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IMPACT OF CO_2 CONCENTRATION AND AMBIENT CONDITIONS ON MICROALGAL GROWTH AND NUTRIENT REMOVAL FROM WASTEWATER BY A PHOTOBIOREACTOR

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Abstract

The increase in atmospheric CO_2 concentration and the release of nutrients from wastewater treatment plants (WWTPs) are environmental issues linked to several impacts on ecosystems. Numerous technologies have been employed to resolves these issues, nonetheless, the cost and sustainability are still a concern. Recently, the use of microalgae appears as a cost-effective and sustainable solution because they can effectively uptake CO_2 and nutrients resulting in biomass production that can be processed into valuable products. In this study single (*Spirulina platensis* (*SP.PL*) and *mixed indigenous microalgae* (*MIMA*) strains were employed, over a 20-month period, for simultaneous removal of CO_2 from flue gases and nutrient from wastewater under ambient conditions of solar irradiation and temperature. The study was performed at a pilot scale photo-bioreactor and the effect of feed CO_2 gas concentration in the range (2.5-20%) on microalgae growth and biomass production, carbon dioxide bio-fixation rate, and the removal of nutrients and organic matters from wastewater was assessed. The *MIMA* culture performed significantly better than the monoculture, especially with respect to growth and CO_2 bio-fixation, during the mild season; against this, the performance was comparable during the hot season. Optimum performance was observed at 10% CO_2 feed gas concentration, though *MIMA* was more temperature and CO_2 concentration sensitive. *MIMA* also provided greater removal of COD and nutrients (~83% and >99%) than *SP.PL* under all conditions studied. The high biomass productivities and carbon bio-fixation rates (0.796 -0.950 $g_{dw}.L^{-1}.d^{-1}$ and 0.542-1.075 $g_C.L^{-1}.d^{-1}$ contribute to the economic sustainability of microalgae as CO_2 removal process. Consideration of operational energy revealed that there is a significant energy benefit from cooling to sustain the highest productivities on the basis of operating energy alone, particularly if the indigenous culture is used.

***Keywords*:** Nutrient removal, growth rate, biomass production, carbon capture, energy balance**.**

1 Introduction

There has been increasing focus on the use of microalgal culture technology (MCT) for both bio-fixation of CO_2 from flue gases (Adamczyk et al., 2016; Al Ketife et al., 2017; Almomani et al., 2017; Razzak et al., 2013; Toledo-Cervantes et al., 2018; Zhou et al., 2017a;

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Zhou et al., 2017b) and removal of nutrients from wastewater (AlMomani and Örmeci, 2016; Arbib et al., 2017; Gao et al., 2018; Sutherland et al., 2014; Sutherland et al., 2015; Zhou et al., 2017a; Znad et al., 2018a), with the technical and cost implications of the combined process also recently considered (Judd et al., 2017; Kasprzyk and Gajewska, 2019). The use of biology for carbon capture and direct generation of useful products, predominantly biofuel (Bai and Acharya, 2017; de Godos et al., 2014; Fernández et al., 2012; Kassim and Meng, 2017; Singh et al., 2016; Zhang et al., 2011), obviates the energy-intensive solvent regeneration step of the conventional absorption process for carbon capture (Hammond and Spargo, 2014; Wang et al., 2017; Wilberforce et al., 2019). Moreover, the removal of nutrients from wastewater is considered an essential requirement for the approval of treatment facilities (Almomani et al., 2014; Nourmohammadi et al., 2013). Nutrients in wastewater leads to eutrophication (Blaas and Kroeze, 2014; Schneider et al., 2013), increases the growth of unwanted plants and poses a more toxic environment to fish and aquatic organisms (Allagui et al., 2014; Anis et al., 2015; Kang et al., 2019; Liang, 2009; Ziegler et al., 2016). It has been proven that untreated nutrients in wastewater run-offs hinder the efficiency of disinfection processes and increase the chlorine demand (Farrell et al., 2018; Martin et al., 1985). As a result, it has become necessary to find a successful treatment process that can remove nutrients before the discharge of treated wastewater. MCT offers a single-step alternative to classical biological nutrient removal (BNR) technologies for wastewater treatment (Judd et al., 2015), which are generally simple and effective for removal of nitrogen (N) but more complex for phosphorus (P) removal.

MCT thus provides a potentially low-energy means of achieving both carbon capture and nutrient removal in a single process (Cabello et al., 2017; Wang et al., 2018). However, the economic viability of the process is highly sensitive to the rate of CO_2 fixation, in CO_2 mass per day captured per unit of volume of biomass, and the corresponding algal growth rate. Published works on the use of MTC have thus far predominantly been at the bench scale, for short time and using artificial light during cultivation periods (Abou-Shanab et al., 2013; Abreu et al., 2012; Al Ketife et al., 2017; AlMomani and Örmeci, 2016; Marbelia et al., 2014; Znad et al., 2018a). Published works that deal with algae growth under natural solar irradiation are limited and in most cases deal with carbon capture (Lam and Lee, 2014; Li et al., 2013) or nutrient removals (Sutherland et al., 2014; Zhimiao et al., 2016) individually, the latter mainly relating to biofuel production (Do et al., 2018). Moreover, based on our literature review, no work was published on the cultivation and use of microalgae in single process for carbon capture and nutrient removal under the prevailing favorable ambient conditions of the Arabian Gulf, where natural light levels are high and wastewater temperatures predominantly in the 20-30°C range known to favor algal growth (Bouterfas et al., 2002). Moreover, few such studies encompassed a comparison of different microalgae strains to improve the performance of the MTC process.

Accordingly, the current study addresses the above-identified knowledge gaps, in terms of evaluating the potential use of microalgae as MTC technology for simultaneous removal of CO_2 and nutrient under different seasonal conditions and for an extended time. The work was conducted at pilot scale for a period of 20 months, during this time CO_2 bio-fixation capacity and growth of two algal strains (single strain Spirulina platensis (*SP.PL*) and mixed indigenous microalgae (*MIMA*)) and concomitant removals (nutrients and organic matter)from wastewater were studied. The seasonally affected algal growth, CO_2 bio-fixation, and nutrient removal rates were then used to assess the overall energy benefit or penalty of maintaining the optimum temperature during the mild season. The appraisal was limited to determining the difference in

energy capacity between the hot and mid seasons, all other energy contributions (pumping, mixing, etc) being considered unchanged between seasons.

2 Materials and methods

2.1 Algal culture

Both a single-strain microalgae species (Spirulina platensis, *SP.PL*, UTEX Culture Collection of Algae, University of Texas) and a mixed indigenous microalgae (*MIMA*) culture were used in the study. The *MIMA* was collected from a secondary basin of Doha South wastewater treatment plant (WwTP), and was washed thoroughly with distilled water to remove residual bacteria prior to cultivation without further characterization. Stock solutions of the microalgae were grown at room temperature under continuous fluorescent light providing an irradiance of 180 $\mu E.m^{-2}.s^{-1}$, and mixed by aeration with filtered air. The growth media was informed by Zarrouk (1966) and comprised (in g/L): 16.8 $NaHCO_3$, 2.5 $NaNO_3$, 0.5 K_2HPO_4, 1 K_2SO_4, 1 NaCl, 0.2 $MgSO_4.7H_2O$, 0.04 $CaCl_2.2H_2O$, 0.01 $FeSO_4.7H_2O$ and 0.08 EDTA, yielding a pH of 9.5±0.4. The required pH values for *SP.PL* and *MIMA* are 9.5±0.4 and 7.5±0, respectively. The pH of the medium was adjusted as required with 1M solutions of NaOH or HCl.

The carbon content of microalgae biomass was determined using a Flash EA1112 CHNS analyzer (Thermo Finnigan CE Instruments, Italy) equipped with a gas chromatography column and a thermal conductivity detector. Algae samples were incinerated under controlled conditions, followed by catalytic oxidation and reduction. The gases generated were separated by gas chromatography and measured with a thermal conduction detector (TCD). Tests were performed following the methodology described previously (Gonçalves et al., 2016).

