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Optimization of carbon dioxide supply in raceway reactors: influence of carbon dioxide molar fraction and gas flow rate.

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Abstract

Influence of CO_2 composition and gas flow rate to control pH in a pilot-scale raceway producing *Scenedesmus* sp. was studied. Light and temperature determined the biomass productivity whereas neither the CO_2 molar fraction nor the gas flow rate used influenced it; because pH was always controlled and carbon limitation did not take place. The CO_2 molar fraction and the gas flow rate influenced carbon loss in the system. At low CO_2 molar fraction (2-6%) or gas flow rate (75-100 $l \cdot min^{-1}$) the carbon efficiency in the sump was higher than 95%, 85% of the injected carbon being transformed into biomass. Conversely, at high CO_2 molar fraction (14%) or gas flow rate (150 $l \cdot min^{-1}$) the carbon efficiency in the sump was lower than 67%, 32% of the carbon being fixed as biomass. Analysis here reported allows the pH control to be optimized and production costs to be reduced by optimizing CO_2 efficiency.

1. Introduction

Microalgae have great biotechnological potential in producing valuable substances for the pharmaceutical, nutraceutical, food and feed industries (Spolaore et al., 2006; Koller et al., 2014). Moreover, their usefulness has been proposed for other purposes such as CO_2 mitigation, wastewater treatment and biofuel production (Oswald, 1991; Muñoz and Guieysse, 2006; Chisti, 2008; Acién et al., 2012). Whatever the final application, two main photobioreactor types are used to produce microalgae: (i) closed photobioreactors, where high-value-added products are produced from strains that are highly sensitive to contamination, and (ii) raceway reactors, which are simpler and less expensive, and where contamination-proof strains can be utilized (Posten, 2009; Acién et al., 2013). Worldwide, more than 95% of commercial microalgae production is carried out in raceway reactors due to their low construction cost, easy scale-up and low energy requirements (Benemann, 2013).

Although raceway reactors theoretically have the potential to produce more than 40 $g \cdot m^{-2} \cdot day^{-1}$ of microalgal biomass, equivalent to 146 $tonne \cdot ha^{-1} \cdot year^{-1}$ (Brennan and Owende, 2010), reported productivities are generally far lower. Richmond estimated that annual raceway productivity was between 16-19 $g \cdot m^{-2} \cdot d^{-1}$ (Richmond et al., 1990). A maximum productivity of 21 $g \cdot m^{-2} \cdot d^{-1}$ was reported for *Spirulina* (Vonshak and Guy, 1992) and 13.2 $g \cdot m^{-2} \cdot d^{-1}$ for *Chlorella* (Hase et al., 2000). One reason for this is that, under phototrophic growth conditions in open raceways, microalgal cultures can become carbon limited due to the low amount of CO_2 being transferred directly from the atmosphere (de Godos et al., 2015). To maximize biomass productivity in raceway reactors, it is essential to supply carbon as a nutrient source at the same rate as that required for biomass production (Stengel and Soeder, 1978). Carbon constitutes approximately 50% of the microalgal biomass; therefore, to achieve the potential of raceway reactors, the carbon supply capacity must be up to 20 $g \cdot m^{-2} \cdot day^{-1}$. This carbon can be supplied to the culture either as carbon dioxide or bicarbonate (Grobbelaar, 2013), both of which increase the microalgae biomass production costs, even more so if some of the carbon provided is lost to the environment. It has been estimated that the cost of providing CO_2 is approximately 30% of the total microalgal production system cost (Benemann, 1997). In order to reduce this cost, utilizing flue gases as the carbon source has emerged as an economically and

environmentally feasible option to control pH and to provide the necessary CO_2 for biomass production - this has been widely demonstrated (Laws and Berning, 1991; Putt et al., 2011; González-López et al., 2012; de Godos et al., 2014).

