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Optimisation and growth kinetic analysis of Microalgae, *Arthrospira platensis* in 2-L Photobioreactors

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Optimisation and growth kinetic analysis of Microalgae, *Arthrospira platensis* in 2-L Photobioreactors

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Abstract. In recent years, photoautotrophic microalgae are widely recognised due to their diverse yet significant natural values, particularly in healthcare, including pharmaceuticals, cosmetics and feedstock industries. One of the most exploited blue-green microalgae, *Arthrospira* sp. has been addressed as a potent superfood. However, the microalgal mass-production requires suitable inoculum strain and controlled cultivation conditions for enhanced growth performance. Hence, this study aimed to maximise biomass of *Arthrospira platensis* chosen strain in a 2 litres indoor photobioreactor under three different parameters which were aeration rate, light intensity and pH of the medium. In the present study, a comparative study of growth performance between helical (S1) and straight form (S2) of *A. platensis* was conducted and the results revealed that morphological differences did not affect growth performances. Meanwhile, the optimisation based on the parameters studied shows that cultivation of *A. platensis* with aeration of 0.5 L/min and medium of pH 9.0 yielded the highest biomass production which were 1.500 ± 0.049 g/L. Under different light intensity, *A. platensis* produced the highest biomass and maximum specific growth rate of 1.142 ± 0.037 g/L and 0.716 ± 0.018 1/day, respectively when cultivated under irradiance of 6000 Lux. In conclusion, compared to before optimisation, biomass and maximum specific growth rate after optimisation was 137% and 24% increased, respectively.

1. Introduction

The influence and enhanced awareness of modern healthy lifestyle has drastically increased the global demand of *Arthrospira* sp. also known as Spirulina as a food supplement in food industries due to its promising therapeutic and nutritive values. *Arthrospira* sp. has blue-green unbranched and non-heterocystous filaments that consists of vegetative cells involved in binary fission in a single plane, perpendicular to the main axis [1]. Nevertheless, *Arthrospira* sp. has a unique helical shape and regularly coiled trichome (filaments) morphology. These helical trichomes, however, can lose their original helical forms and convert to abnormal morphologies under the adverse conditions of temperature and pressure [2], affecting the overall growth performance and biomass production.

In mass-cultivation process, factors such as climate change and limited land area have hindered large-scale productions of *Arthrospira* sp. through conventional open pond system [3]. As a result, a closed bioreactor system with better-controlled parameters, optimisation platform and a productive cultivation medium is needed [4]. In term of laboratory cultivation, the production of *Arthrospira* sp. is highly dependent on the major environmental factors including luminosity, pH, inoculation size, mixing and macro and micronutrient composition in media [5]. Among all nutritional media,



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Zarrouk's medium (ZM) is the most widely used standard media for the cultivation of *Arthrospira* sp. due to the high alkalinity which is around 9.0-9.5 [6]. However, the medium pH may be reduced significantly due to the increase of concentration of carbon dioxide in the system, particularly for the culture with small size [7]. Therefore, the bicarbonate buffering system is commonly applied in the nutritional medium to buffer the changes of the pH medium. Other than that, light and photoperiod play important role in *Arthrospira* sp. cultivation where the high values of light intensity promote biomass production whilst low value promotes phytopigment production [8]. Lastly, aeration and mixing are important in ultrahigh density culture as they give a homogenous distribution of the microalgae filaments throughout the growth system which allows them to receive adequate exposure of light and nutrients [5]. In this study, those 3 factors have been studied in optimising the biomass production of *Arthrospira* sp. for mass-cultivation in indoor conditions.

2. Materials and Methods

2.1. Preparation of the medium and inoculum

The cultures of two morphological forms (S1: coiled trichome forms, S2: straight trichome) of *Arthrospira platensis* were purchased from the laboratory of University of Texas (UTEX-LB2340) located at Austin, Texas. Meanwhile, the medium used as the main cultivation for both strains of *A. platensis* was Zarrouk's medium (ZM) [9]. Upon arrival, the strains were cultured under irradiance of light intensity of 3000 Lux at room temperature ($30\pm 2^\circ\text{C}$). Then, the 10% (v/v) of the stock culture from each strain were transferred into fresh ZM medium and the absorbance reading of both inoculums were then monitored daily for cellular growth rates by measuring the optical density (OD) using UV-Vis spectrophotometer at the measurement wavelength of 680 nm.