2.2 Pilot plant photobioreactor design

The 250L-capacity PBR pilot plant (*Greenline*, Valorsabio, Santa Cruz, Portugal) was configured as eight, 100 mm-diameter high-grade polyethyl terephthalate columns operating in series (Fig. 1). The tubes were interconnected by collectors at both ends and individually fitted with CO_2 gas injection ports and sampling points. pH, temperature and dissolved oxygen (DO) sensors were fitted at the outlets of tubes 1, 4 and 8, and connected to a data-logger. The algal biomass was circulated with a variable speed pump, and the return water blended with fresh feed in a separate 10 L mixing tank. The algal biomass was recovered using a simple clarifier coupled with a membrane separation unit. The plant was installed at the Qatar University campus in Doha (25.2854° N, 51.5310° E) in a partially shaded area.

2.3 Pilot plant operation

Trials were conducted over a period of 20 months (May 2016 - Dec 2017) under ambient conditions of light and temperature. The wastewater used (Table 1) was secondary wastewater from Doha North WwTP. Experimental conditions were recorded four times daily and segregated according to quarterly time periods; Period #1; Jan-Mar, Period #2; Apr-Jun, Period # 3; Jul-Sep and Period #4; Oct-Dec . The operational periods are characterized by ambient temperature (T_A, °C), PBR pilot plant temperatures (T_B, °C), number of hours of daylight (τ, h), light intensity (I_{avease}, $\mu E.m^{-2}\ s^{-1}$) and total light received (I'_{ave}, $E.m^{-2}$). Table 2 shows the maximum, minimum and average key operating parameters (T_A, T_B, τ, I and I') during the operation periods of the PBR. The averages light intensities were determined as the mean of daily measurements recorded over the stated periods. At least four I measurements were carried

out in each day using NIST Radiometer (International light. Model IL 400A). Measurements were taken at the surface of polyethyl terephthalate columns and reported an average value at 95% confidence level (α=5%). T_A, T_B, and pH were measured continuously using electrodes and thermometers connected to data-logger and reported as an average value at α=5%. Under all studied conditions, it was observed that the $T_{B,ave}$ is 1-3.7°C lower than $T_{A,avg}$. The ambient temperature.

Commissioning proceeded by circulating the secondary effluent through the reactor for one hour before adding 7.5 L of the algal inoculation media. Cultures were allowed to grow for seven days at (i) initial biomass (cell dry weight) concentration of 4.4x 10^{-3} $g_{DW}.L^{-1}$, (ii) an initial pH of 9.5±0.4 and 7.5±0 for *SP.PL* and *MIMA*, respectively, and (iii) ambient temperatures and light intensity corresponding to cultivation period τ. The algae cultures were pre-adapted at a CO_2 feed gas concentration ($C_{c,g}$) of 2.5% v/v to overcome the environmental stress induced by the higher CO_2 dosages (5–20%) during startup.

At the end of the seventh day, the pilot plant was filled with fresh secondary effluent WW. The wastewater was circulated inside the reactor for one hour and the biomass concentration inside the pilot plant was adjusted to 0.012±0.02 $g_{dw}.L^{-1}$ by adding 7.5 L of concentrated algae before starting the reactor operation. The effluent was then replaced with a fresh sample and CO_2-enriched air injected into the base of the reactor tubes at concentrations of 2.5-20% v/v and a normalized flow rate of 0.4 vvm (volume of gas per working volume per minute) divided equally between all eight cylinders. The CO_2 concentrations were obtained by mixing atmospheric air with CO_2 at proportions informed by mass flow meters (RS Components, Madrid, Spain), and the gas stream 0.22 μm-filtered prior to daily injection. As the main objective of this study is to utilize MCT for bio-fixation of CO_2 from flue gases, analysis of flue gas samples from local industries showed CO_2 concentrations in the range 4 to 17% v/v. Accordingly, it was decided to use in this study CO_2 concentrations in the range of 2.5 to 20% and investigate if microalgae can tolerate concentrations similar to that in flue gases or higher. Samples were collected daily for determination of algal growth rate, organic matter and nutrient percentage removals, biomass productivity and CO_2 bio-fixation rate. pH and CO_2 partial pressure were continuously monitored over the course of the experiments using sensors.

2.4 Analyses

Algal growth was determined by measuring the increase of the growth medium optical density at 690 nm (OD_{690})(Almomani and Örmeci, 2018). Samples were collected daily from the PBR equalization tank for OD_{690} determination using a spectrophotometer (VARIAN 100 Bio UV-visible spectrophotometer, USA). The measured OD (OD_{690}) was converted to biomass concentration (X, $g_{dw}.\ L^{-1}$) according to the calibration curve:

$$X = 0.652.OD_{690nm} - 0.0021 \quad (1)$$

The specific growth rates (μ, d^{-1}) and biomass productivities (P_{bio}, $g_{dw}\ L^{-1}\ d^{-1}$) were then determined according to:

$$\mu\ (\mathrm{day}^{-1}) = \frac{\ln(X_2) - \ln(X_1)}{\Delta t} \quad (2)$$

where X_1 and X_2 represent the initial and final biomass concentration (in $g_{dw}\ L^{-1}$) over the time period Δt (in days) of the initial period of the exponential growth phase.

$$P_{bio}\ (g_{dw}.L^{-1}d^{-1}) = \frac{X_f - X_i}{t_f - t_i} \quad (3)$$

where X_f and X_i correspond to biomass concentration (in $g_{dw}.\ L^{-1}$) at times t_f and t_i (in days), these being the end and beginning of cultivation time respectively.

Carbon dioxide fixation rate (R_{CO_2}, in $g_C.L^{-1}.d^{-1}$) was determined from the ratio of microalgae carbon content to average biomass productivities:

$$R_{CO_2} = C_C.P_{bio}.\frac{M_{CO_2}}{M_c} \quad (4)$$

where C_C is the carbon content of the microalgal biomass (in % w/w) determined using the Flash CHNS analyzer, P_{bio} the average biomass productivity, and M_{CO2} and M_C the respective CO_2 and carbon molecular weights ($g.mol^{-1}$).

2.5 Net energy penalty/benefit determination

Significant energy dissipation takes place in the cultivation process from water evaporation and convection from air bubbles. The determination of the benefit or penalty of cooling the system during the hot season can be achieved if a few simplifying assumptions are made, specifically:

a. losses due to minor differences in temperature between water PBR and water vapor in the air near the liquid surface can be neglected, such that radiation can be ignored,
b. the water vapor temperature is the same as that of air in the PBR enclosure,
c. all other energy contributors (mechanical mixing, liquid and biomass transfer, etc) are unaltered.

The evaporative heat loss W_{EV} ($kJ.h^{-1}$) is then given by:

$$W_{EV} = \Delta H_V \times R_{EV} \quad (5)$$

where ΔH_V is the heat of evaporation in $kJ.kg^{-1}$ and R_{EV} is the water evaporation rate in $kg.h^{-1}$, estimated from(Rafferty and Culver, 1998):

$$R_{EV} = 0.00753 \times A \times (P_W - P_A) \quad (6)$$

P_w, P_A in the above equation respectively represent the saturated water vapour and dew point pressure (in mmHg), and A is the surface area of the PBR.

The convective heat loss W_C in $kJ.h^{-1}$ is given by:

$$W_C = h_c \times A \times [T_w - T_a] \quad (7)$$

where T_w and T_A are the water and air temperature respectively and h_c the heat transfer coefficient in $W.m^{-2}$ which can be estimated from (Stoever, 1941):

$$h_c = 5.7 + 3.8v \quad (8)$$

where v is the air speed in $m\ s^{-1}$.

The heat transferred over the whole cultivation period is thus given by $W_{EV} + W_C$. This energy transfer is then balanced against the potential energy of the generated biofuel over the same period, a conversion factor of 7.55 kWh per kg algal biomass (Beal et al., 2012) being used to convert from kg_{DW} biomass to kWh potential energy.

