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Outdoor Cultivation of the Biodiesel Promising Microalga *Scenedesmus obliquus* in Municipal Wastewater: A Case Study

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Abstract: Despite the promising research findings on microalgae as a feedstock for biodiesel, reduction of its production cost is still a challenge. One possible solution to overcome this problem is outdoor biomass production using wastewater as a growth medium. The present study aimed to evaluate the climate key parameters for *Scenedesmus obliquus* outdoor biomass production during different seasons, as one of the promising microalgae for biodiesel production. *S. obliquus* was grown continuously in municipal wastewater using bubble column photobioreactors (PBR) made of plastic sleeves arranged vertically in a pilot area of 6m^2 with a total working volume of ≈850 L. Biomass productivity showed positive correlation with light intensity and temperature (0.824 and 0.697, respectively). On the other hand, a negative correlation was recorded between biomass productivity and rainfall (-0.520). The average monthly biomass productivity increased from 13.6 to 20.9g dry weight (DW) m^{-2} d^{-1} by increasing of light intensity/temperature from 6.8 MJ m^{-2}/14.4°C to 15.8 MJ m^{-2}/29.6°C, respectively. At high rainfall of 14.5 mm d^{-1}, the biomass productivity was reduced to 14.0 g DW m^{-2} d^{-1}. The monthly biomass productivity ranged between 13.6 up to 20.9g DW m^{-2} d^{-1} with annual mean productivity of 17.8±2.8g DW m^{-2} d^{-1}. Although the used system showed high efficiency for algae cultivation, high light fluctuation in Shenzhen climate requires continuous regulation of biomass concentration in PBR to enhance the biomass productivity.

Keywords: Microalgae, *Scenedesmus obliquus*, Biofuel, Outdoor, Biomass, Photobioreactors, Wastewater

1. Introduction

Recently, and due to energy shortage, extensive research has been done to find potential biomass feedstocks from different sources for bioenergy production. Various biomasses have been identified as alternate source of energy fuels; including various kinds of bio-wastes, e.g. food wastes, municipal wastes, agricultural wastes; edible and non-edible energy crops; and various aquatic oleaginous plants. However, much thrust has been put on using microalgae as a source of biofuel and biogas for energy applications [1, 2]. Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that are able to adapt to live in an extremely broad spectrum of environments due to their unicellular or simple multicellular structure. They grow photoautotrophically using carbon dioxide (CO_2) producing approximately half of the atmospheric oxygen [3]. In terms of abundance, Sheehan et al. [4] and Chen et al. [5] concluded that the four most important divisions of microalgae are Cyanophyta (blue-green algae), Chlorophyta (green algae), Bacillariophyta (diatoms) and Chrysophyta (golden algae). Among different algal species, those that provide possible commercial applications have been found to be used in aquaculture, human food, value added products for pharmaceutical purposes and as energy feedstocks.

