. However, high urea concentrations are hazardous to *Spirulina* sp. due to ammonium overproduction from microbial urea conversion (Nur et al., 2023). The optimum light intensity for biomass, C-PC production, and COD removal was also investigated since it determines sufficient light absorption for microalga to photosynthesize while cultivated in the wastewater. The result can be seen in Table 1.

There were no discernible variations in the rate of microalgae development at different urea concentrations and light intensities ($P > 0.05$). When 60 ppm urea was added to the microalgae culture, there was a notable variation in the biomass production ($P < 0.05$). Meanwhile, the results for C-PC showed a significant difference at the lowest (20 ppm) and highest (100 ppm) urea levels under various irradiance levels ($P < 0.05$), demonstrating that while in an extreme situation, C-PC in *Spirulina* sp. was dependent on both nitrogen and light availability. At different urea addition and light intensities, COD removal efficiency showed a noticeable difference at all urea concentrations and irradiance levels ($P < 0.05$).

Effect of Light Irradiation and Urea Concentration on Biomass Concentration

The biomass productivity of *Spirulina* sp. cultured on Zarrouk medium with AW differs significantly ($P < 0.05$) only at 60 mg/L of urea at various light intensities (6,000-10,000 lux) as shown in Table 1 and Figure 1. The biomass did not, however, demonstrate a significant difference at other urea fractions including control ($P > 0.05$). The supplementation of 20 – 40 and 80 – 100 mg/L revealed insignificant effects under different irradiances ($P > 0.05$). The lowest biomass concentration was found at 0 mg/L urea addition with maximum light intensity ranging from 0.58 ± 0.40 to 0.60 ± 0.47 mg/L.

Table 1. Effect of urea and light intensity on specific growth rate, biomass, phycocyanin and COD removal efficiency at exponential phase