3 Results and discussion

3.1 Algae growth and productivity

3.1.1 Seasonal impacts

Examples of growth of *SP.PL* and *MIMA* during the four cultivation periods are given in Fig. 2 for the reference feed gas concentration $C_{c,g}$ = 2.5% CO_2. Both algae strains followed the typical growth curve of lag, exponential growth, and stationary phases. The lag time for *SP.PL* was longer than *MIMA* and for both algae the lag phase highly dependent on cultivation period. For *SP.PL* lag phase durations of 1.5, 0.75, 1.75 and 1.25 d were observed for Periods #1-4 respectively, compared with shorter lag phases (~0.25-0.5 d) for *MIMA* cultures for all periods. The subsequent exponential growth phase differed less consistently between the two algal

strains, the durations during Periods #1-4 being 5.5, 3.5, 3.3 and 9.5 d for *SP.PL* and 4.0, 4.0, 3.5 and 4.25 d for *MIMA* respectively. The short lag-phase for *MIMA* reflected the expected increased tolerance of the locally-acclimatized mixed culture to changes in conditions.

At the same feed gas concentration $C_{c,g}$ of 2.5% CO_2, the μ values, as determined during the first three days of the exponential growth phase, and biomass productivity were found to vary significantly with the season for both algal strains (Fig. 3a). The most rapid growth and productivity (P_{bio}) was recorded at the mild temperatures (20-25°C) and moderate total light intensities (I'_{ave} = 4.64-8.47 $E.m^{-2}$) associated with Periods #1 and #4. The 5°C lower temperature of Period #1 only marginally reduced P_{bio} compared with Period #4, whereas the 5°C increase in bioreactor temperature (from 25 to 30°C), and accompanying 4% increase in received irradiation dose (Table 2), produced a 30-50% drop in growth rate between Periods #4 and #2. Growth was correspondingly up to 20% higher for the *MIMA* strain for Periods #1 and #4, compared with a small difference for Periods #2 and #3 (Fig. 3a). This to some extent corroborates previous reports of major biomass loss from exceeding the optimum temperature by only 2-4°C (Moheimani and Borowitzka, 2007; Singh and Dhar, 2011), and very low growth rates reported at temperatures higher than 35°C (Teoh et al., 2004) which some authors have attributed to the reduced CO_2 solubility in the liquid which affects the available inorganic carbon and the growth (Lam and Lee, 2013). A similar trend with the season was noted at higher $C_{c,g}$ values (Figs. 3b-d).

Reported values of μ in the literature have ranged from 0.22 and 0.41 d^{-1} for light intensity (*I)* values between 68 and 85 $\mu E.m^{-2}.s^{-1}$(Kumari et al., 2014; Singh et al., 2016; Sydney et al., 2010) and as high as 1.4 d^{-1} at intensity of 174 $\mu E.m^{-2}.s^{-1}$(Xue et al., 2011). Many studies of microalgae generally have demonstrated that increasing the light intensity beyond the so-called saturation point, the maximum light intensity the algal biomass is able to harness (Richmond, 1999), may lead to photo-oxidation that damage the light receptors and so impair photosynthesis and algal productivity (Brock and Brock, 1969; Ota et al., 2015; Singh and Singh, 2015). In the present study, although the mean light intensity values at the PBR surface almost doubled from 115 to 220 $\mu E.m^{-2}.s^{-1}$ in Periods #1 and #4 respectively, the growth rates increased only by 18% and 16 % for *SP.PL* and *MIMA*, respectively. Light intensities providing a reasonable specific growth rate (μ) and biomass productivity (P_{bio}) reported for *Chlorella vulgaris* (*C.V*), the most commonly studied strain, varied widely at 40-1240 $\mu E.m^{-2}.s^{-1}$ (Abou-Shanab et al., 2013; Abreu et al., 2012; AlMomani and Örmeci, 2016; Lam and Lee, 2013; Li et al., 2003; Marbelia et al., 2014; Ruiz-Martinez et al., 2012; Znad et al., 2018a). According to the *C.V* literature at an optimum light intensity of 100 $\mu E.m^{-2}.s^{-1}$ and 5% $C_{c,g}$, the associated maximum μ, and P_{bio} values are around 1.17 d^{-1} and 0.74 $g.L^{-1}.d^{-1}$ respectively at a temperature of 24°C for a batch cultivation process (Abreu et al., 2012; Al Ketife et al., 2017; Li et al., 2013). This compares to a reasonable growth rate at a narrower range of 68-400 $\mu E.m^{-2}.s^{-1}$ for *SP.PL* (Ho et al., 2018; Kumari et al., 2014; Liu et al., 2018; Singh et al., 2016; Xue et al., 2011; Yuan et al., 2011; Zhou et al., 2017b).

It has been noted that the simple organic matter in municipal effluent generally provides more rapid growth than a CO_2 carbon source alone, due to mixotrophic growth (Znad et al., 2018a). Nonetheless, the maximum μ values recorded in the current study were somewhat higher than those reported under similar conditions for *C.V*. For example, μ values for *C.V* cultivated in secondary wastewater (SWw) have previously been reported as between 0.186 and 1.86 d^{-1} (AlMomani and Örmeci, 2016; Ruiz-Martinez et al., 2012; Znad et al., 2018a; Znad et al., 2018b).

3.1.2 Feed gas CO_2 concentration impacts

Changes in growth and productivity were evident across the feed gas CO_2 concentration range studied (Figs. 3a-d). Over the 2.5-20% range of $C_{c,g}$ values studied there was a maximum in both P_{bio} and R_{CO2} at 10-15% (Fig. 4) across all seasons. The *SP.PL* trend indicated a clear maximum R_{CO2} for Period #4 at $C_{c,g}$ = 10%, whereas the maximum for Periods #1, #2 and #3 occurred at around 15%. For *MIMA* the maximum R_{CO2} for periods #2 and #4 was at 10%, and for Periods #1 and #3 at 15 %. Values of R_{CO2} increased by 35-42% by increasing $C_{c,g}$ from 2.5 to 10% v/v across all seasons.

The overall trend suggests growth may be carbon limited at the lower gas concentration but inhibited (though still reasonably high) at elevated CO_2 levels, since across all $C_{c,g}$ values the algae carbon content changed little (from 41.5±1.2 to 51.5±2.1%w/w for *SP.PL,* and from 44.2±1.3 to 56.2±3.1%w/w for *MIMA*). As with the growth data (Fig. 3), seasonal impacts are significant with reference both to the optimum values and the trend. As indicated in Figure 3, P_{bio} is increased by 42% by an increase in $C_{c,g}$ from 2.5 to 10% v/v for all seasons. However, the extent of the fall in P_{bio} and R_{CO2} beyond the optimum concentration also appears to be season dependent, with a greater rate of decline during Period #3 (the hot season, represented be the dashed lines in Fig. 4) than Period #4 (the mild season) for the *MIMA* strain in particular. The results are comparable to values reported in the literature (Gonçalves et al., 2016; Tang et al., 2011)

The decreased bio-fixation efficiency at higher $C_{c,g}$ values has been attributed to diminution of the photosynthetic action of the selected microorganisms (Al Ketife et al., 2016; Znad et al., 2018b) as a consequence of the reduced pH and CO_2 mass transfer, the latter pertaining to the relatively slow rate of hydrolysis of CO_2 to H_2CO_3 (Silva and Pirt, 1984; Sung et al., 1999). In the current study the introduction of CO_2 temporarily marginally depressed the pH, to 8.2± 0.1 for *SP.PL* and 6.8± 0.2 for *MIMA*, with recovery to the normal operational range of 8.6-9.6 (8.9± 0.2 on average) and 6.8-7.5 (7.2± 0.1 on average) for the two respective cultures taking up to 7 hrs. Notwithstanding this, and as with the growth data, the carbon bio-fixation values reported in this study are slightly higher than the values reported in the literature for the *C.V.* strain (Ruiz-Martinez et al. 2012, Li et al. 2013).