2.2. Growth profile of both strains

To study the growth profile of *A. platensis*, the cultures were cultivated using 500 mL of sterilised Erlenmeyer flask with 250 mL of working medium [10]. Then, 10% (v/v) of the previously prepared inoculum was introduced into fresh ZM and incubated for 18 days under the same condition as previous with maintained pH value of the medium throughout the incubation period. The growth rate of each *A. platensis* culture was monitored daily and the strain which has higher performance was used for the following experiments. All experiment was conducted in triplicates and the mean of triplicate results was used to present the results obtained.

2.3. Optimisation of biomass production

The influence of different parameters (aeration rate, pH and light intensity) on the biomass production were studied by cultivating *A. platensis* in 2 L photobioreactor (bubble column) with 1.5 L of ZM as shown in Figure 1. Similarly, 10% (v/v) of inoculum were grown in the photobioreactor at room temperature with aeration of 0.5 L/min using air pump under the irradiance of 3000 Lux ($42\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$) with 0 h dark and 24 h light cycle for 18 days for each experiment unless otherwise stated.

For the study of aeration rates, the *A. platensis* was cultured in the photobioreactor with three different aeration rates (0.1 L/min, 0.5 L/min and 1.0 L/min) and incubated under the standard condition. Meanwhile, the evaluation of the influence of pH on the *A. platensis* biomass production was conducted in ZM with different pH (pH 8.5, pH 9.5 and pH 10) that were prepared by adjusting the pH using concentrated hydrochloric acid and sodium hydroxide under the monitor of pH. Lastly, the study of light intensity affecting the cultivation of *A. platensis* was done by cultivating the culture under the illumination of white fluorescence lamp at three different light intensity (4000 Lux, 5000 Lux and 6000 Lux). Likewise, the cultures were incubated and analysed for the optical density daily. Then, the optimum condition from each experiment was used for the next parameter. Next, a standard linear regression was constructed based on the observed optical density readings and microalgal dry cell weight and was used to analyse the dynamic growth curves of microalgae.



Figure 1. The 2 L photobioreactor used in the cultivating microalgae to optimise the biomass production of *A. platensis* under specific conditions.

2.4. Growth Kinetics Analysis

The calculation of cell growth kinetic which includes maximum growth rate (μ_m) and cell productivity (P_x) were calculated using the dry cell weight data.

The maximum specific growth rate of *A. platensis* was calculated as (2.1) where X_1 and X_2 are biomass concentration at time interval t_1 and t_2 hours;

$$\mu_m = 2 \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (2.1)$$

The cell productivity of the microalgae was calculated as (2.2) where $(x_m - x_i)$ is the variation in cell concentration, T_c is the cultivation time;

$$P_x = \frac{x_m - x_i}{T_c} \quad (2.2)$$

3. Results and Discussion

3.1. Growth analysis of different microalgae strains

The study of growth profile between *A. platensis* strains with coiled trichome (S1) and straight trichome (S2) showed both morphological forms exhibit similar readings in term of maximum cell concentration (OD_{680nm}), cell productivity (P_x) and maximum specific growth rate (μ_m) based on Table 1. The maximum P_x for S1 (0.048 ± 0.001 g/L/day) was found lower compared to S2 (0.050 ± 0.002 g/L/day). In terms of μ_m , it can be seen that S2 (0.393 ± 0.012 1/day) was slightly higher than S1 (0.381 ± 0.004 1/day) and same trend observed with maximum cell concentration. Although S2 was slightly higher than S1, the one-way ANOVA analysis showed that there was no significant difference ($P > 0.05$) between S1 and S2. On the contrary, Figure 2 shows S1 was found to have higher daily P_x and μ than S2, in spite of lower overall P_x and μ_m as shown in Table 1.

These findings are in lines with the previous results obtained by [6], who obtained similar growth kinetics patterns for *A. platensis*. In their study, the low concentration of culture *A. platensis* in the initial culture had resulted in a noticeable net decrease in growth rate and a further decrease after achieving peak value was then recorded due to the low amount of light penetration because of high cell density. Therefore, since S2 achieving slightly higher P_x and μ_m compared to S1, strain S2 has been chosen as the main experimental microalgae strain for the upcoming studies. As has been described by previous study [11], the gradual fragmentation and a decrease in trichomes size, as well as increased coiling of trichomes of spirulina, would negatively affect the harvest efficiency. Therefore, S2 with straight trichomes which more mechanically rigid, and no coiling is comparatively a better candidate for large-scale industrial cultivation. In addition, straight variants with increased

survival capacity have better growth performance and viability than helical variants in both indoor artificial growth conditions as well as in cryopreservation storage [2, 12].