Whatever the CO_2 source may be (pure CO_2, mixtures with air or flue gases), to maximize raceway reactor productivity, it is necessary to engineer a CO_2 supply to the raceway that meets the culture's carbon requirements. The engineering aspects of raceway reactors were previously studied (Weissman et al., 1988), these have now been revised to improve the efficiency of this technology (de Godos et al., 2014; Sompech et al., 2012; Chiaramonti et al., 2013; Ketheesan and Nirmalakhandan, 2013; Mendoza et al., 2013a,b). The preferred method of increasing the CO_2 absorption capacity in raceway reactors is by including a channel sump, where gas is bubbled in at the bottom, the greater depth allowing an increased contact time and hence the mass transfer between the gas and the liquid phase (Weissman et al., 1988; Mendoza et al., 2013b; Benemann et al., 1987; Park et al., 2011; Craggs et al., 2012). Alternative systems have also been proposed including an airlift-driven raceway reactor, for which a maximum CO_2 utilization efficiency of 33% has been reported (Ketheesan and Nirmalakhandan, 2012); and the use of an external carbonation column (Putt et al., 2011) although this leads to higher power consumption as the culture has to be circulated through the absorption unit. More recently, a detailed analysis of the optimal sump design and the operational conditions to maximize the CO_2 absorption from flue gases containing 10% CO_2 has been reported (de Godos et al., 2014). Nonetheless, very few studies have analyzed the influence of operational CO_2 supply system conditions on the performance of microalgae cultures in raceway reactors, especially the variation in system efficiency according to environmental and operational conditions.

In this work, we have performed a detailed analysis on the influence of the CO_2 supply system's operating conditions; that to say (i) the CO_2 molar fraction in the gas supplied and (ii) the gas flow rate, on the performance of *Scenedesmus* sp. cultures developed in a pilot-scale (100 m^2) raceway reactor operated in semicontinuous mode for one year. The CO_2 molar fractions were modified in the range reported for flue gases, ranging from 2% when natural gas was used to 15% when coal was used (US DOE, 2010). The objective of the work is to demonstrate the capability of the on-demand injection of CO_2–rich gases to meet the requirements of microalgae cultures produced in raceway reactors, and to discover, at the pilot scale, if there are any adverse

effects taking place as a result of excessive pH gradients or carbon availability. Because carbon is a major nutrient-limiting factor in the production of microalgae biomass, the uncovering of inadequate operating conditions will improve the performance of these reactors. Moreover, because CO_2 supply is a relevant cost at the commercial scale, optimizing CO_2 utilization will allow us to reduce the production costs. Both an increase in productivity and a decrease in production costs will enhance the system's profitability and help to expand the development of this biotechnology.

2. Materials and methods

2.1. Microorganism and culture medium

The microalga strain *Scenedesmus sp.* was used. Our group previously isolated this strain from freshwater used in greenhouse fertigation. *Scenedesmus* strains have been widely reported on for outdoor production because of their tolerance to adverse conditions. Experiments were performed using Arnon medium, prepared using fertilizers instead of pure chemicals, to give the following ionic concentrations (mmol l^{-1}): NO_3^- (9.49); NH_4^+ (0.59); $H_2PO_4^-$ (1.00); K^+ (1.60); Ca^{2+} (5.00); Mg^{2+} (3.25); SO_4^+ (1.20); HCO_3^- (2.80); Na^+ (8.20); Cl^- (11.10) Fe^{3+} (2.00); Mn^{2+} (0.84); Zn^{2+} (0.56); B^{3+} (0.49) and Cu^{2+} (0.08).

2.2. Raceway reactor and operating conditions

Experiments were performed in a pilot-scale raceway reactor located at the Estación Experimental Las Palmerillas of the Fundación CAJAMAR (Almería, Spain). The reactor comprise two 50 m length, 1 m wide channels connected by 180º bends, with a total surface area of 100 m^2 (Figure 1). The raceway reactor is equipped with a 0.59 m^3 sump (0.65 m length x 0.90 m width x 1 m depth) located 1.8 m downstream of the paddlewheel. The entire reactor, including the sump, is made of white 3 mm-thick fiberglass. The liquid in the raceway was circulated by a marine plywood paddlewheel, with a 1.2 m diameter, driven by a three-phase electric motor (W12 0.35 kW, Ebarba, Barcelona, Spain) with gear reduction (I ¼ 25 0.37 kW 90º; 0.50 HP, WEG Iberica, S.A., Barcelona, Spain). The motor velocity was controlled by a frequency inverter (CFW 08 WEG Iberica, S.A., Barcelona, Spain). Removable deflectors made of stainless steel were placed in the bends at intervals of one quarter of the channel width to optimize the fluid-dynamics in the reactor and to reduce power consumption. The