3.2 Organic carbon and nutrient removal

Examples of organic carbon and nutrient removal trends for *SP.PL* (Fig. 5a) and *MIMA* (Fig. 5b) for Periods #3 and #4, respectively representing the hot and mild seasons, mirror the trends in growth and productivity. Percentage removals of organic carbon (as chemical oxygen demand, COD), total inorganic nitrogen (TIN) and total phosphorus (TP) gradually decreased over the first 3-10 days of cultivation to reach an equilibrium value. Equilibration was most rapid for phosphate (3 days) followed by ammonia (5 days), COD taking around 10 days to reach the maximum removal value. The equilibrium effluent TIN species (ammonia, nitrate and nitrite) concentrations ranged from 0 to 7.3± 0.1 mgN L^{-1}, and those of TP from 0 to 3.0± 0.1 mg L^{-1}. Control experiments were conducted to measure the degree of ammonia removal by volatilization at working pH values. The results reveal that the percentages of ammonia removals due to volatilization at pH of 8.9± 0.2 and 7.57.2± 0.1 were 6% and 2 %, respectively, confirming that the observed ammonia removals were due to the microalgae uptake. Temperature and pH fluctuated around an average value with no noticeable increase or decrease over the course of the tests conducted within a specific period apart from a brief decrease during

the daily injection of CO_2. Dissolved oxygen (DO) values throughout the study ranged from 7.6 ± 0.1 to 8.6 ± 0.1 $mg.L^{-1}$, which was not considered sufficiently high to cause photosynthesis inhibition. There was no statistically significant difference between the three measuring locations of DO confirming that the growth, CO_2 capturing and contaminates removals are homogenous all over the PBR. Moreover, it was observed the average DO values for periods # 2, 3 and 4 are within 95 to 98% of the corresponding oxygen-water saturated values. Period #1 showed DO value of 92% of the saturation limit.

A control experiment conducted in the absence of the algal biomass indicated less than 4% and 3% COD and ammonia removals. It was thus surmised that most of the observed ammonia and organic carbon removal was by algae assimilation.

Trends in equilibrium carbon and nutrient removal with $C_{c,g}$ followed those of the growth, productivity and bio-fixation, peaking at 10% for all algal and wastewater species under all ambient conditions, other than for COD and TP removal by *MIMA* during the mild season (Periods #1 and #4) when removal peaked at 15% feed gas concentration (Fig. 5). For both strains, COD removal decreased during the hot season of Periods #2 and #3, ranging from 47 to 60% for *SP.PL* compared with 71-90% during Periods #1 and #4. The corresponding values for *MIMA* were in the range 59-70% for Periods #2 and #3 and 77-99% for Periods #1 and #4. TIN and TP followed the same trends as COD removal and were removed to roughly the same extent for a specific set of operating conditions. TIN removal by *SP.PL*, for example, decreased from 70-93% during Periods #1 and #4 to 49-59% during Periods #2 and #3.

Whilst some authors have reported negligible removal of COD by algae (Wang et al., 2009), the application of *SP.PL* to wastewater purification generally has been widely studied (Table 3). Across a number of studies, Li et al. 2013, AlMomani and Örmeci 2016, Znad et al. 2018 showed average removals of COD, TN and TP from municipal primary and secondary wastewater (PWw and SWw respectively) have varied between 22 and 95%. The wide variation is associated largely with key factors such as incubation time, temperature and algal species. The outcomes also again reflect the impact of mixotrophic rather than autotrophic growth, i.e. the energetic preference for dissolved organic carbon assimilation compared with CO_2 fixation (Lalucat et al., 1984), as dictated by photo-limitation during periods of darkness (Hatnagar et al., 2010).

3.3 Energy balance

Whilst the productivity of the PBR is reasonable during the hot season under optimal conditions of 10% CO_2 feed gas concentration, the maximum P_{bio} value attainable under the mild season conditions is around 80% higher for the *MIMA* culture. This being the case, the option of implementing cooling for the PBR during the hot season should be considered.

A very simple approach can be taken to estimate the energy benefit or penalty on implementing cooling based on two assumptions:

a. The improved productivity during the mild season is primarily associated with the lower temperatures, and
b. Productivity is considered roughly linearly related to solar irradiation intensity.

Any error associated with the second assumption is likely to be small if the benchmark data used for the comparison are the mean values for Periods #2 and #3 (hot season) and those of Period #4 (mild season). The average value of I_{ave}' for Period #2-3 is 9.4 $E.m^{-2}$, compared with a value of 8.47 for Period #4 – a difference of only 10%. Moreover, data from Period #1 suggest that light intensity is not a significant contributory factor, given that mean productivity is only

~20% less than the maximum value associated with Period #4 despite having an I_{ave} value little more than half that of Period #4. There is thus a maximum possible error of 10% from the second assumption.

The calculation proceeds by making basic assumptions concerning the prospective biofuel content of the culture, and the associated energy, and the energy demanded for chilling the culture as given by W_{EV} and W_C (Section 2.5). Accordingly, based on Equations 4 and 5, the PBR configuration described in Section 2.2, and the seasonal ambient conditions summarised in Table 2, values for W_{EV} and W_C of 0.92 and 0.10 kWh.m^{-3} culture for the cultivation period can be calculated for the cooling energy requirement. This compares with a value of 4.6 and 5.4 kWh.m^{-3} for the latent biomass energy for the *SP.PL* and *MIMA* cultures respectively. Thus, even when an electrical: cooling power conversion efficiency of 50%, the option of cooling the culture during the hot season results in a 2.6-4.4 kWh.m^{-3} net increase in recovered energy overall.

4 Conclusions

An extended, 20-month study of two algal species, a monoculture (*Spirulina platensis*, *SP.PL*) and mixed indigenous culture (*MIMA*), in microalgae culture technology (MCT) configured as a photobioreactor (PBR) has been undertaken. The study was conducted at pilot-scale using a 60 L-capacity column reactor installed outdoors in the Arabian Gulf and subject to uncontrolled ambient conditions of light and temperature. PBR performance was appraised with reference to the dual functions of CO_2 mitigation by bio-fixation (and the associated algal growth) and wastewater treatment, the latter with reference to the 'key wastewater quality components of chemical oxygen demand (COD), the nutrient content as represented by the total nitrogen (TN), and total phosphorus (TP). The reactor was fed with secondary municipal wastewater (SWw) and a gas stream having CO_2 concentrations ($C_{c,g}$) between 0 and 20%. Accordingly, the following outcomes were obtained:

1. The mixed indigenous culture performed significantly better than the monoculture, especially with respect to growth and CO_2 bio-fixation, during the mild season; against this, the performance was comparable during the hot season.
2. Both CO_2 bio-fixation and wastewater purification were significantly impaired during the hot season, the bio-fixation rate decreasing by 30-60% and the wastewater contaminates decreased in a similar amount.
3. Optimum performance was observed at a $C_{c,g}$ value of 10% v/v for all parameters other than COD and TP removal during the mild season for the *MIMA* culture, where the optimum was 15%.
4. The performance of the *MIMA* culture was more sensitive to both $C_{c,g}$ and season, all performance parameters showing a steep decline both from Period #4 (the mild season) to Periods #2-3 (the hot season) and from the optimum $C_{c,g}$ value (10 or 15%) to higher values.
5. Based solely on a consideration of operating energy, there a significant net energy benefit (of up to 4.4 kWh.m^{-3} for the mixed culture) from cooling the biomass during the hot season to sustain the highest productivities, when reasonable assumptions are made concerning evaporative cooling and losses.

There is significant benefit from employing the indigenous mixed culture and optimizing both the feed gas concentration and the culture temperature. Maintaining culture temperature incurs energy demand for cooling during the hot season, which a rudimentary energy balance suggests it is more than compensated for the embedded energy of the algal biomass generated.