Urea (mg/L)	Light Intensity (Lux)	μ_{max} (d)	Biomass (mg/L/d)	C-PC (%)	COD (mg/L)	COD Removal Efficiency (%)
0	6000	0.09 ± 0.33^{a}	0.58 ± 0.40^{a}	0.74 ± 0.11^{a}	2,341^{d}	35.70
0	7000	0.09 ± 0.71^{a}	0.58 ± 0.51^{a}	0.75 ± 0.21^{a}	2,100^{c}	42.11
0	8000	0.09 ± 0.96^{a}	0.59 ± 0.69^{a}	0.75 ± 0.26^{a}	2,054^{b}	42.17
0	9000	0.11 ± 0.86^{a}	0.60 ± 0.64^{a}	0.80 ± 0.10^{a}	1,981^{a}	43.02
0	10000	0.12 ± 0.91^{a}	0.60 ± 0.47^{a}	0.86 ± 0.12^{a}	1,986^{a}	42.47
20	6000	0.10 ± 0.33^{a}	0.61 ± 0.60^{a}	0.90 ± 0.30^{a}	2,219^{d}	39.06
20	7000	0.11 ± 0.71^{a}	0.61 ± 0.73^{a}	0.95 ± 0.31^{a}	1,967^{c}	45.78
20	8000	0.12 ± 0.96^{a}	0.61 ± 0.53^{a}	1.65 ± 0.24^{b}	1,865^{b}	47.49
20	9000	0.12 ± 0.86^{a}	0.61 ± 0.54^{a}	1.89 ± 0.12^{b}	1,713^{a}	50.73
20	10000	0.12 ± 0.91^{a}	0.62 ± 0.63^{a}	1.88 ± 0.17^{b}	1,722^{a}	50.12
40	6000	0.11 ± 0.54^{a}	0.61 ± 0.71^{a}	1.45 ± 0.20^{a}	1,641^{d}	41.18
40	7000	0.11 ± 0.68^{a}	0.62 ± 0.76^{a}	1.50 ± 0.71^{a}	1,350^{c}	48.86
40	8000	0.13 ± 0.78^{a}	0.63 ± 0.61^{a}	1.58 ± 0.67^{a}	1,300^{b}	50.06
40	9000	0.13 ± 0.94^{a}	0.63 ± 0.47^{a}	1.89 ± 0.66^{a}	1,246^{a}	51.29
40	10000	0.13 ± 1.02^{a}	0.63 ± 0.31^{a}	1.74 ± 0.54^{a}	1,305^{b}	47.38
60	6000	0.13 ± 1.02^{a}	0.66 ± 0.26^{a}	1.74 ± 0.43^{a}	964^{e}	62.45
60	7000	0.14 ± 0.95^{a}	0.67 ± 0.08^{a}	1.84 ± 0.42^{a}	871^{d}	65.41
60	8000	0.18 ± 1.28^{a}	0.71 ± 0.14^{b}	2.16 ± 0.62^{a}	650^{a}	73.25
60	9000	0.15 ± 1.57^{a}	0.68 ± 0.22^{a}	2.22 ± 0.61^{a}	663^{b}	72.94
60	10000	0.15 ± 1.13^{a}	0.68 ± 0.26^{b}	2.20 ± 0.41^{a}	670^{c}	72.69
80	6000	0.13 ± 0.87^{a}	0.68 ± 0.31^{a}	3.58 ± 0.52^{a}	215^{e}	89.80
80	7000	0.13 ± 0.67^{a}	0.69 ± 0.27^{a}	4.21 ± 0.32^{a}	70^{a}	96.51
80	8000	0.12 ± 0.53^{a}	0.69 ± 0.11^{a}	3.89 ± 0.37^{a}	89^{b}	95.83
80	9000	0.04 ± 0.76^{a}	0.61 ± 0.37^{a}	3.89 ± 0.70^{a}	97^{c}	95.54
80	10000	0.04 ± 0.71^{a}	0.61 ± 0.26^{a}	3.12 ± 0.81^{a}	137^{d}	93.71
100	6000	0.03 ± 0.32^{a}	0.60 ± 0.16^{a}	2.75 ± 0.29^{a}	432^{e}	80.43
100	7000	0.04 ± 0.41^{a}	0.60 ± 0.18^{a}	3.53 ± 0.19^{b}	231^{a}	89.31
100	8000	0.04 ± 0.48^{a}	0.60 ± 0.11^{a}	3.15 ± 0.12^{ab}	278^{b}	87.24
100	9000	0.03 ± 1.22^{a}	0.59 ± 0.24^{a}	3.11 ± 0.24^{ab}	340^{c}	85.18
100	10000	0.02 ± 1.08^{a}	0.59 ± 0.31^{a}	2.89 ± 0.28^{a}	412^{d}	81.03

Note:
Average values are shown (n = 3)
Sharing letters indicate there is a significant value ($P < 0.05$)
The same letter indicates there is no significant value ($P > 0.05$)

Effect of Light Irradiation and Urea Concentration on Phycocyanin Production

Figure 2 shows how the growth medium's phycocyanin levels changed when urea was added under different irradiances. At a light irradiation of 7,000 lux, the maximum C-PC production was obtained by adding 80 ppm urea accounting for 4.21 ± 0.32 ppm. The observation findings showed that, at different light intensities, the addition of 80 ppm urea produced the maximum C-PC output compared to other concentrations, with a value range of 3.12 ± 0.81 - 4.21 ± 0.32 ppm. In contrast, the lowest C-PC yield (0.74 ± 0.11 - 1.89 ± 0.66 ppm) was achieved when the additional urea ranged from 0-40 ppm. Conversely, the light irradiance impact indicates that when the additional urea concentration was high (80–100 ppm), the C-PC concentration achieved decreased with increasing light intensity. However, when the additional urea was less than 80 ppm, the yield of C-PC tended to rise with increasing light intensity.

Effect of Light Irradiation and Urea Concentration on COD Removal

The impact of urea and light intensity on COD levels in AW is depicted in Figure 3. Microalgae consumed the waste's proteins, carbohydrates, and oil, which caused the COD levels in the media to decrease. Similar to the C-PC yield, the maximum COD reduction percentage of 96.51%, or a final COD concentration of 70 ppm, was obtained when 80 ppm of urea was added at a light intensity of 7000 lux. However, with a COD concentration of 1,246–2,341 ppm, the addition of 0–40 ppm urea to different irradiances was only able to lower COD by 41.18–51.29%.