reactor is equipped with four dissolved oxygen probes (OD5120, Crison, Spain), and four pH and temperature probes (pH5083T, Crison, Spain), connected to a multimeter (MM44, Crison, Spain) and data acquisition software (Daqfactory, Azeotech, Arizona, USA) for data acquisition and control. The probes were located at the beginning of each section into which the reactor can be divided: the sump, channels and the paddlewheel. A layout of the raceway reactor and the position of the probes is shown in Figure 1. The raceway reactor was additionally equipped with a gas injection system to pump CO_2 into the sump through three plate membrane diffusers (AFD 270). The gas flow rate entering the reactor was measured by a mass flow meter (PFM 725S-F01-F, SMC, Tokyo, Japan).

The raceway was first inoculated using culture from a 3.0 m^3 tubular photobioreactor. For this, 5 m^3 of fresh medium was added to the reactor. Then 3 m^3 of inoculum from the tubular photobioreactor was also added. Finally, the raceway reactor was topped up with fresh medium to a water depth of 15 cm (overall volume 15 m^3). The initial biomass concentration was 0.2 $g \cdot l^{-1}$ and the raceway was operated in batch mode until a biomass concentration exceeding 1.0 $g \cdot l^{-1}$ was reached - from this point the reactor was operated in semi-continuous mode at a dilution rate of 0.15 d^{-1}. Steady state was reached after ten days, whereby the biomass concentration and the overall behavior of the reactor remained constant (only modified as a result of environmental conditions i.e. light and temperature variation during the day) - all the experiments were performed under these conditions. The reactor was operated under these same conditions for one year with no modifications or reinoculations.

During the experiments, the culture temperature was a function of the ambient temperature and the incident solar radiation, no temperature control mechanisms were used in the culture. The dissolved oxygen varied throughout the day - also as a function of the temperature and solar radiation on the reactor surface; likewise, this was not controlled. In contrast, the culture pH was kept at 8.0 by on-demand CO_2 injection into the sump. Experiments were performed modifying the CO_2 molar fraction entering the sump gas inlet in addition to the gas flow rate supplying the sump. Thus, to evaluate the effect of the CO_2 molar fraction in the gas on system performance, a mixture of pure CO_2 and air was used to simulate flue gases with different CO_2 proportions. The CO_2 concentration in the flue gases was a function of the fuel used; thus it was possible to find concentrations from 2% (if natural gas was used) up to 14% CO_2 for “dirty” gases

corresponding to coal-fired power stations. In practice, the CO_2 molar fractions evaluated here were 2%, 6%, 10% and 14% CO_2 at a gas flow of 100 l min^{-1}. In a second set of experiments, the effect of different gas flow rates was evaluated, performed at gas flow rates of 70, 100, 130 and 150 l min^{-1}, and at CO_2 molar fractions in the gas of 6% and 14%. Under whichever condition tested, the CO_2 and air flow rate was monitored using flow meters (FR4500, Key Instruments, USA) while the CO_2 supply and air were regulated by on/off solenoid valves, switched automatically through the computer control system.

2.3. Analytical methods

The biomass concentration (Cb) dry weight was measured by vacuum filtration of 250 ml of culture through 0.45 μm filters, drying it in an oven at 80 °C until constant weight was achieved. The total carbon concentration (TC) in the liquid phase was determined by spectrophotometric measurements using total inorganic carbon (TIC) and total organic carbon (TOC) kits (Hach-Lange, LKC 381). The culture medium inlet to the reactor, the culture harvested from the reactor, and the culture broth (after biomass removal by filtration) were all analyzed. To determine the CO_2 concentration in the gas entering and leaving the sump, a CO_2 probe (GMT220, Vaisala, Finland) connected to a data acquisition system was used. To measure the CO_2 concentration in the outlet gas, the gas reaching the surface above the sump was collected via a fume hood.