However, a full techno-economic analysis (TEA) is needed to comprehensively appraise the implications of these operational parameters.

5 Recommendations and future perspective

Several attempts have been made to develop effective carbon capture and storage technologies (CCSTs) as well as advanced wastewater treatments with performance and economic feasibility barriers affect their practical applications. However, the dual action of microalgae for both bio-fixation of CO_2 from flue gases and removal of nutrients from wastewater offer a substantial economic alternative. In this scenario, CO_2 and wastewater contaminates are reduced from ecosystem producing algae strains that can be converted afterward to biofuels and high-value-added products. Microalgae, naturally grown in water bodies, have the advantage of adding value to the treated wastewater and not competing with freshwater resources

Upon completion of this study, the following is recommended;

1- Large scale pilot plants testing is pivotal to generate the required data using various algae strains;
2- Assess the economic feasibility and Life Cycle Analysis of the system in large scale operations;
3- Given the discrepancy between the bench and pilot scale testing results for microalgae growth rates, biomass productivity, and CO_2 bio-fixation rate; it is highly recommended that extrapolations bench-scale tests are avoided. Proper scale-up studies should be considered at a near life pilot scale systems using data generated at the laboratory level;
4- Apply full-scale operation on systems that incorporate wastewater treatment and reduce GHG emissions from industrial flue gases while producing biofuel;
5- Develop an effective biomass harvesting technologies;
6- Focus and provide more in-depth analysis of energy value and economic viability of the biofuel and products generated from microalgae when using CO_2-wastewater as a feed to the MTC process.

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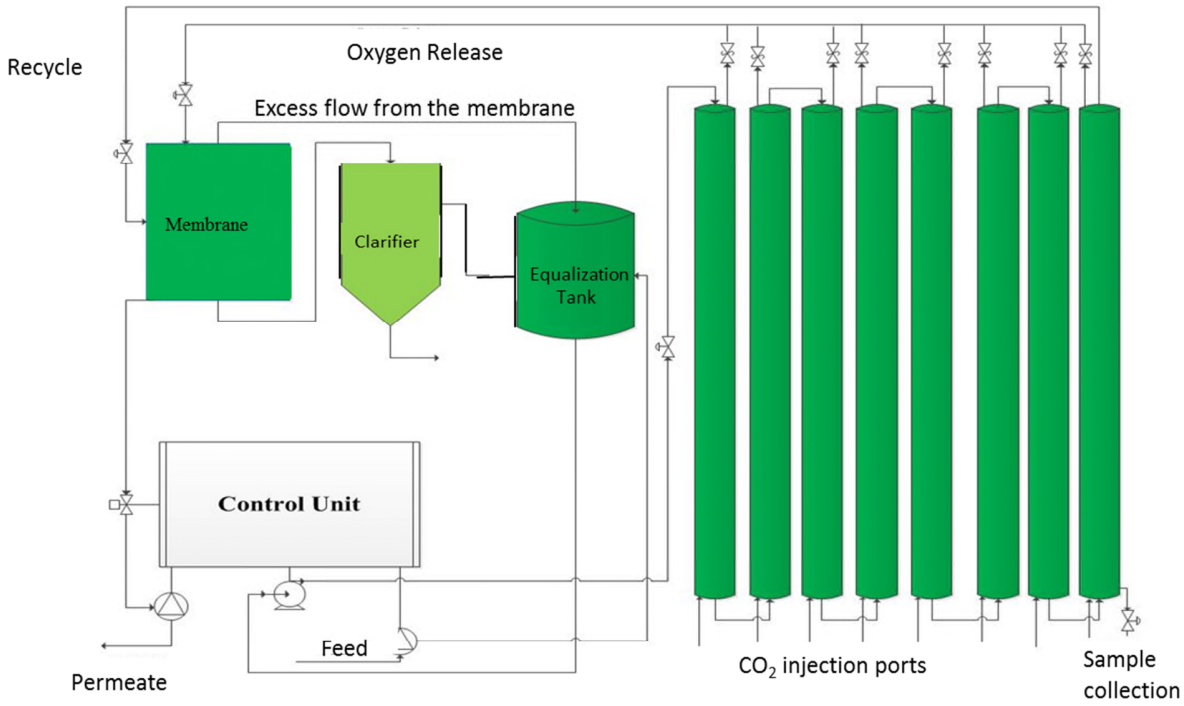