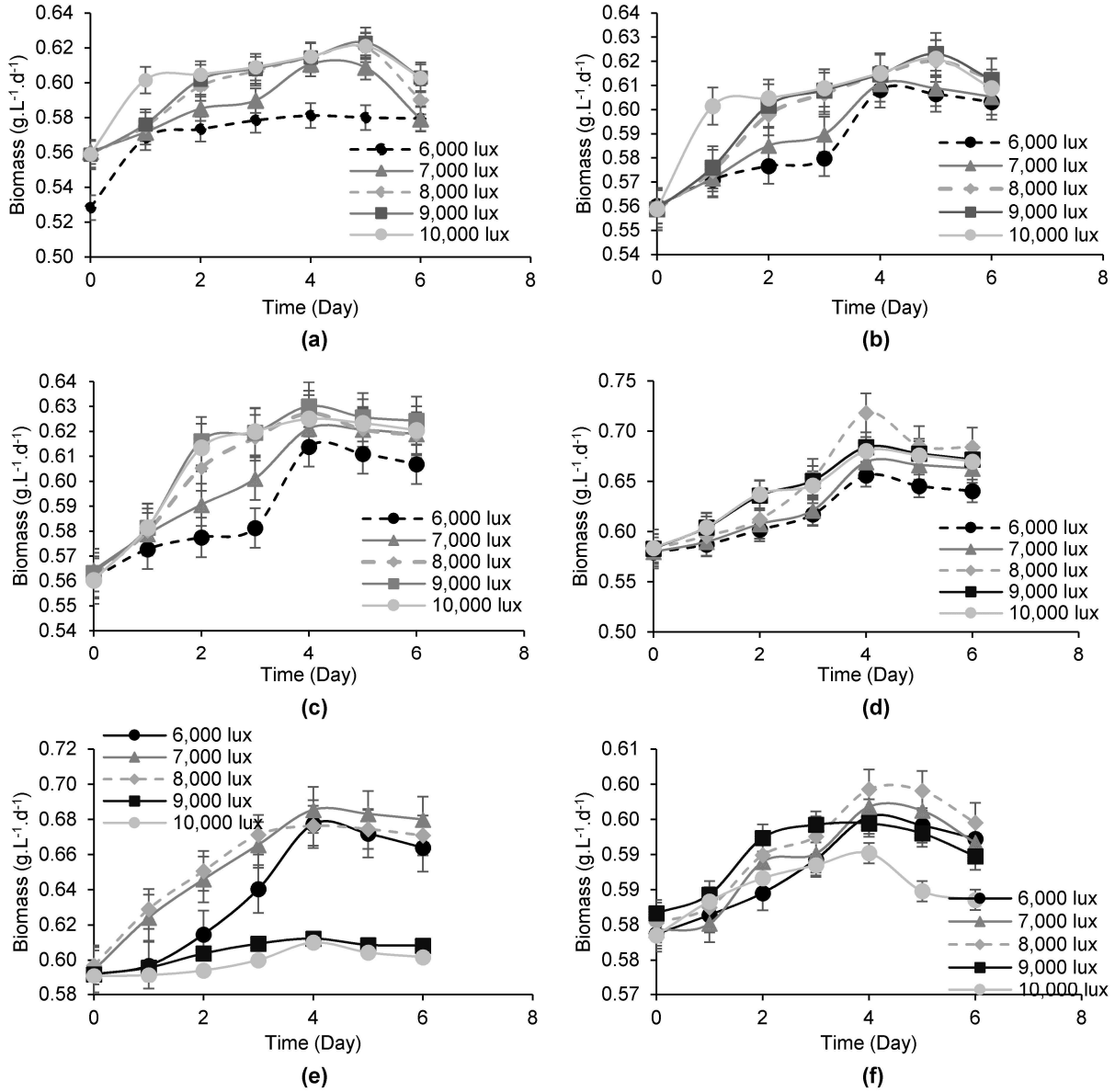

Figure 1. Effect of urea and light intensity on biomass production in AW. (a) Urea 0 ppm; (b) Urea 20 ppm; (c) Urea 40 ppm; (d) Urea 60 ppm; (e) Urea 80 ppm; (f) Urea 100 ppm. All values are an average of means ± SD.

