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Impact of Photoperiod on the Carbon Metabolic Pathways of *Chlorella Vulgaris* for Biomass Production and Nutrient Removal in Treating Nutrient-Rich Wastewater

Nur Afiqah Mohamad Saman, Wai Hong Leong, Hemamalini Rawindran, Mardawani Mohamad, Muslim Abdurrahman, and Jun Wei Lim

Abstract Cultivation of *Chlorella vulgaris* has been done in different photoperiod to study the effect of photoperiod cycles on the on the carbon metabolic pathways of the microalgae species in order to enhance its biomass and lipid productions. Autotrophic, heterotrophic and three different photoperiod cycles of mixotrophic growth mode were evaluated for its biomass growth, carbon and nutrient uptakes and lipid yield. The studied photoperiods for mixotrophic growth are 16:8, 12:12 and 8:16 h (light:dark hours). Heterotrophic condition produced much lower microalgal biomass and lipid yield compared to microalgae grown under autotrophic and mixotrophic condition. There is no significant difference in microalgal biomass yields observed under continuous illumination between 16:8 h, 12:12 h and 8:16 h photoperiods. All studied parameters showed a near complete removal of COD on the second day of cultivation. Higher nitrogen removal was observed at longer photoperiod conditions.

Keywords Microalgae · Photoperiod · Biomass · Carbon · Nitrogen

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1 Introduction

Microalgae is steadily gaining attraction as the preferred feedstock for sustainable biofuel productions termed as the third-generation biofuels. Moreover, microalgae have the ability in fixing atmospheric carbon dioxide for its growth and produce a variety of end products [1]. Production rate of biodiesel from microalgae is about 5000–20,000 gal/acre/year which is comparatively high than oil production from other feedstocks [2]. In addition, some microalgae strains are very versatile that they can be grown in extreme environments and conditions e.g. high nutrient wastewater loadings and saline water bodies [3–5].

While the merits of using microalgae as a potential biofuel feedstock are undeniably promising, the commercialization of microalgae-based biofuels is rather uncertain [6]. Thus, leading to extensive studies to further enhance the biomass and lipid productivities for eventual exploitations as microalgal biomass feedstock. These include manipulation of cultivation parameters and cultivation modes. The main obstacle that impedes the commercialization of microalgal biodiesel production is the low lipid productivities due to the low cell biomass and lipid production. Owing to the low biomass yield, the harvesting process become difficult which can subsequently lead to high unit cost of downstream process. Various studies into optimizing or modifying the cultivation conditions have been investigated to induce both the microalgal biomass and lipid productivities [7, 8]. As microalgae are known to be able to utilize various carbon metabolic pathways, namely, autotrophic, heterotrophic and mixotrophic, for its growth, manipulating its growth metabolic pathways perhaps could provide an insight into inducing the cellular biomass and lipid productions [9].

Other than that, photoperiod is one of the important physicochemical factors affecting the growth of microalgae as it regulates the cell division in microalgae reproduction. The influence of this photoperiod on biomass and lipid productivity of microalgae has not been clearly established. To further improve the biomass yields of microalgae, it is crucial to optimize the photoperiod to achieve high microalgal biomass growth productivities. Hence, this study is aimed to investigate the carbon metabolic pathways through varying the light and dark cycles i.e., photoperiod for optimizing the microalgal biomass production along with the nutrient removal from nutrient-rich wastewater. Thus, able to provide an insight on the dynamics of microalgal biomass growth in relation to the carbon and nitrogen metabolic pathways under different photoperiod conditions.

2 Materials and Methods

2.1 Wastewater Medium Preparation

Prior to the experiment, a synthetic wastewater medium simulating a high strength municipal wastewater was prepared based on a modified wastewater composition

from Leong et al. [10] yielding concentrations of chemical oxygen demand, COD and ammonium-nitrogen, NH_4^+–N at 145 mg/L and 48 mg/L, respectively. The following were compositions (mg/L) for the wastewater: sucrose (109), $FeCl_3.6H_2O$ (10), $CaCl_2$ (42), $(NH_4)_2SO_4$ (226), K_2HPO_4 (180), KH_2PO_4 (35) and $MgSO_4$ (49).