Although the potential of microalgae to contribute to the world energy demand is high, there is a large gap between the current available technologies and the one needed to supply the potential world demand. Biomass production technology must be scaled-up to significantly contribute to the market with lower production costs. Algae can be cultivated in the traditional cultivation systems known as "open ponds", or in closed systems known as "photobioreactors (PBRs)". Open ponds are the oldest and simplest systems for microalgae cultivation and are usually established as shallow ponds designed for outdoor microalgae cultivation [6]. They are less expensive to set-up since they require constant less maintenance [1]. The two main used prototypes of open ponds are circular ponds and raceway ponds. The former ones are mainly utilized for cultivation of *Chlorella* in Asian countries. However, raceway ponds are widely used for the large-scale commercial production of *Spirulina* and *Dunaliella* [6]. The main disadvantages of open ponds are water loss by evaporation, due to their opening feature, and susceptibility of contamination by unwanted species. Although open ponds are typically inoculated with the desired algal species, undesired organisms will certainly be introduced overtime and can severely reduce the biomass yield of the inoculated species. Once a significant competitor has taken residence in the pond, it is enormously difficult to eliminate. Out of 3000 microalgal species collected through aquatic species program, none of them was able to constantly dominate in an open pond with desirable biofuel properties [4]. Recently, closed PBRs have been successfully used for high microalgal biomass production. Unlike open ponds, PBRs allow unialgal dominant culture growth for prolonged durations. In addition, they have minimum contamination with other organisms while having the advantage of efficient using the solar light and a higher amount of CO_2 [7, 8]. However, the comparison between PBRs and open ponds may not be easy, as the evaluation depends on several factors; such as algal species, cultivation conditions, and the method used to calculate the productivity. There is still a gap between designing a cheap PBR on one hand, and building a suitable one that meets all demands of the algal cells, for enhancing the economic viability of the process, on the other hand. However, the most important advantage of closed PBRs, which might make them the system of choice for biomass production, is that they support over ten-fold higher volumetric productivity than open ponds; consequently they have a smaller "footprint" on a biomass productivity basis. It means that a higher bioreactor costs can be compensated by higher biomass productivity, which is a very important goal to collect as much solar energy as possible from a given land area. The most common closed PBRs geometries are flat-plate reactors, tubular reactors and bubble column reactors [9-12]. The tubular system is the most efficient PBR geometry, which facilitates the diffusion of CO_2, maximizes the use of solar light, by avoiding large areas of shade, as well as controlling the given temperature [12, 13].

In addition, mass culture of microalgae requires a large amount of water and nutrients, leading to increased production costs [14]. If organic wastewater can be used for cultivation of algae, it serves as a good alternative to reduce costs as well as helps in wastewater treatment [15-17]. Although wastewater supplements algae with nutrients and lesser amounts of utilizable organic carbon, there are several environmental factors that limit algal growth. Light and temperature are ones of the key factors that affect microalgal photosynthesis and growth. In a previous screening study, *Scenedesmus obliquus* was nominated as a promising microalga for outdoor large-scale biodiesel production because of its high biomass production which resulted in high lipid and fatty acid productivities [18]. In the present study, raw municipal wastewater was used to cultivate the green microalga *S. obliquus* in outdoor bubble column PBR continuously for one year. The influence of temperature, light intensity, and rainfall rate on its biomass productivity, as a key factor for high lipid productivity, was studied. Although cultivation of microalgae on wastewater has been studied at the laboratory scale [19-22], the present study evaluates the actual outdoor benefits that could be obtained by using these supposedly low-cost options.

2. Materials and Methods

2.1. Photobioreactors Design

In order to select the suitable material for the designed PBR, two kinds of plastic sleeves (P1 and P2) made of polyethylene with different transparency (80 and 90%, respectively) were tested. From each kind, two diameters (6 and 10 cm) with material thickness of 250 µm were used. The bubble column PBR was constructed at Harbin Institute of Technology Shenzhen Graduate School (HITSZ), South China (22°35'30"N, 113°57'45"E) as previously described by Abomohra et al. [27]. Up to 70 cylinders were hanged on a metal stand in 5 lines, covering $6m^2$ with a total volume of 850±50 L of microalgal suspensions. Each cylinder was filled with about 11.5 L of municipal wastewater and 500 mL of 6 days-old inoculums. The geometry of the bioreactor is 2 m height, 2 m width, and 3 m length. All cylinders were connected together from the bottom with a rubber hose, and each 3 successive bags were controlled with a valve. Compressed air was periodically injected at the bottom of each cylinder through air stones with a mean air flow of 0.177±0.015 L L^{-1} culture medium min^{-1} for mixing and aeration.

2.2. Microalga and Culture Conditions

The green microalga *S. obliquus* was obtained from Freshwater Algae Culture Collection, Institute of Hydrobiology, China (strain number FACHB-276). For pre-culture production, the strain was grown in 12 L of BG11 medium [23]. After 8 days of laboratory cultivation, the inoculum was added to the outdoor system. The PBR was operated throughout the year starting from April 2015 to March 2016 in batch mode with each batch lasting 6 days with initial optical density (OD_{680}) of 0.25±0.07 for each batch. Municipal wastewater was pumped out from the sewage pipeline at Shenzhen University Town, China and standing still for 1 h to simulate primary clarifier the effluent.

