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Optimizing photoperiods for enhanced microalgae-based phycoremediation of food wastewater in Malaysia

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Abstract. Food processing wastewater poses significant environmental challenges due to high pollutant levels, necessitating effective treatment methods. While *Chlorella vulgaris* has shown promise in wastewater treatment, the optimal light/dark photoperiod regimes for maximizing biomass growth, nutrient removal efficiency, and COD reduction remain underexplored. This study evaluates the effects of 12H:12H light/dark and 24H light photoperiods on the performance of *C.vulgaris* in treating food processing wastewater. The 12H:12H photoperiod achieved 73% COD removal with a biomass yield of 0.44 g/L, while the 24H light achieved 75% COD removal with a biomass yield of 1.02 g/L. Both photoperiods fully removed ammonia by day 12. Although the 24H light period increased biomass production, it is energy-intensive, whereas the 12H:12H photoperiod is more energy-efficient but requires further evaluation. These findings highlight the importance of optimizing photoperiod regimes to enhance the efficacy of microalgae-based wastewater treatment. This research supports sustainable wastewater management in the food industry and aims to meet regulatory standards through tailored photoperiod strategies.

1 Introduction

Food processing wastewater, laden with high concentrations of pollutants, presents substantial risks to human health, aquatic ecosystems, and the quality of water for drinking and agriculture if left untreated; furthermore, it can compromise food safety by introducing pathogens or harmful residues into food products [1]. Efforts in wastewater treatment aim to meet Environmental Quality Act standards before discharged [2]. While biological

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treatment using bacteria is widely adopted in the industry for its cost-effectiveness and sustainability, it is associated with the release of carbon dioxide, contributing to global warming and climate change [3]. Microalgae-based bioremediation offers an alternative, with *Chlorella vulgaris* showing high nutrient removal efficiency [4]. Previous studies have demonstrated the effectiveness of *C. vulgaris* in removing COD and other pollutants from various wastewater types, including brewery Cleaning in Place (CIP) wastewater achieved a COD removal rate of 75% and phosphate removal efficiency of 79% [5].

Microalgae can be cultivated using four methods photo-autotrophic, heterotrophic, photo-heterotrophic, and mixotrophic with mixotrophic cultivation favoured in wastewater treatment for its superior biomass production efficiency [6]. The study conducted by Cao and colleagues (2023) highlighted mixotrophic cultivation's superior biomass productivity at 0.777 g/L/d, surpassing heterotrophic (0.687 g/L/d) and photoautotrophic (0.171 g/L/d) methods [7]. These insights underscore the importance of cultivation methods in enhancing biomass productivity, offering valuable implications for sustainable biofuel production.

This study investigates optimizing photoperiods to improve *C. vulgaris* performance in treating food processing wastewater by comparing the effects of 12H:12H light/dark and 24H light photoperiod on biomass growth, COD reduction, and nutrient removal efficiency. In Malaysia, abundant sunlight and nutrient availability support continuous microalgae production, making light period optimization essential for effective nutrient removal and productivity. Although sunlight lasts about 12 hours daily, adding nighttime lighting increases costs. Thus, examining the impact of different photoperiods on microalgae-based wastewater treatment is crucial. This research assesses how varying light durations affect mixotrophic microalgae treatment using *C. vulgaris* with consistent LED grow lights. Improving *Chlorella*-based bioremediation under different photoperiods aims to enhance wastewater management in Malaysia's food processing industry, addressing environmental concerns and meeting Department of Environment (DOE) treatment requirements.

2 Materials and methods

Food processing wastewater, from a local Malaysian facility, underwent primary treatment to reduce suspended solids and oil/grease before experimentation. *C. vulgaris* stock was cultured for 3 weeks in a fertilizer-enriched medium. Following sedimentation, 15 ml of the resulting microalgae paste was added to 500 ml conical flasks and mixed with the wastewater of 250 ml volume. Two photoperiods (12H:12H light/dark and 24H light) were tested with continuous aeration for 12 days at ambient temperature. Continuous aeration was maintained using air pumps connected to each flask. LED grow lights were placed above the flasks to provide the required illumination for the specified photoperiods, ensuring reproducibility of the experimental conditions. Parameters including ammoniacal nitrogen, COD and orthophosphate were measured using colorimetric methods (HACH Methods 8155, 8000 and 8048 respectively) on days 0, 2, 5, 7, 9, and 12. The spectrophotometer DR3600 was employed for analysis. Microalgae biomass was assessed by centrifuging the sample and drying it at 60°C overnight. The dry mass was weighed using an analytical balance.

3 Results and discussion

3.1 Characterization of food wastewater

The presence of microalgae culture led to fluctuations in ammonia levels, necessitating treatment to comply with the discharge limit set by the Department of Environment

(DOE)[8], given the initial COD concentration of 1500 mg/L.