2.2 Chlorella Vulgaris *Cultivation Using Synthetic Wastewater Medium*

Samples of the freshwater microalgae, *Chlorella vulgaris* was obtained from the culture collections belonging to the Centre for Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS. The microalgae, *Chlorella vulgaris* was chosen in the study due to its ability to populate ponds naturally combined with its adaptability in tolerating high nutrient loadings in sewage effluents [11]. Stock culture of the microalgae, *Chlorella vulgaris* (500 mL) was inoculated in a 5-L laboratory clear glass bottle containing approximately 4.5 L of the prepared synthetic wastewater medium. The photobioreactor was continuously aerated with compressed air and illuminated with a white light-emitting diode (LED) light at the light intensity of 1200 lx. The initial cultivation pH was adjusted to 7.1 ± 0.1.

2.3 *Photoperiod Experimental Design*

The experimental setup consisted of culturing the microalgae, *Chlorella vulgaris* acclimated to the synthetic wastewater medium under five different photoperiod design settings in 500 mL Erlenmeyer flasks as functional bioreactors. Approximately 50 mL of the *Chlorella vulgaris* inoculum was introduced in each of the bioreactor containing 450 mL of the synthetic wastewater medium. The different photoperiod designs in the study with varying light:dark cycle (h:h) included 24:0 (autotrophic), 16:8, 12:12, 8:16 and 0:24 (heterotrophic) designated as BR-24L, BR-16L, BR-12L and BR-8L and BR-0L, respectively (Table 1). All the bioreactors except for the BR24-L (autotrophic) were supplemented with the additional organic

Table 1 Designated bioreactors under varying photoperiod cultivation conditions

Bioreactor	Photoperiod	
	Light (h)	Dark (h)
BR-24L (Autotrophic)	24	0
BR-16L	16	8
BR-12L	12	12
BR-8L	8	16
BR-0L (Heterotrophic)	0	24

carbon source (sucrose) from the compositions of the synthetic wastewater medium. Each of the bioreactor was continuously aerated with compressed air and the cultivation pH was regulated and maintained at 7.0 ± 0.1 using either 1 N of H_2SO_4 or 1 N of NaOH throughout the study. The experimental study was carried out until the stationary growth phase was achieved and performed in duplicates. Samplings were executed every two days for analyzing the microalgal biomass, COD and NH_4^+–N concentrations.

2.4 Analytical Procedures

The microalgal biomass concentration was determined via spectrophotometry using a UV-VIS spectrophotometer (Shimadzu UV-2600). An analysis of optical density was measured at the wavelength of 688 nm and the microalgal biomass concentration, B (g/L) was calculated using Eq. 1.

$$B = 0.3557 \times OD_{688nm},\ R^2 = 0.988 \tag{1}$$

The concentration of COD was determined using the closed reflux, titrimetric method: 5520 following the Standard Methods for the Examination of Water and Wastewater [12]. The concentrations of NH_4^+–N species were also analysed following the Standard Methods [12], namely, the titrimetric method: 4500-NH_3.

3 Results and Discussion

3.1 Microalgal Biomass Growth

The growth patterns of *Chlorella vulgaris* were visualized in Fig. 1. The initial microalgal biomass in all bioreactors are relatively the same, which was around