Table 1. Maximum cell concentration (OD_{680nm}), cell productivity (P_x) and maximum specific growth rate (μ_m) of *Arthrospira platensis* strains (S1: coiled trichomes; S2: straight trichome).

Strain of <i>A. platensis</i>	Max cell conc. (680 nm)	P_x (g/L/day)	μ_m (1/day)
S1	1.287 ± 0.019	0.048 ± 0.001	0.381 ± 0.004
S2	1.318 ± 0.059	0.050 ± 0.002	0.393 ± 0.012

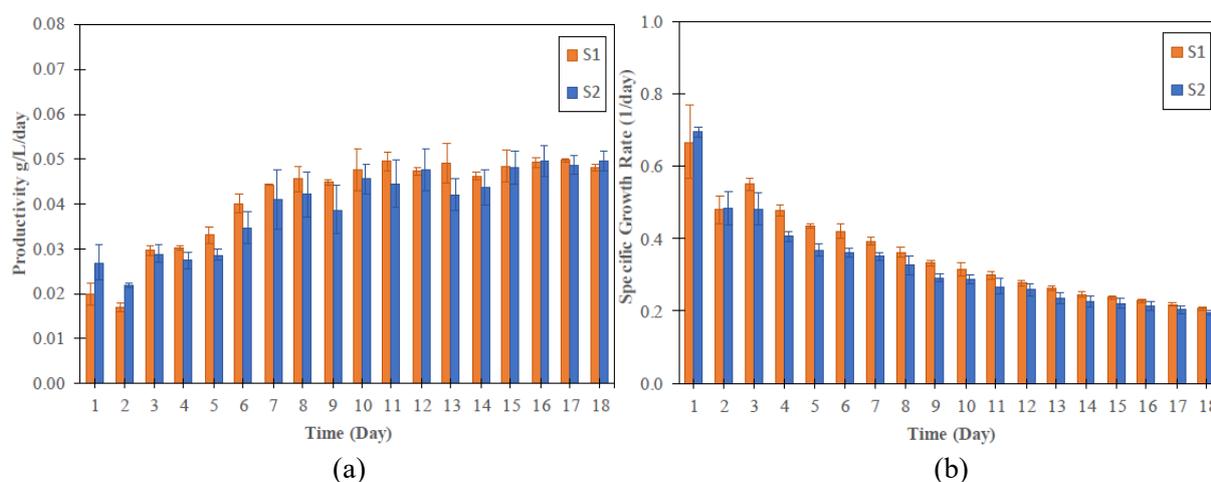


Figure 2. Variation in (a) cell productivity, P_x and (b) specific growth rate, μ of *A. platensis* in unaerated batch culture. Each value is the mean of three samples. Bars represent the standard deviation of the mean.

3.2. Effect of aeration rate on microalgae growth

The growth rates of *A. platensis*, expressed as dry cell weight (g/L), under the unaerated condition (Control) and the aerated condition (0.1, 0.5 and 1.0 L/min) are illustrated in Figure 3 (a). In general, the growth curves for different aeration rate of *A. platensis* cultures showed similar growth trend. Besides, a rapid increase in biomass values was reported at all aeration rates. On the contrary, the biomass increased gradually with increasing incubation time in unaerated condition, giving the maximum biomass values (X_m) at 0.919 ± 0.041 g/L on the 18th day. The difference in the dry cell weight may due to the *A. platensis* cultures bubbled with ambient air at different aeration rates increased significantly as the cultivation time increased. Despite that, all cultures showed a linear growth pattern independent of different aeration rates. The increased growth rate was associated with the increase in aeration rate up to 0.5 L/min. Meanwhile, the further increase in aeration rate to 1.0 L/min resulted in comparatively reduced growth rate as compared to other aeration rates. As mentioned by Priyadarshani et al. [13], aeration plays a key role in keeping the microalgae in suspension, and thus giving the *Arthrospira* sp. filaments a homogenous distribution for adequate exposure to illumination throughout the cultivation.