2.4. Carbon balance

To determine carbon utilization efficiency, when modifying the CO_2 molar fractions in the gas or the gas flow rate injected into the sump, a complete mass balance was performed on the reactor overall. Regarding the gas phase, the CO_2 molar fractions in the sump's gas inlet and outlet were measured. With regard to the liquid phase, the carbon concentration in the culture medium inlet to the reactor, the culture broth outlet from the reactor, and the biomass outlet from the reactor were measured. These measurements allowed us to perform the carbon mass balance for the entire system (Eq. 1), where $mC_{input\ gas}$ is the total mass of carbon entering with the gas (g/day), $mC_{input\text{-}liq}$ is the total mass of carbon entering with the fresh medium (g/day), $mC_{output\text{-}gas}$ is the total mass of carbon that escapes to the atmosphere from the sump (g/day), $mC_{output\ liq}$ is the total mass of carbon in the culture leaving the reactor with the harvested liquid

(g/day), $mC_{biomass}$ is the total mass of carbon in the culture leaving the reactor with the harvested biomass (g/day), and $mC_{outgassing}$ is the total mass of carbon desorbed from the culture to the atmosphere during its circulation around the raceway, mainly in the channels and the paddlewheel (g/day). Because all the carbon fluxes were experimentally measured, except for the losses caused by stripping, Equation 1 was used to calculate these losses.

$$mC_{input\ gas} + mC_{input\ liq} = mC_{output\ gas} + mC_{output\ liq} + mC_{biomass} + mC_{outgassing} \quad (1)$$

The carbon efficiency in the sump was calculated from the ratio between the mass of carbon absorbed from the gas to the liquid phase ($mC_{input\ gas} - mC_{output\ gas}$) and the mass of carbon injected into the sump ($mC_{input\ gas}$) (Eq. 2). The carbon efficiency in the biomass was calculated as the ratio between the mass of carbon assimilated by the biomass ($mC_{biomass}$) and the mass of carbon injected into the sump ($mC_{input\ gas}$) (Eq. 3). Finally, carbon losses from stripping were calculated as the ratio between the mass of carbon lost by stripping into the channels and the paddlewheel ($mC_{outgassing}$) and the mass of carbon injected into the sump ($mC_{input\ gas}$) (Eq. 4).

$$Sump\ efficiency = \frac{mC_{input\ gas} - mC_{output\ gas}}{mC_{input\ gas}} \quad (2)$$

$$Biomass\ efficiency = \frac{mC_{biomass}}{mC_{input\ gas}} \quad (3)$$

$$Losses\ from\ stripping = \frac{mC_{outgassing}}{mC_{input\ gas}} \quad (4)$$

3. Results and discussion

To evaluate the influence of CO_2 supply on raceway reactor performance, experiments were performed for one year modifying the CO_2 molar fraction in the sump's gas inlet and the gas flow rate supplied to the sump - a total of 26 different culture conditions were evaluated, all of them in triplicate. During the experiments, the environmental conditions (sunlight and temperature) were also modified. To do this, a variance analysis was first performed to identify the relevant variables. Results showed that solar radiation and temperature had a major influence on the cultures' biomass productivity whereas neither the CO_2 molar fraction in the inlet gas nor the gas flow rate

entering the sump had any effect on biomass productivity (Figure 2). The positive influence of solar radiation and temperature on biomass productivity was directly related to the improvement in culture conditions, which allowed an increase in the steady-state biomass concentration. Regarding the null effect of the CO_2 molar fraction in the gas and the gas flow rate, this was due to the on-demand CO_2 injection system used, which allowed the pH and CO_2 availability to be kept at adequate levels for the CO_2 molar fraction and gas flow rate values tested.