2.3. Determination of Biomass

Culture samples were taken every morning around sunrise as triplicates from each line, and the mean value was used. Biomass production was determined from the increase in the DW which was estimated by measuring the OD_{680}. Later, the optical density readings were converted to cellular dry weight using predetermined calibration curves (R^2 = 0.958). The volumetric productivity (g L^{-1} d^{-1}) was calculated from the increase in DW during each batch run time (6 days) according to Abomohra et al. [18]. The areal productivity was calculated from the volumetric productivity as follow;

$$\text{Areal productivity } (gm^{-2}d^{-1}) = \frac{volumetric\ productivity}{total\ area} \times total\ volume \quad (1)$$

2.4. Data Analysis

Results are presented as mean ± standard deviation (SD). The statistical analyses and 3D mesh figures were carried out using SPSS-20 and SigmaPlot-10, respectively. Data obtained were analyzed statistically to determine the degree of significance using least squares means test (LSMEANS) at a probability level (P) ≤ 0.05.

3. Results

A preliminary outdoor experiment was performed to select the suitable material and diameter of the plastic sleeves. Results showed that the plastic bags of 80% transparency with 10 cm diameter (P1-10) showed the highest growth rate (Figure 1 and Table 1). It showed the maximum significant volumetric biomass productivity of 0.176 g DW L^{-1} d^{-1}, which was 23% higher than that of P2-10; and consequently it was used for further cultivation.

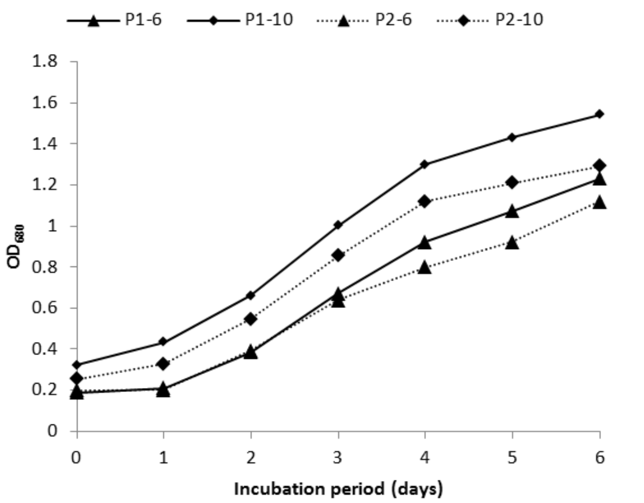

Figure 1. Growth pattern of Scenedesmus obliquus in municipal wastewater using plastic sleeves with different transparencies/diameters.

Table 1. Growth parameters of Scenedesmus obliquus after 6 days of growth in municipal wastewater using plastic sleeves with different transparencies/diameters.

Bags kind	Growth rate (d^{-1})	Dry weight (g L^{-1})	Biomass Productivity (g DW L^{-1} d^{-1})
P1-6	0.192±0.014[a]	0.810±0.032[a]	0.135±0.004[a]
P1-10	0.224±0.060[b]	1.056±0.056[b]	0.176±0.012[b]
P2-6	0.165±0.011[c]	0.724±0.065[a]	0.121±0.006[c]
P2-10	0.194±0.017[a]	0.857±0.098[a]	0.143±0.005[a]

Each value is the mean of three replicates ± SD.
Means with the same letter in the same column are insignificant at $P \leq 0.05$.

In order to find out the effect of environmental factors/conditions on biomass productivity of microalgae, *S. obliquus* cultivated in plastic sleeves bubble column PBR was studied throughout 12 months during 2015/2016 at HITSZ covering all seasons. The annual air temperature range was 9.8-30.5°C, with an average value of 23.9±5.8°C. Daily rainfall showed maximum value of 149.5 mm d^{-1} in September 2015; while rainfall at the days of cultivation showed a maximum value of 57.9 mm d^{-1}, with an annual average of 3.7±10.6 mm d^{-1} (Figure 2A). However, the recorded minimum light intensity was 2 MJ m^{-2} and the maximum value was 29 MJ m^{-2} with an annual average of 13.2±5.1 MJ m^{-2} (Figure 2B).