Table 1. Characterization of food wastewater.

Parameters	Food wastewater (mg/L)	DOE discharge limit (mg/L)
COD	1500	200
pH	6.8	5.5-9.0
Orthophosphate	123	-
Nitrate	1	-
Nitrite	500	-
Ammoniacal nitrogen	4	2

3.2 Growth and nutrient removal of microalgae in food wastewater by different photoperiods

The study delved into the biomass growth curves of *Chlorella vulgaris* over a span of 12 days under two distinct light conditions: 24H light and 12H:12H light-dark photoperiod, as depicted in Figure 1(a). *C. vulgaris* reached the early stationary phase on day 9, marking an opportune time for harvesting. The microalgae culture subjected to 24H sustained growth until the 12th day, with the maximum biomass concentration recorded at 1.02 g/L on the 12th day, compared to 0.44 g/L on the 9th day in the 12H:12H condition. Notably, Leong and friends reported parallel results when cultivating *C. vulgaris* in municipal wastewater under continuous light (24-h) compared to three light/dark regimes (8h/16h, 12h/12h, and 16h/8h) [9]. The decrease in cell biomass observed under the 12-hour light condition could be due to biomass loss through respiration under darkness [10].

The study of Cosgun and Wong stated that the relationship between photoperiod and light intensity can impact microalgae growth, where the increment of the photoperiod is more beneficial for microalgal growth when at the light intensity below than the optimal [11-12]. Conversely, when the light intensity reaches or exceeds the optimal level, the increment of the photoperiod is detrimental to microalgal growth [13-14]. Liu and coworkers found that microalgae exhibit photoinhibition under conditions of high light intensities and prolonged light periods [15] . In this study, a light intensity of 3000 lux, suitable for microalgae growth, was used, promoting higher biomass under 24-hour light conditions. Another result also showed by Putri et al., who demonstrated that a light intensity of 35000 lux and 24-hour light led to higher biomass compared to light/dark regimes (20h/4h, and 16h/8h) [16].

The COD removal efficiency trends are notably similar, with the 12H:12H light/dark photoperiod consistently achieving high levels, including an intense COD removal of 73% (reduced to 380 mg/L COD from 1500 mg/L COD) on day 2, reach the peak at 78% on day 5. The 24H closely follows, displaying a significant removal of 75% (reduced to 360 mg/L COD from 1500 mg/L COD) on day 2. It's worth noting that day 2 coincides with the exponential phase of microalgae growth (refer to Figure 1(b)), where the culture actively consumes the carbon source. Statistically, there is no significant difference on the COD removal after day 2 for both illuminations. The result indicates that for 24H doesn't notably enhance algae's COD degradation suggesting 12H:12H is sufficient for effective COD removal. This result is consistent with previous studies that found minimal improvement in COD degradation with extended 24-hour photoperiods, as COD removal was influenced by light intensity rather than duration, which controlled the light-dark cycle in algal metabolism to break down organic substances [17-18]. The incomplete COD removal is due to the presence of non-biodegradable matter and a poor nutrient ratio in the food wastewater [19]. In this study the C:N:P ratio of wastewater was 12.2:4.1:1, results oppose the traditional Redfield C:N:P ratio of 106:16:1 endorsing the need for optimizing the C:N:P for wastewater for better nutrient removal result [20].

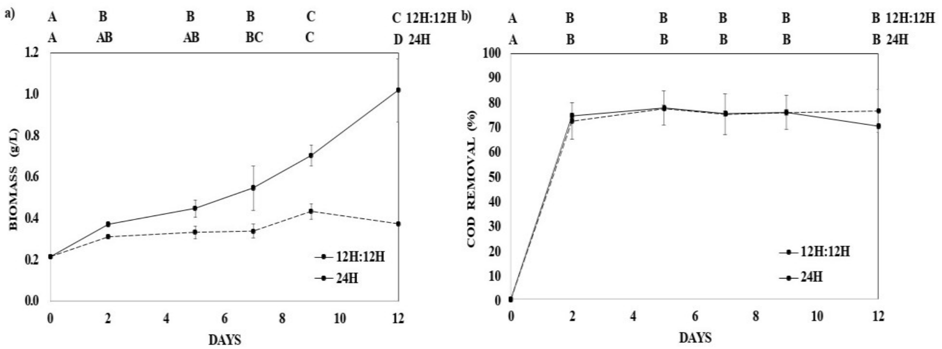

Fig. 1. The (a) growth curve and (b) COD removal efficiency of the *C. vulgaris* microalgae in food wastewater under different illumination duration of 24H light and 12H:12H light-dark photoperiod with continuous air supply. Statistically significant was evaluated based on concentration in one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