Because environmental conditions influence biomass productivity, and consequently, the culture's carbon requirements, at first we only analyzed data from the winter time (when biomass productivity was constant) in order to study the influence of the CO_2 molar fraction in the gas and the gas flow rate supplied to the sump for pH control. In this sense, the winter time data showed that whatever the CO_2 molar fraction in the gas, the major contribution to the carbon balance was the carbon supplied to the sump (an average of 72%) and the carbon fixed in the biomass (on average 56%), followed by carbon losses from stripping (an average of 27%) (Figure 3A). The carbon input with the culture medium (an average of 28%) was lower than the carbon output in the culture broth (on average 13%), indicating that carbon was removed from the culture medium in spite of the CO_2 supply. With regard to the carbon losses in the sump, their contribution to the overall carbon balance was really low (on average 3%) due to the highly efficient gas supply system used. By analyzing the influence of the CO_2 molar fraction in the sump's gas inlet, results showed that only the carbon flows related to gas-liquid mass transfer were modified (sump and stripping) (Figure 3A). The data showed that the higher the CO_2 molar fraction in the gas, the higher the amount of carbon supplied to the sump, even when the same on-demand CO_2 injection system was used and the culture remained under the same culture conditions and at the same biomass productivity level. Moreover, the higher the CO_2 molar fraction in the gas, the higher the carbon losses both in the sump and from stripping to the atmosphere in the channels and at the paddlewheel. These results can be explained by increased culture oversaturation when large CO_2 concentrations are used in the gas, leading to higher losses when the culture is in contact with air. No relevant differences were observed in the carbon input with the culture medium, nor the output with the culture broth or the biomass when modifying the CO_2 molar fraction in the gas.

Regarding the influence of the gas flow rate, experiments were performed at two different CO_2 molar fractions in the gas phase, 6% (Figure 3B) and 14% (Figure 3C). The results were analogous to those previously observed when the CO_2 molar fraction in the gas was modified - the variation in the gas flow rate did not modify the carbon flows in the liquid phase but it did change the carbon flows in the gas phases. Thus, the higher the gas flow rate entering the sump, the higher the carbon supplied to the sump and also the higher the carbon losses from the sump, as well as from stripping in the channels and paddlewheel. This can be explained by the overabundance of CO_2 supplied when operating with large gas flow rates providing more carbon than the culture requires, thus carbon remains in the culture and is lost to the atmosphere. This is confirmed when analyzing the results obtained using different CO_2 molar fractions in the gas. So, when using 6% CO_2 in the gas, the carbon losses in the sump, and from stripping, were 3% and 17%, respectively; whereas using 14% CO_2 in the gas, the carbon losses were higher - 5% and 23% for the sump and from stripping, respectively.

According to these results, the CO_2 injection system used was sufficient to supply the carbon required by the culture even when only 2% CO_2 was used in the gas phase and with a gas flow rate of 100 $l \cdot min^{-1}$. Utilizing larger CO_2 molar fractions in the gas phase, or higher gas flow rates, did not improve biomass productivity in the cultures but simply increased carbon losses to the atmosphere, both in the sump and from stripping in the channels and the paddlewheel. However, this behavior can vary greatly according to the variation in the culture's carbon demand if higher productivity is achieved under more suitable culture conditions. To study the system's efficiency when facing higher carbon demand, experiments were repeated during the summer time, maintaining the experimental conditions used in the winter. Results confirmed that the carbon demand in summer was far higher than in the winter time - 2.8 times higher, but the overall distribution of carbon balance was quite similar to that obtained during the winter (Figure 4A). Thus, in summer time, the carbon supplied to the sump represents 86% of the carbon input whilst the carbon supplied with the culture medium was only 14% of the overall input. Regarding output, 48% of the carbon was removed with the biomass, whereas 22% was lost to the atmosphere from stripping in the channels and the paddlewheel. Carbon losses in the sump represented 14% of the overall carbon whereas carbon output in the culture broth accounted for 16% of the overall carbon (Figure 4A). By comparing these figures with those obtained in winter time, one can observe that the

carbon supply was far higher in the summer due to the larger demand of the culture to produce biomass; however, at the same time, more carbon was lost from the sump under these conditions. In this case, the carbon mass entering with the culture medium was equal to that removed with the culture broth.

By analyzing the influence of the CO_2 molar fraction in the gas and the gas flow rate on the system performance during the summer time, one can observe similar behavior to that previously observed in results obtained in the winter time (Figure 4B, C). Consequently, the results confirm that in summer, despite the major carbon demand to produce biomass, when the CO_2 molar fraction in the gas phase increases, so does the carbon losses in the sump and from stripping in the channels and the paddlewheel. Regarding the influence of the gas flow rate, we determined a similar trend as previously observed for the winter time - the higher the gas flow rate, the greater the carbon losses both in the sump and the channels and the paddlewheel. It is important to note the large carbon supply values obtained when we used 14% CO_2 in the gas phase and gas flow rates higher than 100 $l \cdot min^{-1}$. Under these conditions, carbon supply exceeded 1500 $g \cdot day^{-1}$, and because biomass productivity did not increase, the carbon losses from stripping to the atmosphere in the channels and the paddlewheel surpassed 40% of the carbon input (Figure 4C).