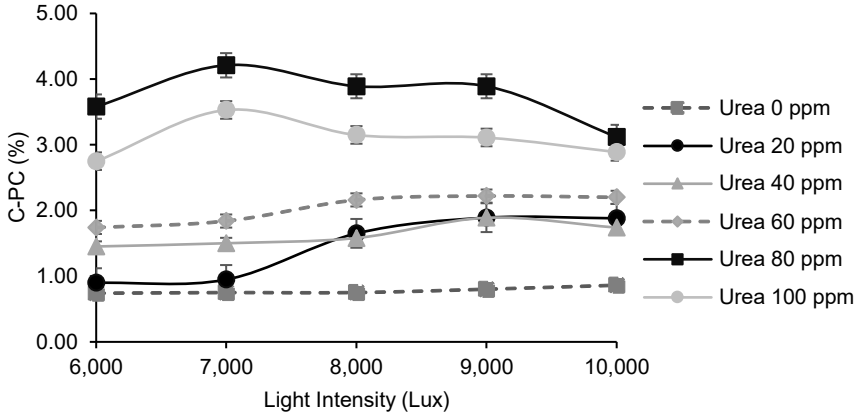

Figure 2. Effect of urea and light intensity on C-PC in AW. All values are an average of means ± SD.

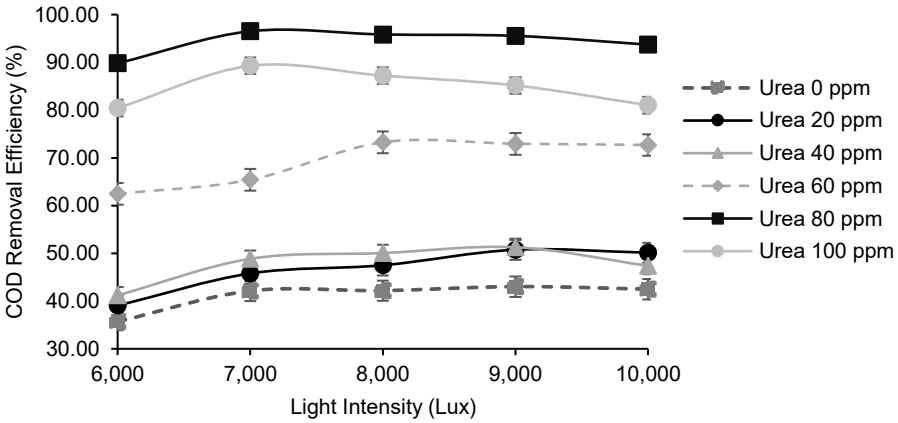

Figure 3. Effect of Urea and Light Intensity on COD Removal Efficiency in AW. All values are an average of means ± SD.

Discussion

Effect of Light Irradiation and Urea Concentration on Biomass Production

The maximum specific growth rate and biomass productivity of *Spirulina sp.* both steadily increased with higher urea addition, as shown in Figure 1. This might happen due more chlorophyll was generated in the cell of microalgae, which was affected by the presence of nitrogen from urea (Sukadarti et al., 2016). In photosynthesis reactions, chloroplasts found in chlorophyll possesses the ability to catalyze the conversion of CO_2 into carbohydrates (biomass). Nevertheless, the growth rate and biomass were prone to decrease when more than 60 mg/L of urea was added. Urea has a tendency to produce ammonia when it dissolves in water at certain temperatures. Carvalho et al., (2004) and Nur et al., (2019) reported that *Spirulina* sp. can withstand an ammonia content of 90 mg/L as evidence in favor of this. Hence, at 100 mg/L, growth was completely halted, indicating *Spirulina* sp.'s tolerance to the harmful chemical present in urea—high ammonia levels. Taufikurahman et al., (2020) found that *Spirulina plantesis* cultivated in dairy manure effluent yielded 0.04 g/L/d of biomass. In this study, the result was ten folds higher, 0.71 ± 0.14 g/L/d due nitrogen supplied from urea affected how chlorophyll was produced. In the meantime, the biomass was prone to fall in the absence of urea supplementation, suggesting a shortage of nitrogen source. As urea is more readily converted into ammonia in alkaline conditions and ammonia is more readily digested by *Spirulina* sp. at a given concentration, it can be concluded that urea is a better source of nitrogen to enhance the growth and biomass productivity.