The presence of ammoniacal nitrogen, including NH_3 and NH_4^+, presents varied toxicological implications [21]. The data in Figure 2(a) suggests that, in the initial stages, the 24H photoperiod exhibits a more rapid removal of ammonium compared to the 12H:12H period. On day 2, the 24H photoperiod demonstrates a substantial decrease of in removal efficiency -39%, while the 12H:12H period shows a more modest reduction of -9%, this indicated that the ammonium in wastewater is not utilised but is released from cell to wastewater due to cell death, which occurs as a natural consequence during the growth phase of the cells causes by imbalance nutrient content in food wastewater. Statistically, both illuminations showed a significant difference, and both photoperiods ultimately reached a maximum removal efficiency of 100% by day 12, highlighting that while the 24H period starts with a more accelerated removal, the overall effectiveness converges with the 12H:12H period over time. Furthermore, a direct correlation emerges between biomass productivity and ammonium uptake, supported by microalgae's preference for ammonium as a nitrogen source [22]. This research ensures that ammonium levels remain within acceptable ranges that promote the flourishing of *C. vulgaris*, deviating from previous findings that indicated inhibitory effects beyond concentrations of 110 mg/L [23].

Moreover, microalgae can directly uptake orthophosphate (PO_4^{3-}) as a phosphorus source for cell growth and nutritional content [24]. In the 12H:12H photoperiod scenario, orthophosphate removal efficiency gradually improves with fluctuations, starting at 3.63% on day 2 and reaching 16.76% by day 12. Conversely, the 24H photoperiod demonstrates a more consistent and pronounced increase, starting at 4.14% on day 2 and steadily climbing to 31.80% by day 9, maintaining a high level on day 12 (31.72%). Despite these variations, no significant difference in orthophosphate removal is evident between the two photoperiods [Figure 2(b)]. The limited capability of microalgae to achieve high orthophosphate removal rates may stem from imbalanced nutrient ratios. Moreover, the correlation between ammoniacal nitrogen and orthophosphate concentrations, as indicated by previous research, suggests an optimal ratio of 35:1 for efficient nutrient removal [25]. However, this study reveals a ratio of 0.2:1, implying that the concentration of ammoniacal nitrogen may not be sufficiently high to facilitate complete orthophosphate removal.

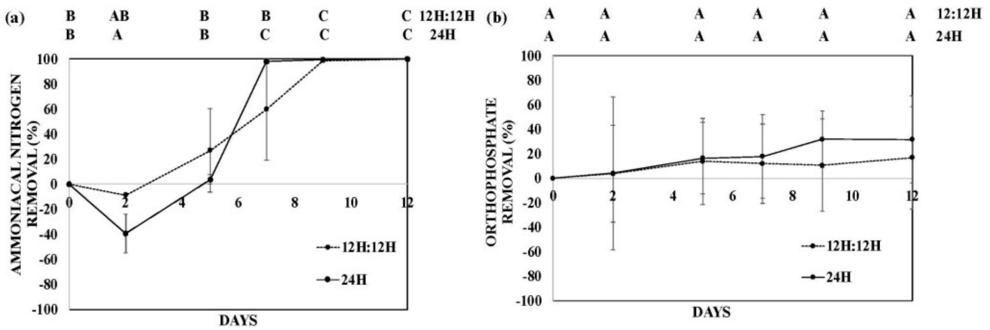

Fig. 2. The (a) ammoniacal nitrogen, (b) orthophosphate removal efficiency of the *C. vulgaris* microalgae in food wastewater under different illumination duration of 24H and 12H continuous air supply. Statistically significant was evaluated based on concentration in one-way analysis of variance (ANOVA) followed by LSD all-pairwise comparison test at $p < 0.05$.

4 Conclusion

This study investigates the impact of imbalanced C:N:P ratios on microalgae-based wastewater treatment. Despite nutrient challenges, a 12H:12H light-dark photoperiod achieved 73% COD removal with sustained biomass growth, while a 24-hour photoperiod resulted in higher biomass production with comparable COD removal efficiency (75%). Both photoperiods achieved complete ammonia removal, though initial removal rates varied, and orthophosphate removal improved gradually, indicating potential nutrient ratio imbalances. Consequently, optimizing nutrient ratios is essential for enhancing treatment efficacy in the food industry. The 12H:12H photoperiod emerges as a viable, cost-effective option for efficient COD removal. Future research should focus on optimizing nutrient ratios and investigating nutrient removal trends. In conclusion, optimizing photoperiods represents a promising strategy for sustainable management of food industry wastewater, ensuring regulatory compliance and addressing environmental concerns effectively.

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