Figure 1: *PBR pilot plant, schematic*

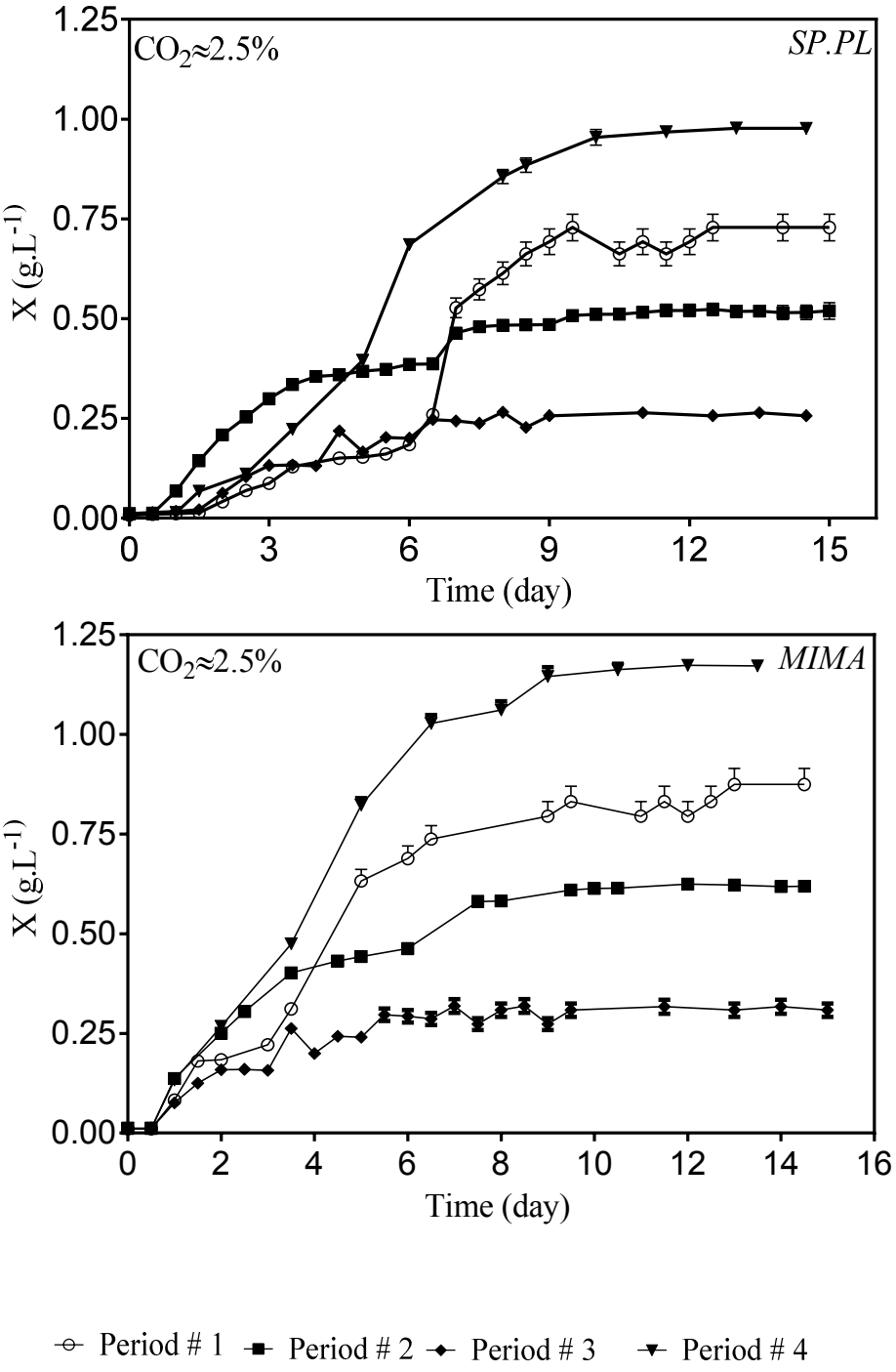
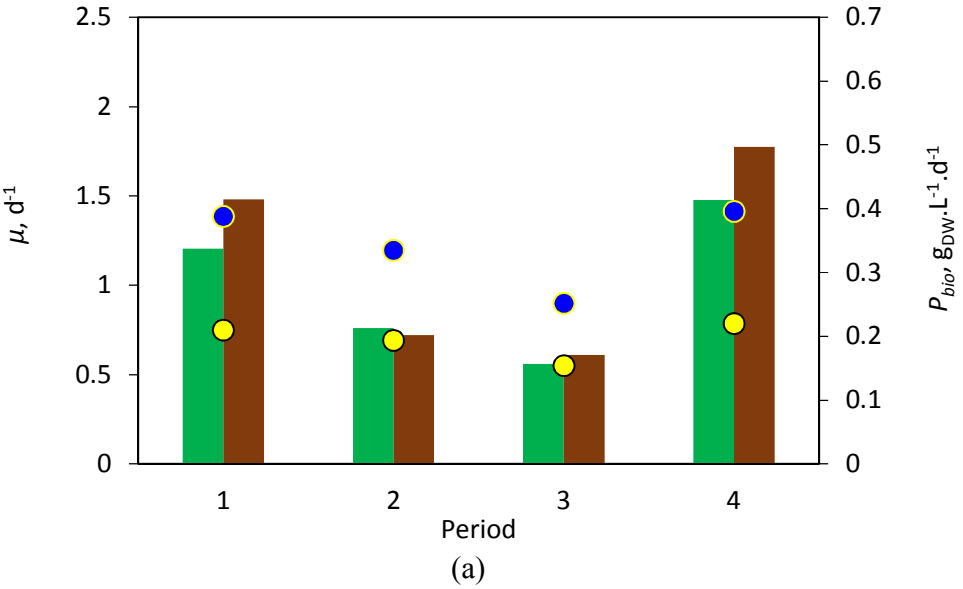
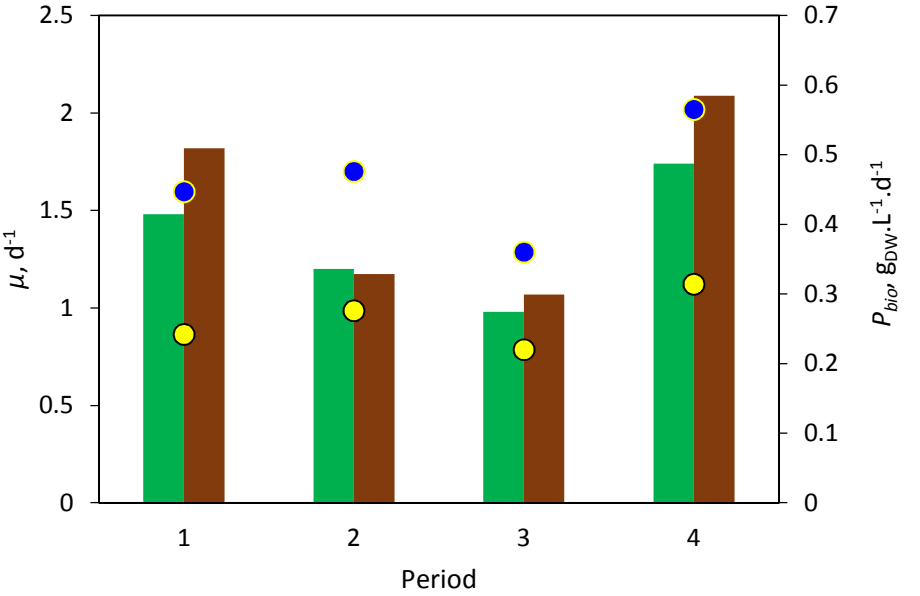


Figure 2: *Biomass concentrations of SP.PL and MIMA biomass as a function of cultivation time at different cultivation periods and a CO_2 dose of 2.5%. Periods: (1) Jan-Mar, (2) Apr-Jun, (3) Jul-Sep and (4) Oct-Dec.*


2.5
2
1.5
1
0.5
0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Period
μ, d-1
Pbio, gDW.L-1.d-1

(a)


2.5
2
1.5
1
0.5
0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Period
μ, d-1
Pbio, gDW.L-1.d-1

(b)

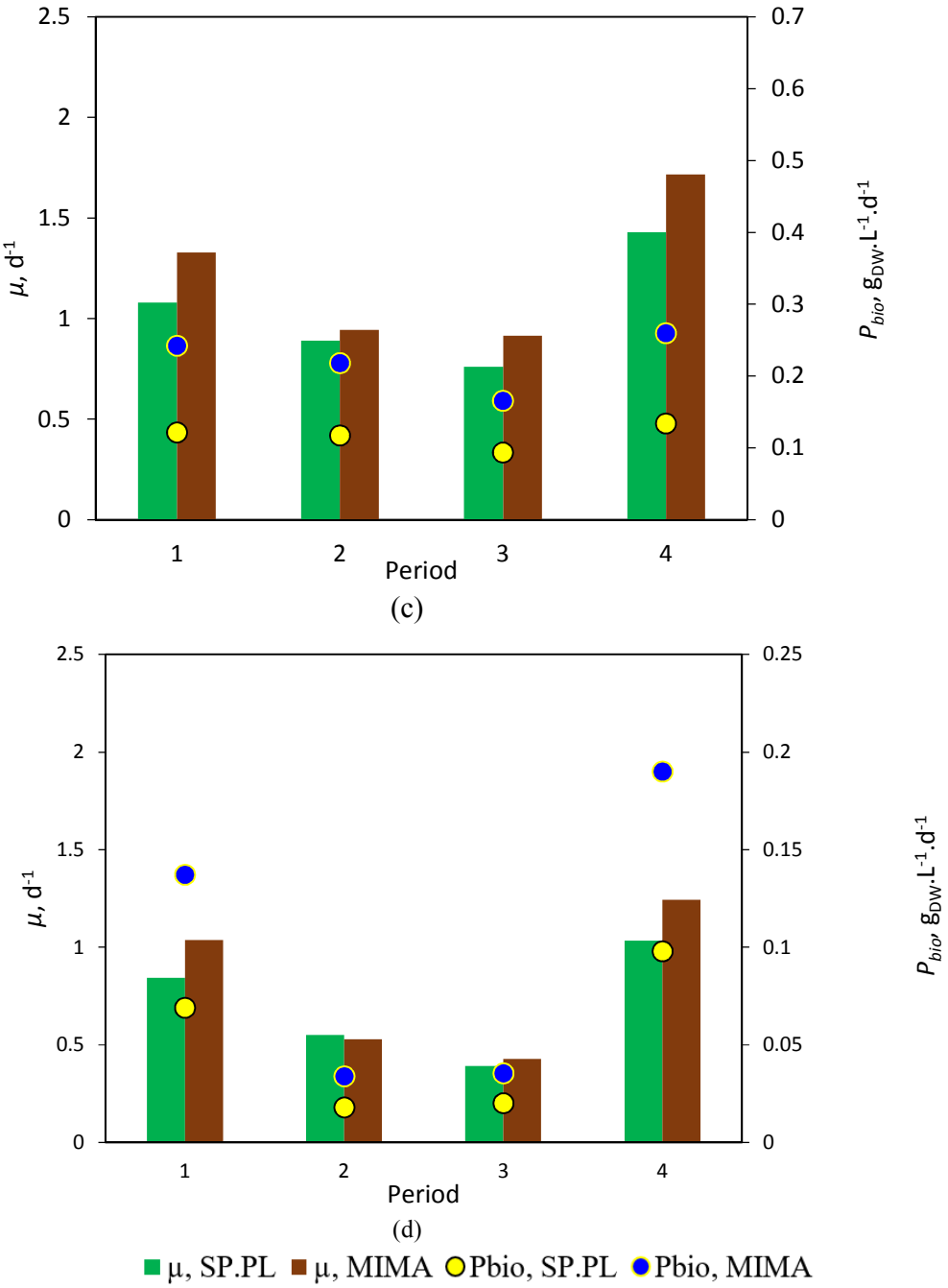
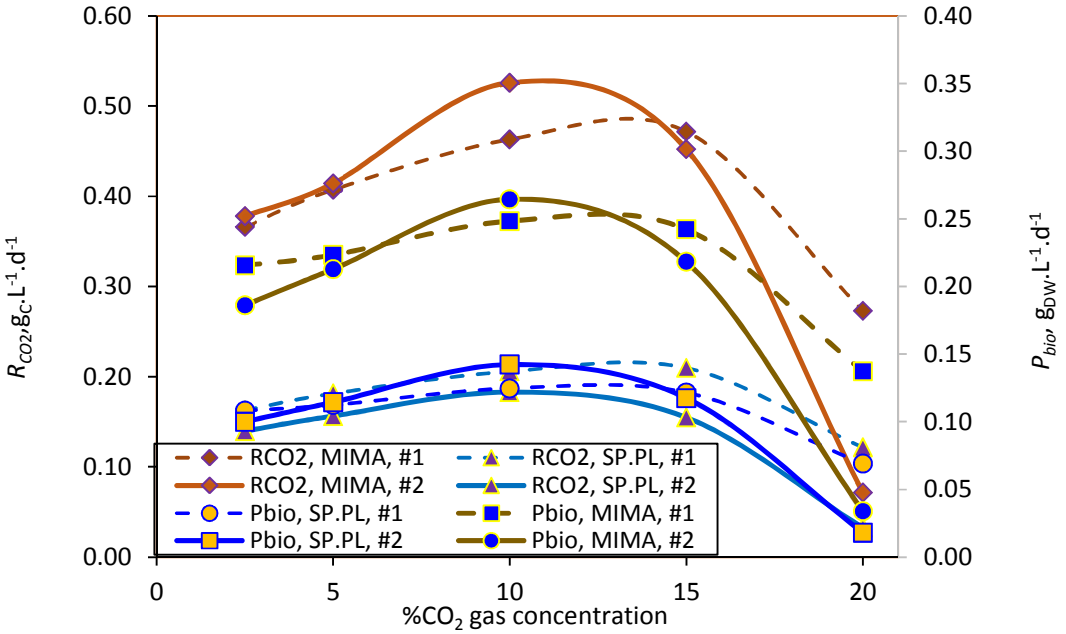