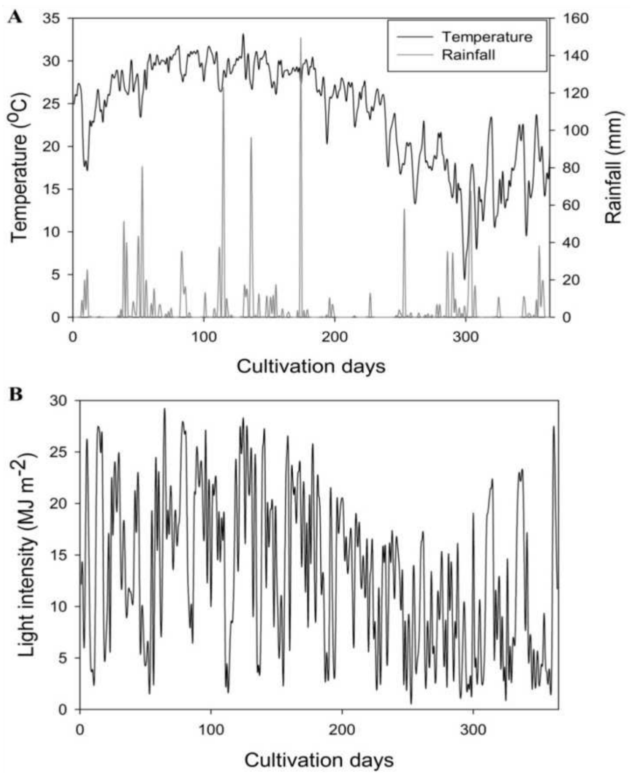

Figure 2. Recorded daily average air temperature, rainfall (A), and light intensity (B) during the cultivation period (April 2015 to March 2016) at HITSZ, China.

During the experiments, the annual areal productivity ranged from 8.5-22.9 g DW m^{-2} d^{-1} with a mean value of

17.9g DW m^{-2} d^{-1}. Results of correlation matrix between biomass productivity and the studied influencing factors are given in Table 2. Averages of daily light intensity and ambient temperature showed a significant positive correlation (0.824 and 0.697, respectively) with biomass productivity. In contrast, a significant negative correlation was found between biomass productivity and average rainfall at the cultivation days (-0.520, Table 2). In order to further elucidate the dependency of biomass productivity on the studied factors, the results from all batches were plotted in three-dimensional graphs (Figure 3). Results confirmed that the maximum biomass productivity was obtained at the maximum light intensity, maximum temperature and minimum rainfall.

Table 2. *Spearman correlation between the variables affecting outdoor biomass production of Scenedesmus obliquus.*

Parameters	Productivity	Growth rate	Temperature	Light	Rainfall
Productivity	1				
Growth rate	0.980**	1			
Temperature	0.697**	0.705**	1		
Light	0.824**	0.796**	0.717**	1	
Rainfall	-0.520**	-0.540**	-0.208ns	-0.312*	1