According to these results, the culture's carbon demand influences the system's design, a far higher carbon supply capacity being necessary in the summer than in the winter time. This larger demand also modifies the system behavior, but the overall phenomena pattern taking place is the same. It is important to note that in the summer time, even when using 2% CO_2 and a gas flow of 100 $l \cdot min^{-1}$, it was possible to control the culture pH although, under these conditions, the system was at the limit of its capacity - the valve regulating CO_2 injection was open for long periods and the pH remained at values above the setpoint a great deal of the time. Table 1 and Table 2 summarize the daily pH and injection time variations during the experiments performed, modifying the CO_2 molar fraction in the gas and the gas flow rate supplied to the sump both for the winter and summer times. Results shows that whichever CO_2 molar fraction was used in the gas phase, the pH was controlled between 7.3 and 8.4; the minimum values being measured at night while the higher values were measured in the daylight period when photosynthesis increased the pH in the cultures by CO_2 consumption (Table 1). However, even though the pH was controlled (even when using 2% CO_2 in

the gas), the time the valve was open largely varied as a function of the CO_2 molar fraction. During the winter time, the variations were lower but in the summer time, the valve was open for 150 min during the daylight period when using 2% CO_2, the pH being outside the control limit especially at noon when the culture received maximum irradiance. On the other hand, the results obtained regarding the modification in the gas flow rate shows that, when using 6% CO_2, the system performed perfectly - with the pH being well controlled and the injection time slightly diminishing when the gas flow rate increased (Table 2). However, when using 14% CO_2 in the gas, the injection time only diminished in the winter time, whereas in summer, we observed no injection time dependency on the gas flow rate. This could be due to the delay time existing in the reactor between the pH measurement and the CO_2 injection - thus, if excess CO_2 (a high CO_2 molar fraction and a high gas flow rate) is provided, the system does not perform well.

The supply of CO_2 to microalgae cultures is a major factor determining system performance, both because the carbon is a main nutrient for microalgae and because the CO_2 supply allows one to control the culture pH at its optimal value. Although constant CO_2 injection (whether pure or in mixtures with air or even flue gases) has been widely studied (Anjos et al., 2013; Fulke et al., 2010), it is not an adequate method to supply CO_2 to microalgae cultures given that excess CO_2 in an air mixture can also overacidify the culture broth; while a large fraction of the CO_2 supplied is not efficiently used and is therefore lost to the atmosphere. The most suitable system for supplying CO_2 to microalgae cultures is the on-demand injection of CO_2-rich gases, although overall efficiency is a function of the design and operating conditions used - maintaining a minimum level of alkalinity in the culture broth being imperative (González-López et al., 2012; de Godos et al., 2014). Thus, whatever the reactor type used, enough CO_2 must be supplied to satisfy the requirements for producing biomass; a minimum of 2 $kgCO_2$ per kilogram of biomass produced being necessary. This amount of CO_2 can be supplied either by using low gas flow rates of highly concentrated CO_2 (including pure CO_2) or alternatively, large gas flow rates of low concentrated CO_2, such as flue gases. Whatever the CO_2 molar fraction in the gas or the gas flow rate may be, system reliability also varies as a function of its capacity to efficiently use the CO_2 supplied in producing biomass while minimizing carbon losses.