The present findings showed similar result to the previous study with μ_m and P_x exhibited linear dependence on the aeration rates. Figure 3 (b) shows the variation in biomass productivity (P_x) and maximum specific growth rates (μ_m) with continuous sparging operation at different aeration rates. The relatively lower values in μ_m and P_x recorded from the culture at aeration of 1.0 L/min after 18 days have confirmed the inhibitory effect of shear stress associated with excessive aeration rate on

microalgal growth. The findings are in parallel with study done where an increase in the aeration rate from 0.2 to 1.2 *vvm* resulted in significant improvements in both μ_m and P_x (2.5- and 3.0-fold higher, respectively), while further increases in aeration did not affect growth nor productivity [14]. Furthermore, as reviewed by many studies, strong agitation rate provided during the cultivation process may cause excessive hydrodynamic stress to the algal growth, causing increased cell mortality, decreased growth rate and cell viability, or even cell lysis [15, 16]. In contrast, sufficient aeration also prevents cell growth on the reactor walls and enables *A. platensis* to rotate about its helix, allowing all cells of the population are equally exposed to the light, promoting the photosynthesis, and subsequently, better growth performance and characteristic green colouration [6, 17].

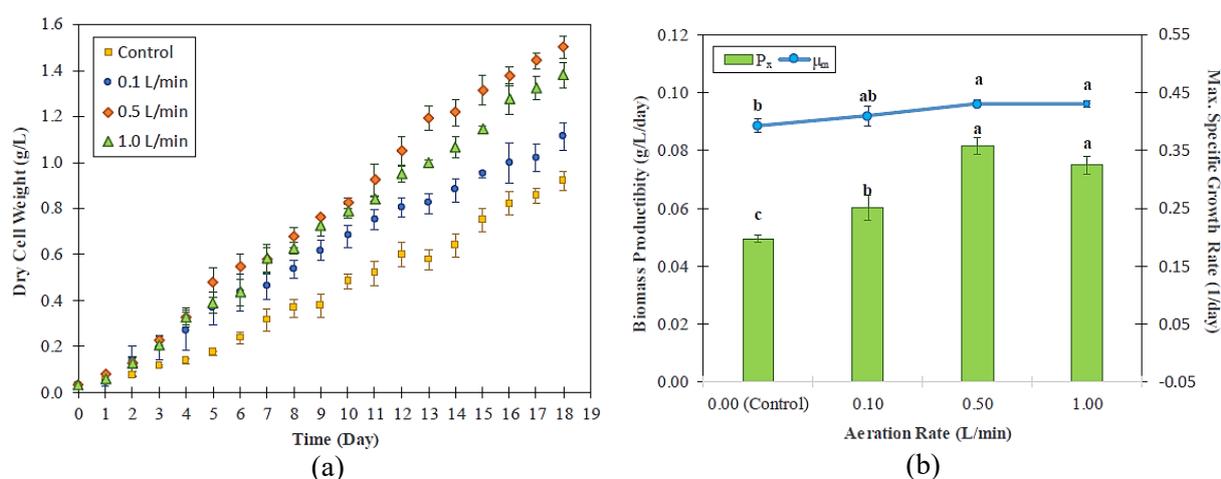


Figure 3. (a) Dry cell weight (g/L) variation at different aeration rates: 0.0 L/min, 0.1 L/min, 0.5 L/min and 1.0 L/min in triplicates. (b) Effect of aeration rate on biomass productivity (g/L/day) and the maximum specific growth rate (1/day). Bars represent the standard deviation of the mean and different superscript letters indicate a significant difference between means ($p < 0.05$).

3.3. Effect of pH of the media on microalgae growth

At the end of the experiment with three varying pH values (pH 8.5, 9.5 and 10.0) under fully controlled conditions, maximum dry cell weights were found to be 1.212 ± 0.034 , 1.370 ± 0.044 and 1.104 ± 0.055 g/L for the treatments, respectively, and 1.500 ± 0.049 g/L for control in Figure 4 (a). Meanwhile, Figure 4 (b) shows the variation in biomass productivity (P_x) and maximum specific growth rates (μ_m) at different pH levels. A significant change was observed between the treatments and control ($p < 0.05$). Similar to the results observed by Madkour et al. [18], all growth curves showed no lag phase in the present experiment and depicted similar growth behaviour and pattern with maximum biomass concentrations being recorded at the 18th day. Despite that, all cultures showed a linear growth pattern, independent of different culture pH. The present study also revealed that the increase in culture pH was associated with the increased growth rate in treatments up to pH 9.0, while the growth rate greatly reduced with the increase in pH of 9.5 and 10.0.