** Significant correlation at $P \leq 0.01$, * Significant correlation at $P \leq 0.05$, ns Non significant correlation.

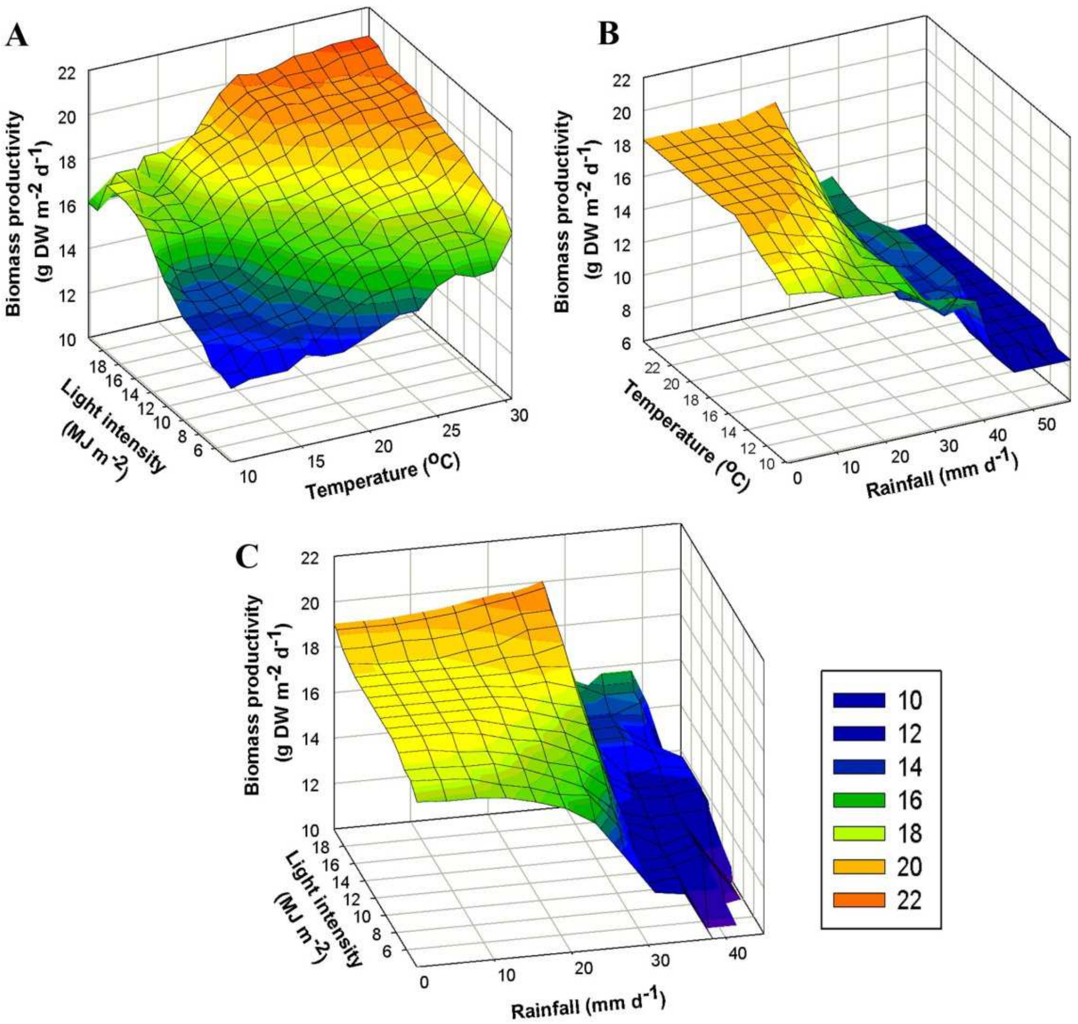

Figure 3. *Three-dimensional graphs showing the effect of temperature/light (A), temperature/rainfall (B) and light/rainfall (C) on areal biomass productivity (Data were analyzed using SigmaPlot-10).*

Monthly biomass productivity showed insignificant changes from April to November, with significant reduction from September to April. The reduction in biomass productivity was attributed to the reduction in light intensity and temperature (Table 3). High ambient temperature and light intensity in spring/summer exerted a decisive effect on the biomass productivity of *S. obliquus*. Upon decrease in temperature during winter, biomass productivity progressively declined to 14.3 g DW m^{-2} d^{-1}, which inferred that the temperature (15.8±1.9°C) and light intensity (9.1±2.4MJ m^{-2}) have almost ceased the growth of the culture (Table 4).