The light intensity influenced the growth rate and biomass concentration when urea was added at a concentration of 20-60 mg/L including control. The phenomenon occurred due the production of chlorophyll was still at low level, which resulted in a slow rate of biomass production. This allows cells to maximize the increase in energy from the light source to convert the available nutrient sources in the medium to enhance biomass generation (Nur et al., 2019). In order to meet the needs of microalgae for light, light intensity also lessened the turbidity factor brought on by waste, therefore, at higher light intensity the biomass yield also increased (Heredia-Arroyo et al., 2011). Meanwhile, the maximum specific growth rate and biomass production were essentially unaffected by the increase in light intensity from 6,000 to 10,000 lux when urea addition is over 80 ppm. As aforementioned, the inhibition due to ammonia presence could also affect this circumstance. At more than 8,000 lux irradiances, algal had lower productivity which was associated with photoinhibition. Microalgae may become photo inhibited at light levels that were excessively high (over 8,000 lux). High light intensity can limit photosynthesis, which can lead to excessive light stimulation that harms chloroplasts (Guidi et al., 2019; Kerena & Krieger-Liszkay, 2011).

Effect of Light Irradiation and Urea Concentration on Phycocyanin Production

A protein called phycocyanin can be discovered in biomass that results from photosynthetic processes (Ridlo et al., 2016). Figure 2 shows how the growth medium's phycocyanin levels changed when urea was added. Urea will increase the amount of nitrogen in the medium, which resulted in more protein being generated (Sala et al., 2018). At 20-60 mg/L urea, increasing light intensity had notable effect on C-PC, however, the concentration was lower compared to

80-100 mg/L urea fraction. Nevertheless, C-PC experienced a slum of decrease when the medium was supplemented by 100 mg/L urea due the inhibition process might take place because of ammonia presence. In addition, at the light intensity above 7,000 lux, C-PC tended to diminish due to photoinhibition which damaged the pigment quality (Kerena & Krieger-Liszkay, 2011). Taufikurahman et al., (2020) mentioned that C-PC of *Spirulina plantesis* in dairy manure effluent was 1.91% at 4,000 lux irradiances. The sufficient of nitrogen source and light intensities could be the reason the level of C-PC in this research was 4-time higher accounting for 4.21 ± 0.32%.

Effect of Light Irradiation and Urea Concentration on COD Removal

Figure 3 shows the effect of urea and light intensity on COD levels in AW. Oil, carbohydrates, and proteins in the waste were consumed by microalgae, which resulted in a drop in COD values in the media. The addition of AW proved that wastewater can be utilized as the medium for microalgae growth in accordance with previous research (Ansari et al., 2017; Gao et al., 2016; Malibari et al., 2018). The amount of COD decreased boosted up with *Spirulina* sp. growth rate. This is the evident that the pace of maximum growth rate matched the rate of COD removal. The optimum COD reduction was achieved by adding 80 mg/L urea at 8,000 lux of light intensity or equivalent to 96.51% elimination. Cheng et al (Cheng et al., 2022) also found that *Chlorella vulgaris* cultivated in mixed swine-fishery wastewater succeeded removing 95.9% COD. Whilst Gao et al (Gao et al., 2016) removed 82.7% COD in aquaculture wastewater using *Scenedesmus obliquus*.

CONCLUSION

Various urea concentrations (mg/L) and light intensities (lux) were employed to culture *Spirulina* sp. in AW for research purposes. Due to its ability to produce 0.71 ± 0.14 g/L/d at 60 ppm and 8,000 lux and a growth rate of 0.18 ± 1.28 1/d, AW has the potential to be utilized as a medium for *Spirulina* sp. growth. The maximum C-PC and COD elimination, however, was attained with 80 ppm urea addition under 7,000 lux intensity, yielding 4.21 ± 0.32% and 96.5%, respectively. In conclusion, it is important to carefully maintain culture conditions at ideal irradiance and nitrogen levels in order to ensure high biomass, C-PC productivity, and COD elimination.

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