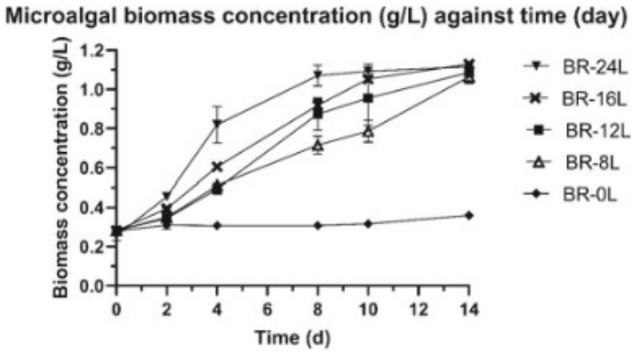

Fig. 1 Microalgal biomass growth cultured under different photoperiods

0.2 g/L. The first 2 days were considered as the lag phase period of microalgae cultured in all bioreactors as demonstrated by a relatively low biomass growth. BR-24L experienced a sudden increase in biomass concentrations (days 2–8) before entering the stationary phase at the 10th day of cultivation. BR-16L experienced a logarithmic growth phase in the 4th–8th day of cultivation whereas BR-12L and BR-8L experienced logarithmic growth phase later in the 8th–14th day and 10th–14th day of cultivation respectively. A trend was observed at which longer photoperiod induces faster microalgal biomass growth. Meanwhile, no logarithmic growth phase was observed in BR-0L indicating that the growth of *Chlorella vulgaris* was inhibited in heterotrophic culture.

At the end of cultivation study (14th day), the final biomass yields of 1.115 ± 0.003 g/L, 1.130 ± 0.001 g/L, 1.087 ± 0.040 g/L, 1.065 ± 0.020 g/L, and 0.359 ± 0.009 g/L were achieved in BR-24L, BR-16L, BR-12L, BR8-L, and BR-0L respectively. Although the final biomasses of BR-24L, BR-16L, BR-12L and BR-8L are comparable, BR-16L attained the highest biomass yield which suggested that under cyclic dark/hour conditions, cell production of biomass is higher.

3.2 Carbon (COD) Removal

COD measurement was carried out in the present study to estimate the carbon removal efficiencies in terms of COD removal from the synthetic wastewater media by *Chlorella vulgaris* at different photoperiods. The amount of organic carbon (sucrose) added to the bioreactors in this study generating an initial COD value of 103.63 mg/L. Figure 2 depicts the removal of COD under different photoperiods.

Rapid removal of COD was observed where about 80% COD removal was attained in all bioreactors on the second day of cultivation where similar COD removal patterns were noticed stipulating that there was no significant relationship between COD removal and cultivation of *Chlorella vulgaris* at different photoperiods. At the end of cultivation, the final concentrations of COD were found to be 6.72 ± 1.33, 8.64 ± 3.991, 13.44 ± 2.661, and 13.44 ± 2.661 mg/L in BR16-L, BR12-L, BR-8L

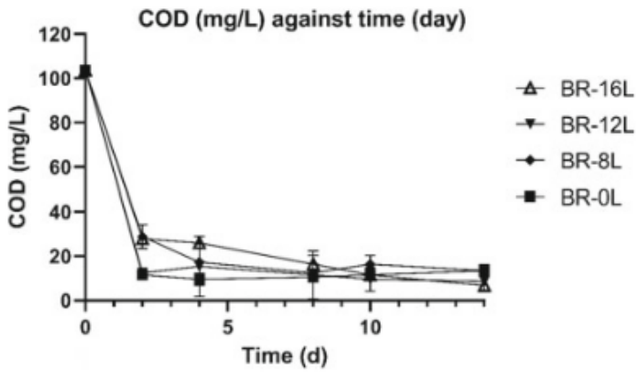

Fig. 2 COD removal by *Chlorella vulgaris* cultivated under various photoperiods

and BR-0L respectively. The almost complete removal of COD from microalgae culture suggested that complete biodegradable COD source was immediately being assimilated by *Chlorella vulgaris.*

3.3 Nitrogen Removal

Microalgae is known to be able to utilize nutrients in particular nitrogen and phosphorus for its growth [13]. Nitrogen could present in various form such as nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+–N), ammonia (NH_3), nitric acid (HNO_3) and nitrogen gas (N_2). In the present study, NH_4^+–N species was introduced in the synthetic wastewater medium. The removal of NH_4^+–N species efficiencies by *Chlorella vulgaris* cultured at different photoperiods is represented in Fig. 3.