Interestingly, *A. platensis* at pH 9.0 (control) recorded the highest growth rate, giving significantly higher biomass ($p < 0.05$) than other culture pH values. On the other hand, pH 10.0 recorded the lowest maximum biomass value (1.104 ± 0.055 g/L). These values correlate fairly well with previous finding where the highest P_x by *A. platensis* was measured as 0.094 g/L/Day at pH 9.0 on the 14th day of incubation [19]. The changes in biomass production of *A. platensis* under variation of culture pH may be related to the influence of pH levels on the bioavailability of nutrients and activity of cell components [20]. As a result, shifting the pH away from the optimum value may significantly impact on the algal metabolisms and ultimately alter its phytochemical and nutritional composition [19, 21] with extreme pH, cultures are subject to undergone shear stress [22]. Hence, it can be suggested that

the pH level of the medium affects the growth of *A. platensis*, and the optimum culture pH for biomass production was recorded at pH 9.0 producing highest biomass of 1.500 ± 0.049 g/L.

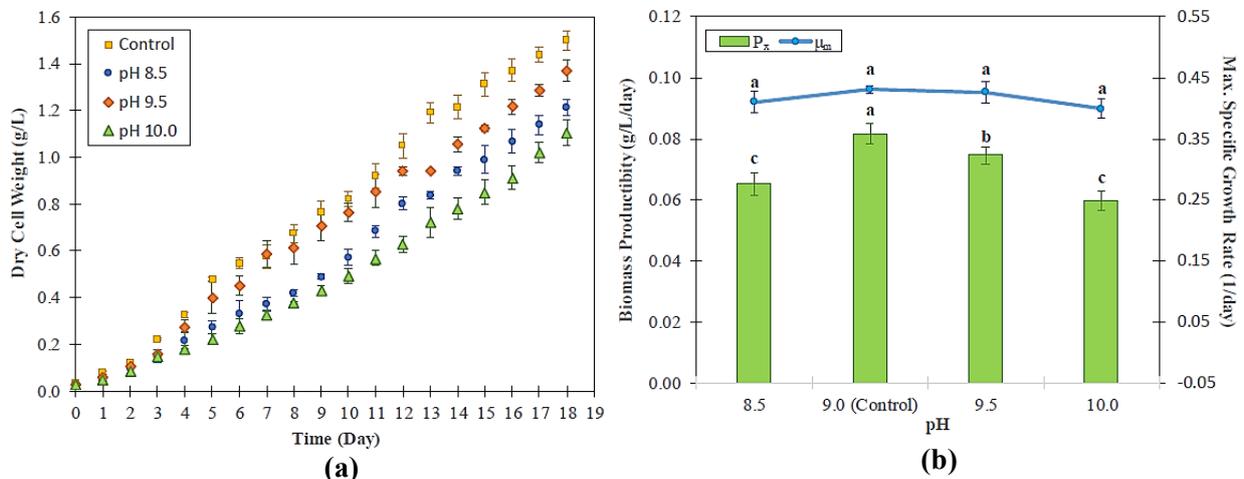


Figure 4. (a) Dry cell weight (g/L) variation at different pH: pH 9.0, pH 8.5, pH 9.5 and pH 10.0 in triplicates. (b) Effect of cultivation pH on biomass productivity (g/L/day) and the maximum specific growth rate (1/day). Bars represent the standard deviation of the mean and different superscript letters indicate a significant difference between means ($p < 0.05$).

3.4. Effect of light intensity on microalgae growth

The growth curve of the S2 *A. platensis* strain expressed as dry cell weight (g/L), illuminated with different light intensities (3000 as control, 4000, 5000 and 6000 Lux) under fully controlled conditions as illustrated in Figure 5. At the end of the experiment, the maximum biomass values were recorded to be 0.942 ± 0.026 , 1.016 ± 0.055 and 1.142 ± 0.037 g/L for the treatments, respectively, and 0.822 ± 0.025 for the control group. All treatments showed similar biomass values during the first 4 days of cultivation as illustrated in Figure 5 (a). According to Danesi et al. [23], at the beginning of cultivation, there are few cells per unit of volume in the vessel and the quantity of light energy received by each cell is higher than the minimum required for the photosynthesis, increasing the cell concentration of the microalgae. However, the mutual shadow effect at the end of cultivation has caused a reduction in energy provided to the cells, limiting the biomass production of microalgae [24].