Figure 3: *Mean μ and P_{bio} values for the two species at different periods at $C_{c,g}$ of (a) 2.5%, (b) 10%, (c) 15% and (d) 20% v/v. Periods: (1) Jan-Mar, (2) Apr-Jun, (3) Jul-Sep and (4) Oct-Dec*

(a)



(b)

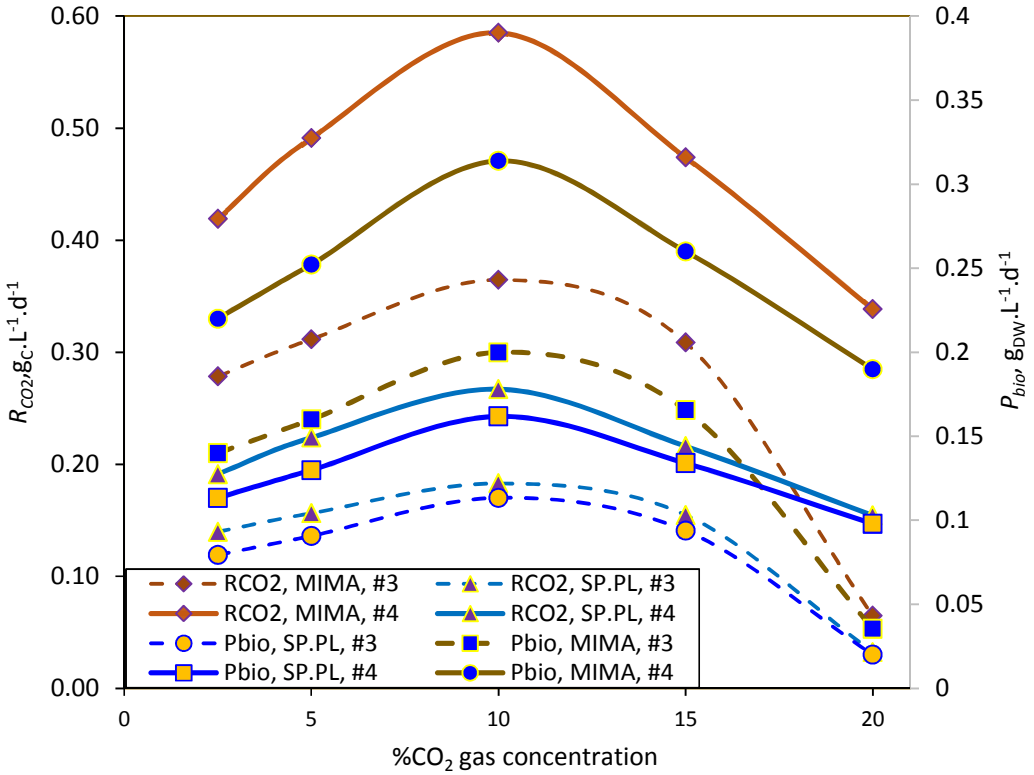


Figure 4: *Carbon fixation rate (R_{CO2}) and productivity (P_{bio}) as a function of feed gas CO_2 concentration for the two algal strains (SP.PL and MIMA) for (a) Periods #1 and #2, and (b) Periods #3 and #4*