The results reported here demonstrate the reliability of the CO_2 supply system used in the pilot-scale raceway reactor, even when modifying the CO_2 molar fraction in the gas from 2% to 14%, or the gas flow rate entering the sump from 75 to 150 $l \cdot min^{-1}$. The range of CO_2 molar fractions in the gas tested is similar to that found in flue gases from different fuels, thus demonstrating that flue gases from natural gas (2-4% CO_2) or coal-fired (10-15% CO_2) power stations can be used to produce microalgae, as long as there are no other contaminant gases contained into the flue gases (NOx, SOx, etc.) which might inhibit microalgae growth. The results demonstrated that modifying the CO_2 molar fraction in the gas or the gas flow rate does not influence the system's biomass productivity; this is mainly because the CO_2 molar fraction in the gas and the gas flow rates tested provide sufficient carbon to avoid limitation, as well as maintaining the pH within a narrow range. With regard to carbon supply and demand, the data show that when using 2% CO_2 in the gas, the amount of carbon supplied operating at a gas flow rate of 100 $l \cdot min^{-1}$ was higher than that required by the culture in winter time (during the central hours of the day); but not in summer time when biomass productivity was higher (Figure 5). Under these conditions, the system was at its limit for pH control, as shown by the longer periods for which the CO_2 injection valve was on. When using large CO_2 molar fractions in the gas, the carbon input was several times greater than that required by the culture, thus the system was more robust and stable; and the CO_2 injection valve only needed to be activated for short periods. Consequently, these data confirm that 2% CO_2 in the gas might not be sufficient to maintain the system under controlled conditions when biomass productivity increases.

With regard to the pH, the widest range in pH variation was from 7.3 to 8.3, but this was across the entire solar period, whereas the variation during daylight hours was far lower, from 7.8 to 8.3, which was no greater than the differences observed when modifying the CO_2 molar fraction in the gas, or the gas flow rate. It has been widely reported that microalgae culture productivity is influenced by the pH to which the cultures are exposed (Costache et al., 2013). Moreover, variations within the reactor can also reduce the cells' performance (Camacho Rubio et al., 1999; Berenguel et al., 2004)due to the existence of pH gradients when CO_2 is injected. However, the results reported here do not show any adverse effects when using a high CO_2 molar fraction in the gas, or high gas flow rates – this is due to the reactor's large volume and the system's inertia to changes in culture conditions prevailing within it. Nonetheless, in

order to avoid this problem occurring, it is necessary to adequately design and operate advanced control strategies such as Model Predictive Control (MPC), which allows one to minimize the pH gradients to which the cells are exposed inside the reactor (Berenguel et al., 2004; Pawlowski et al., 2014). Furthermore, utilizing these advanced control strategies allows one to improve the system's efficiency with respect to the on-off mode used here, thus reducing carbon input and losses while maintaining, or even increasing, the productivity (Berenguel et al., 2004; Pawlowski et al., 2014).

Changes in environmental conditions greatly affect the system's biomass productivity, thus also modifying the cultures' carbon requirements. The CO_2 supply in summer time was, on average, 670 $gC \cdot day^{-1}$ whereas during the winter, the average requirement was 240 $gC \cdot day^{-1}$. In spite of these substantial variations, the ratio of biomass unit produced to carbon taken up ranged from 1.9 to 2.5 $g_{biomass} \cdot gC^{-1}$ whereas a value of 2.2 $g_{biomass} \cdot gC^{-1}$ is theoretically expected if the biomass contains 45% carbon. In terms of the carbon supplied with the gas flow rate entering the sump, the ratio of biomass unit produced to the carbon supplied ranged from 0.5 to 0.9 $g_{biomass} \cdot gC^{-1}$, lower than before due to carbon losses in the system. At any rate, this variation in carbon demand as a function of environmental conditions necessitates the use of flexible CO_2 supply systems, efficient under any conditions. The variation in biomass productivity as a function of environmental conditions also affects overall system behavior. In general, data show that by increasing the CO_2 molar fraction in the gas, or the gas flow rate, carbon losses in the system increase. It was previously reported that by using flue gas with 10% CO_2, CO_2 removal decreased when increasing the gas flow rate or decrease the culture's pH (de Godos et al., 2014). The reduction in CO_2 efficiency at high gas flow rates is related to the retention time reduction of bubbles in the fluid (de Godos et al., 2014). Major carbon losses take place in the sump when the CO_2 supply is greater than the culture broth's capacity to absorb it; likewise, later on, it is lost to the atmosphere in the channels and the paddlewheel when the culture broth has been oversaturated with excess CO_2 in the sump. Carbon losses in the sump, or decarbonization has previously been reported as representing up to 6% of the total inlet carbon, whereas carbon losses from the culture broth represent up to 22% of the total inlet carbon (de Godos et al., 2014). In our work, minimum carbon losses of 16% were achieved only when operating at a low gas flow rate ($\leq$100 $l \cdot min^{-1}$) and using a 6% CO_2

molar fraction in the gas - although average carbon losses in the culture broth were 14%, lower than previously reported.