From Fig. 3, high NH_4^+–N removal is observed in BR-24L followed by BR-12L, BR-16L and BR-0L with final concentration of $0, 0.38 \pm 0.527, 3.15 \pm 0.873, 15.75 \pm 0.873$ and 30.87 ± 0.873 respectively. BR-24L exhibited the fastest NH_4^+–N removal at which complete removal was observed at the 8th day of cultivation at which the microalgal growth has reached stationary growth phase. A similar trend was observed in BR-16L, BR-12L, and BR-8L where high removal of NH_4^+–N species is achieved as the microalgal growth entering stationary growth phase. Contrarily, the NH_4^+–N uptake by *Chlorella vulgaris* grown in BR-0L was impeded as no decline in NH_4^+–N concentrations is seen after second day of cultivation which assumed that NH_4^+–N was not assimilated by microalgae.

It is interesting to note that, although the intake of nitrogen was hindered in BR-0L, a prominent decrease in COD is seen in the second day of cultivation. An assumption was made that a complete consumption of the organic carbon source i.e. sucrose, occurred during the second day of cultivation. The notorious decrease in COD values caused by the complete degradation of organic carbon sources by the microalgae which explained the stagnant growth of microalgae in BR-0L (heterotrophic) even after 14 days of cultivation. The growth of microalgae grown under total dark condition is highly dependent on the presence of organic carbon source. Once the organic

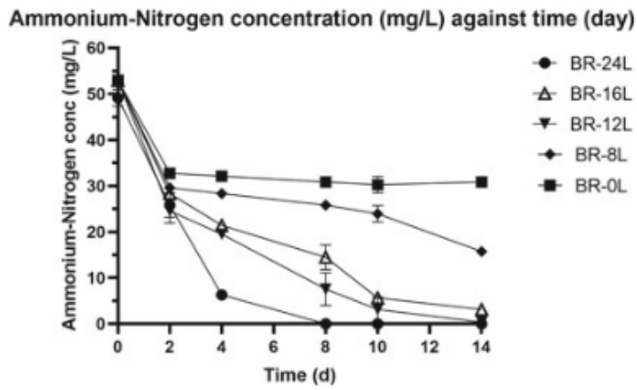

Fig. 3 Ammonium-nitrogen (NH_4^+–N) removal by *Chlorella vulgaris* cultivated under various photoperiods

carbon source was used up, the microalgal growth will be ceased as there is no source of energy left to support the cell metabolism for microalgal growth. The reasoning is further supported by the intake of NH_4^+–N species was prohibited as the assimilation of nitrogen into microalgal cell is largely associated with the availability of organic carbon source that serves as energy to convert NH_4^+–N into biodegradable nitrogen for microalgae assimilation [14]. Nonetheless, other bioreactors continue to demonstrate an increase in biomass growth and reduction in nitrogen concentration although organic compound was fully consumed. It can be inferred that after second day of cultivation, the growth of microalgae in other bioreactors were solely rely on the light energy via the photosynthetic pathway. It is also worth noting that higher NH_4^+–N removal efficiencies were achieved at longer photoperiods which is in tandem with the trend observed in the microalgal biomass growth.

4 Conclusion

This study aimed at finding the optimum photoperiods in enhancing biomass and lipid productions by investigating the effect of photoperiods on the biomass production, carbon and nutrient uptakes and lipid yields. Five different photoperiods were chosen to study in this study. Higher microalgal biomass yield accompanied by higher nitrogen removal by *Chlorella vulgaris* were observed under longer light photoperiod cycles. However, there were no remarkable differences observed in the light cycles of 24 h, 16 h and 12 h in the photoperiod study. Therefore, the 12 hphotoperiod duration is deemed to be the most ideal cultivation condition considering lower energy consumption for illumination viewing from an economic perspective. It can be deduced that the concentration of organic carbon source i.e. sucrose, introduced in the microalgal growth culture is relatively low leading to carbon deficit in the microalgal consortium. Dark/light cycle can counterbalance the shortage of organic carbon source by utilizing carbon dioxide as an inorganic carbon source in the presence of light for photosynthetic metabolic grow. As a result, high biomass growth was achieved under the 16 h, 12 h and 8 h light cycles of photoperiod whereas the growth of the heterotrophic condition (0 h) was totally inhibited.

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