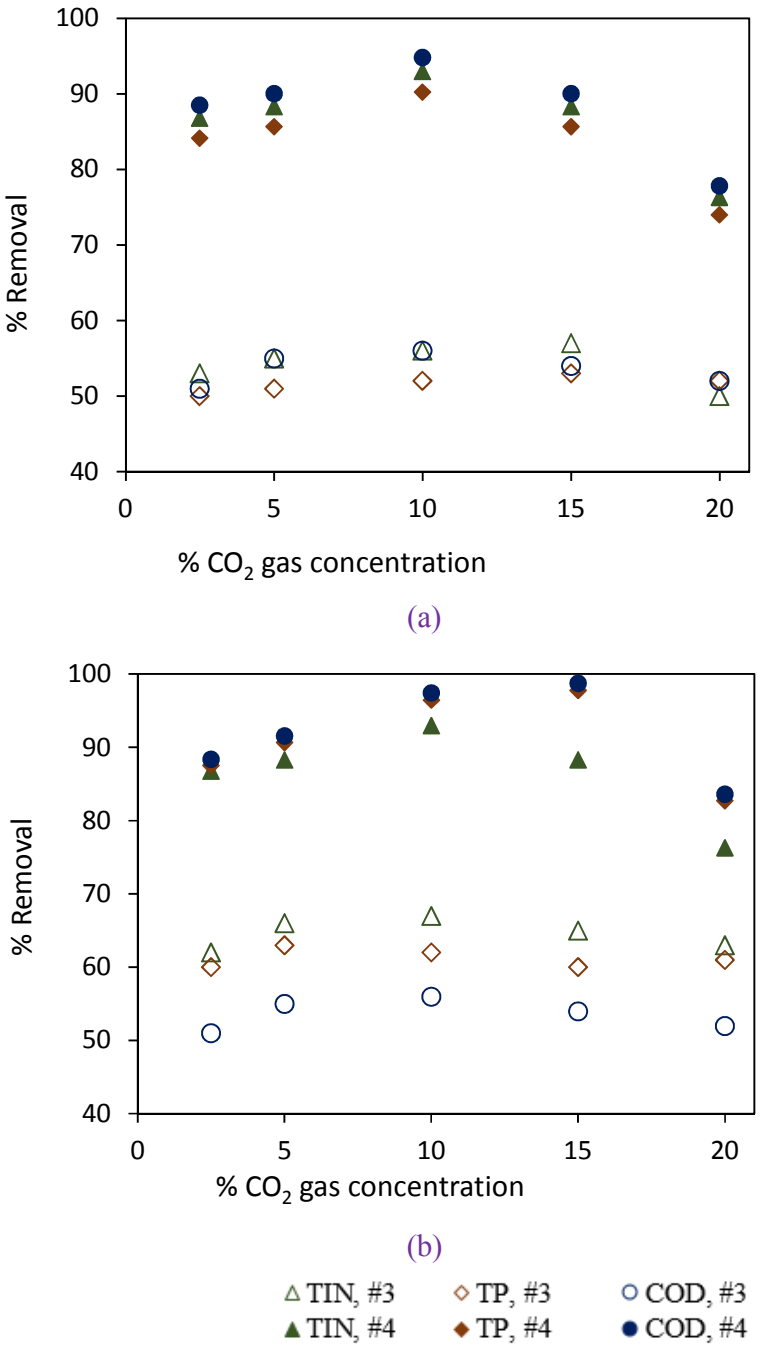


Figure 5: *Nutrient and carbon removal and CO_2 biofixation (R_{CO2}) trends with feed gas CO_2 concentration for (a) SP.PL, and (b) MIMA for Periods #3 and #4.*

Table 1: Characteristics of the secondary effluent used in the experiments

Parameter	***Lower limit***	***Upper limit***	***Average***
COD_s ($mg.L^{-1}$)	50.0 ± 1.5	59.0 ± 0.5	52.0 ± 0.5
NH_4^+ (mg-N/L^{-1})	40.0 ± 0.2	44.0 ± 0.2	42.0 ± 1.5
NO_2^- (mg-N/L^{-1})	0.77±0.06	0.89± 0.06	0.81± 0.06
NO_3^- (mg-N/L^{-1})	9.0±0.1	18.0±0.1	10.5± ±0.1
Total Phosphorus (mg/L^{-1})	8.0±0.2	14.0±0.2	9.3±0.3
N:P ratio	5.95:1	4.50:1	5.73:1
C:N:P ratio	6.25:5.95:1	4.2:4.50:1	5.6:5.73:1
pH	7.2	7.53	7.1

Table 2: Key operating parameter values of the PBR

Period	***Months***	$T_{A,\,low}$, °C (Std.Dev)^{90}	$T_{A,ave}$, °C (Std.Dev)^{90*}	$T_{A,\,high}$, °C (Std.Dev)^{90}	$T_{B,\,Low}$, °C(Std.Dev)^{90}	$T_{B,\,ave}$, °C(Std.Dev)^{90}	$T_{B,\,high}$, °C(Std.Dev)^{90}	T_{low} h(Std.Dev)^{90}	τ_{ave}, h	T_{high}, h(Std.Dev)^{90}	I_{low}, µE m^{-2}s^{-1}	I_{ave}, µE m^{-2}s^{-1}	I_{high} µE m^{-2}s^{-1}	I'_{ave}, E m^{-2}
1	Jan-Mar	18.1 (1.7)	21.6 (2.5)	24.3 (2.6)	16.0 (1.9)	18.3 (1.3)	20.4 (2.2)	10.5 (0.8)	11.2 (0.7)	11.8 (0.8)	105±6	115±10	135±6	4.64
2	Apr-Jun	28.7 (3.2)	30.0 1.6)	36.0 (3.6)	26.5 (2.1)	29.0 (1.1)	35.2 (2.4)	12.9 (0.6)	13.0 (0.4)	13.3 (0.7)	170±7	189±10	240±10	8.85
3	Jul-Sep	32.5 (1.3)	36.2 (1.3)	41.2 (0.9)	27.8 (1.2)	32.5 (1.5)	38.0 (0.8)	12.6 (0.3)	12.9 (0.5)	13.1 (0.5)	185±7	210±15	265±10	9.75
4	Oct-Dec	22.7 (3.5)	25.0 (1.8)	31.3 (4.5)	20.3 (1.1)	23.0 (2.0)	25.5 (0.9)	10.6 (0.2)	10.7 (0.1)	11.0 (0.2)	165±7	220±10	235±10	8.47

$T_{A,ave}$ ambient temperature; $T_{B,ave}$ bioreactor temperature; τ number of hours of daylight; I_{ave} average light intensity; I'_{ave} total light received, Std.Dev: Standard deviation, * number of data used in calculations

Table 3: Spirulina platensis nutrient removal efficiencies (TN, TP and COD percentage removals) reported for various effluents

Mode of operation - C source	***TN_{in} mg L^{-1}***	***TP_{in} mg L^{-1}***	***COD_{in} mg L^{-1}***	***TIC_{in} mg L^{-1}***	***$C_{c,g}$ %v/v***	***I $\mu E\ m^{-2}\ s^{-1}$***	***T, °C***	***pH, (-)***	***C/N/P or N/P ratio***	***TN (%)***	***TP (%)***	***COD (%)***	***Refs***
BioFlo-F_B - MBM & ZM	nr	nr	nr	nr	15	68.2	nr	7.2	nr	nr	nr	nr	(Sydney et al. 2010)
PBR_B – SWw	nr	nr	nr	nr	6	84.2	27	nr	nr	nr	nr	nr	(Singh et al. 2016)
$FPBR_B$ - MZM	618	89	nr	600	2.5	400*	28-30	9.4	600:07:01	nr	nr	nr	(Ho et al. 2018)
$EFPBR_B$ - SWw	130	15	900	nr	nr	90	25	7.79	900:7.79:1	93	80	90	(Zhou et al. 2017b)
$CPBR_C$ - ZM	444	82.5	nr	2400	5	180± 5	30	nr	2400:05:01	nr	75	nr	(Liu et al. 2018)
$CPBR_B$ - ZM										nr	67	nr	
$BAPBR_C$ - SYWw	412	90	nr	nr	0.03	400	20	9 -10	4.5:1	49	81	nr	(Yuan et al. 2011)
$FPBR_B$ - ZM	407	115	nr	2373	10	174	33	9.2	2373:04:01	nr	nr	nr	(Xue et al. 2011)
AA- SDCG-BC_B - CF	76	89	nr	2340	100	nr	nr	9-10	2340:01:01	70	19	nr	(Kumari et al. 2014)

BioFlo-F_B BioFlo-fermenter; MBM Modified Bristol medium; ZM; Zarrouk medium; PBR_B Photobioreactor; SWw Secondary wastewater; $FPBR_B$ Flat-Plate Photobioreactor; MZM Modified Zarrouk medium; $EFPBR_B$ Erlenmeyer flask Photobioreactor; $CPBR_C$ Cylindrical/column Photobioreactor; $BAPBR_C$ Bench-scale airlift photobioreactor; SYWw Synthetic wastewater; AA –SDCG - BC_B Air agitated - Sintered disk chromatographic glass bubble column-; –CF Complex fertilizer. Symbols: Rc = CO_2 fixation rate estimated from Chisti ratio $CO_{0.48}H_{1.831}N_{0.11}P_{0.01}$; $R_X = 1.88 \times P_X$; P_X = biomass productivity estimated from $\Delta X/\Delta t$; Subscripts: $_L$ lab-scale; $_P$ pilot-scale; $_B$ batch; $_C$ continuous; $_s$ semi-continuous; $_X$ = Mixtrophic growth cultivation mode

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Impact of CO2 concentration and ambient conditions on microalgal growth and nutrient removal from wastewater by a photobioreactor

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