Table 3. *Monthly weather conditions, growth rate and biomass productivity of Scenedesmus obliquus grown outdoor at HITSZ, China from April 2015 to March, 2016.*

Month	Temperature (°C)	Light intensity (MJ m^{-2})	Rainfall* (mm)	Growth rate (d^{-1})	Biomass productivity (g m^{-2} d^{-1})
April	23.8±2.9^{a}	15.2±2.6^{ab}	0.3±0.6^{a}	0.185±0.020^{a}	19.9±1.7^{a}
May	27.4±0.9^{bc}	12.2±4.1^{bc}	11.0±21.6^{a}	0.162±0.048^{ab}	17.9±5.1^{ab}
June	30.0±0.1^{c}	19.4±1.4^{a}	3.6±6.0^{a}	0.185±0.037^{a}	19.9±3.4^{a}
July	29.6±0.9^{bc}	15.8±4.4^{ab}	0.2±0.5^{a}	0.198±0.015^{a}	20.9±1.7^{a}
August	29.5±1.0^{bc}	16.0±4.8^{ab}	2.9±5.7^{a}	0.183±0.045^{a}	19.4±4.7^{a}
September	28.8±0.4^{bc}	17.9±2.7^{ad}	4.4±8.8^{a}	0.185±0.034^{a}	19.8±3.8^{a}
October	26.5±1.2^{abc}	13.2±3.7^{bde}	0.1±0.2^{a}	0.187±0.012^{a}	19.8±1.5^{a}
November	24.3±2.3^{a}	11.5±2.6^{bf}	0.0±0.0^{a}	0.180±0.012^{a}	19.2±1.2^{a}
December	18.5±1.4^{d}	7.7±1.7^{cfg}	14.5±29.0^{a}	0.134±0.036^{bc}	14.0±4.0^{bc}
January	14.4±3.6^{e}	6.8±2.1^{cf}	5.5±7.9^{a}	0.130±0.025^{bc}	13.6±2.7^{bc}
February	15.2±2.3^{ef}	11.6±6.0^{bg}	0.2±0.2^{a}	0.145±0.018^{ac}	15.5±2.0^{ac}
March	18.0±2.6^{df}	8.9±5.3^{cef}	6.0±7.2^{a}	0.132±0.042^{bc}	13.9±4.6^{bc}
Average	23.8±5.8	13.0±4.0	4.0±4.7	0.166±0.026	17.8±2.8
F-value	36.680	4.602	0.669	2.806	2.779
P-value	0.0000	0.0002	0.7577	0.0096	0.0101

*It was calculated for the rainfall records at the day of cultivation.
Values with the same letter in the same column showed insignificant difference (at $P \leq 0.05$)

Table 4. *Seasonal average weather conditions and biomass productivity of Scenedesmus obliquus grown outdoor at HIT-Shenzhen, China.*

Season	Temperature (°C)	Light intensity (MJ m^{-2})	Rainfall* (mm)	Biomass productivity (g DW m^{-2} d^{-1})
Spring	27.0±3.1^{ab}	15.6±3.6^{ab}	4.9±5.5^{a}	19.2±1.2^{a}
Summer	29.3±0.4^{a}	16.5±1.2^{a}	2.5±2.1^{a}	20.0±0.8^{a}
Autumn	23.1±4.1^{b}	10.8±2.8^{bc}	4.9±8.3^{a}	17.7±3.2^{ab}
Winter	15.8±1.9^{c}	9.1±2.4^{c}	3.9±3.2^{a}	14.3±1.0^{b}
F-value	14.102	5.618	0.137	5.801
P-value	0.0015	0.0227	0.9349	0.0209

*It was calculated for the rainfall records at the day of cultivation.
Values with the same letter in the same column showed insignificant difference (at $P \leq 0.05$)