In the current study, maximum P_x shows *A. platensis* occurred in the light intensity of 6000 Lux, where 0.111 ± 0.004 g/L/Day was achieved and minimum P_x of 0.079 ± 0.003 g/L/Day was achieved in the light intensity of 3000 Lux. Meanwhile, the biomass productivity was increased significantly by approximately 40% from 0.079 ± 0.003 g/L/Day to 0.111 ± 0.004 g/L/Day by increasing the light intensity from 3000 Lux to 6000 Lux based on Figure 5 (b), Hence, it can be suggested that the higher the light intensity, the faster the growth. A similar observation was reached by [25, 26], who recorded a linear relationship between growth rates and light intensity. Nevertheless, exposing the algae with light intensity above the saturating limits induce the photoinhibition and reduce the photosynthetic efficiency as well as limit the biomass production [20]. Zhang et al. [26] reported that the light intensity should not higher than ~ 8600 Lux to avoid the occurrence of photoinhibition. Therefore, by optimising the light available to algae cells, the potential photosynthetic activity at high-density algae can be maintained at an optimum level and thus, the biomass productivity can be increased.

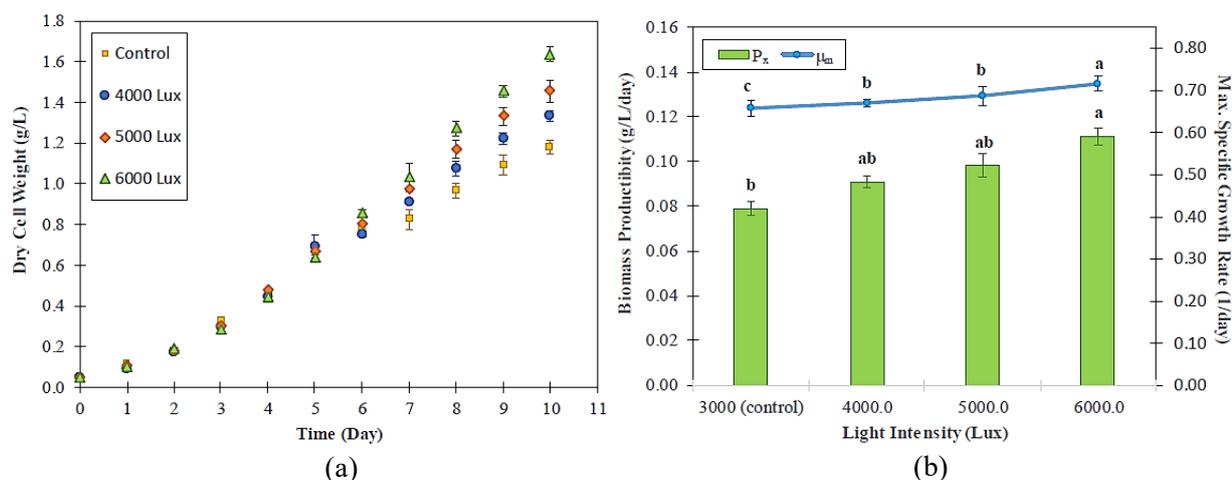


Figure 5. (a) Dry cell weight (g/L) variation at different light intensity: 3000 Lux, 4000 Lux, 5000 Lux and 6000 Lux in triplicates. **(b)** Effect of cultivation pH on biomass productivity (g/L/day) and the maximum specific growth rate (1/day). Bars represent the standard deviation of the mean and different superscript letters indicate a significant difference between means ($p < 0.05$).

4. Conclusion

The comparative study on the growth performance of coiled-form (S1) and straight-form (S2) *Arthrospira platensis* indicated that morphological differences between S1 and S2 did not significantly affect their growth performance due to the similarity in growth pattern, growth characteristics and growth kinetics. Meanwhile, the *A. platensis* biomass production could be maximised by supplying aeration rate up to 0.5 L/min achieving biomass of 1.500 ± 0.049 g/L. However, high aeration rate limits the increase biomass production of *A. platensis* due to generation of high shear stress. Besides that, pH 9.0 was the optimal cultivation pH for *A. platensis* and shifting the pH away from the optimal value could reduce the biomass production. Next, cultivation of *A. platensis* under irradiance of 6000 Lux gave the highest dried cell weight of 1.142 ± 0.037 g/L, improving the biomass production by compared to 3000 Lux. Therefore, compared to before optimisation, biomass and maximum specific growth rate after optimisation was 137% and 24% increased, respectively.

5. Acknowledgement

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