4. Discussion

Interestingly, utilization of microalgal biomass can provide different functions including; 1) removal of CO_2 from industrial flue gases through CO_2 bio-fixation [24], reducing the green house gases emissions of a company or process while producing biodiesel [25]. 2) Wastewater treatment by removal of ammonia, nitrate and phosphate making algae to grow for dual propose to produce biomass and to treat wastewater [2, 16, 24]. 3) Due to its high N:P ratio, the residual algal biomass after oil extraction can be processed into biogas, used as biofertilizers or for animal feeding [26, 27]; 4) Depending on the microalgal species, other valuable compounds used in different industrial sectors; such as polyunsaturated fatty acids, natural dyes, pigments, antioxidants, high-value bioactive compounds may also be extracted [28]. As a general statement, choice of the suitable cultivation system is situation-dependent, dictated by both the final intended purpose of biomass and the used algal strain. However, attention has shifted mostly on closed PBRs, because of the need of accurate control which impaired the use of open ponds [1, 6]. Despite several research efforts to design and operate different PBRs; developing of a cost effective reactor with optimum cultivation procedures is a major challenge for the industrial microalgal biomass production. The present study aimed to construct a cost-effective system for cultivation of the biodiesel promising microalga *S. obliquus* using cheap materials and municipal wastewater as nutrients source. Cultivation in plastic sleeves has advantages since the damaged bags can be cheaply replaced and the technology employing plastic sleeves is simple and scalable enough to support the commercial production of a new algae-based biofuels. Statistical analysis revealed that rainfall was negatively correlated to growth rate and biomass productivity. This leads to the conclusion that high rainfall negatively influences the concentration of nutrients in the supplied municipal wastewater.

Although high light intensities load the photosynthetic photosystems with energy, they can lead to low yield efficiency, photoinhibition or even photo-bleaching [29, 30]. Consequently, microalgae have photoprotective mechanisms which rapidly squander the excess energy as fluorescence and heat. This energy wastage can be decreased or even avoided by exposing the algal cells into low light/high light cycles. As a result, the algal cells behave similarly as if they are exposed to constant moderate light [31]. The flashing light effect can be fully controlled by mixing through the optimized cell transfer from bright zones to dark zones and vice versa in a regular mode [32, 33]. This aspect might explain the higher growth rate of *S. obliquus* in 10 cm diameter tubes than the thinner ones.

The effect of environmental factors/conditions on microalgal biomass productivity grown outdoors in synthetic growth media was reviewed and elucidated by other researchers [11, 27, 34]. The preliminary experiment in the present study showed volumetric biomass productivity of 0.176 g L^{-1} d^{-1} after 6 days of cultivation, with specific growth rate of 0.224 d^{-1} which were 2.2 and 3.7 times higher than those obtained by Bhowmick et al. [35]. Of the different parameters presently under study; light, wastewater composition and temperature were identified as the parameters which mostly affect the productivity in outdoor cultivation. Therefore, to find out the effect of these environmental factors on biomass productivity, experiments were carried out using *S. obliquus*. Values of solar radiation differ with the weather condition and latitude [34, 36]. The changes in temperature showed high influence on the biomass productivity performance of outdoor cultures. The reported optimum temperature range for maximum algal growth rate normally lies between 20 and 30°C [13]. Therefore, the significant low biomass productivity recorded during winter might be attributed to the influence of low temperature. The present study showed annual biomass productivity range of 8.5-22.9 g m^{-2} d^{-1} which was higher than that obtained by Ortega and Roux [37] who reported annual biomass productivity range for *Chlorella pyrenoidosa* of 2.5–4.0 g m^{-2} d^{-1}. Moreover, Hindersin et al. [34] recorded annual biomass productivity ranges for *S. obliquus* grown in flat panel solar tracked PBR using synthetic growth media from -5 up to 30 g DW m^{-2} d^{-1} with a mean productivity of 9±7 g DW m^{-2} d^{-1}. However, the annual mean productivity of plastic sleeves PBR in our study (17.8 g DW m^{-2} d^{-1}) was 98% higher than that reported by Hindersin et al. [34].

5. Conclusion

The growth of *S. obliquus* was year-round studied in bubble column PBR using municipal wastewater with a view to achieve high biomass productivity as a feedstock for biofuel production. Results confirmed that microalgae can be grown efficiently in Shenzhen climatic conditions from spring to autumn with seasonal insignificant changes in biomass productivity (max. of 20.0±0.8 g DW m^{-2} d^{-1}). However, a significant decrease in biomass productivity was recorded during winter. This investigation helped to identify the correlation between some environmental factors and microalgal outdoor biomass productivity. The later showed positive correlation with light intensity and temperature, and negative correlation with rainfall. Further studies are in progress to examine the influence of the studied environmental factors on fatty acid profile of *S. obliquus* and, consequently, on the quality of the produced biofuel.

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