In our experiments, the carbon efficiency in the sump ranged from 67% when using 14% CO_2 in the gas, with a gas flow rate of 150 $l \cdot min^{-1}$, to 98% when using 2% CO_2 in the gas with a gas flow rate of 75 $l \cdot min^{-1}$. A maximal efficiency of 94% was reported when flue gas from diesel combustion was used (de Godos et al., 2014). In contrast, it was also reported that carbon efficiency varied from 59%, to 51% and 46% when the CO_2 molar fraction in the gas changed from 1%, to 5% and 10%, respectively (Ramanan et al., 2010). The system's carbon efficiency varies substantially depending on the bubbling system used; hence in the same raceway reactor the carbon efficiency was 26% using a sump whereas it increased to 82% when using a bubble column connected to the raceway reactor – a result of the better design efficiency of bubble column used (Putt et al., 2011). Moreover, for the same bubbling system, the liquid to gas ratio (L/G) determines the carbon efficiency; it being reported that L/G ratios greater than 20 allows one to achieve carbon efficiencies above 90% (de Godos et al., 2014). A similar trend was observed in our study, demonstrating that at L/G ratios below 15 (with gas flow rates above 130 $l \cdot min^{-1}$), the carbon efficiency in the sump dropped below 80%. Overall, the carbon losses from the sump reported here are similar to those previously reported in closed tubular photobioreactors, ranging from 20% to 5% when using on-off and model predictive control, respectively (García et al., 2003). A CO_2 recovery efficiency of 16% was reported for *Chlorella* sp. using a 15% rich-CO_2 gas, whereas this increased to 56% when a 2% rich-CO_2 gas was substituted (Chiu et al., 2008). Cheng demonstrated that increasing the CO_2 retention time in the photobioreactor significantly enhanced the CO_2 fixation efficiency (Cheng et al., 2006). When the entire liquid surface of a raceway was covered with a plastic sheet, the maximum CO_2 efficiency when 15% rich-CO_2 gas was continuously added was reported to be 64%. This efficiency increased to 95% when intermittent injection was used to better match the carbon requirements of the microalgal culture (Li, Luo and Guo, 2013). In our case, the main reason that the reported data are so high is a result of the sump configuration used - which had previously been demonstrated to be optimal for this raceway reactor, although this might require modification if other reactor parameters changed (length, width, etc.) (de Godos et al., 2014).

To analyze the influence of the studied variables (season, CO_2 molar fraction in the gas and the gas flow rate) on sump performance, the biomass' carbon uptake and the losses from stripping in the channels and paddlewheel, a variance analysis was performed. Table 3 shows that the sump's efficiency at capturing the CO_2 supplied was high, up to 94%, whichever culture condition was used; however, this reduced when the culture's carbon demand was higher, i.e. in summer time, as the valve to provide the required amount of CO_2 was open longer. The sump's carbon efficiency likewise reduced when the CO_2 molar fraction in the gas, or the gas flow rates, increased - to minimum values of 84%. Regarding the system's efficiency to transform the supplied carbon into biomass, this was lower due to losses within the culture medium and from stripping. Its efficiency was higher in winter than in summer time, with values up to 81% and 54%, respectively - due to the lower carbon input. The carbon to biomass efficiency also reduced when the CO_2 molar fraction in the gas, or the gas flow rates, increased - up to values of 55%. With regard to carbon losses caused by stripping to the atmosphere (in the channels and the paddlewheel), the values varied from 32% to 29% from winter to summer time, respectively. In contrast, carbon losses from stripping increased when the CO_2 molar fraction in the gas, or the gas flow rates, increased - up to values of 37%.

In summary, in order to optimize raceway reactor operation when producing microalgae, it is essential to have an accurate estimation of the culture's carbon demand. This carbon demand, along with the composition of the CO_2-rich gas available, determine the minimum gas flow rate to be used for the on-demand injection of CO_2 for pH control and carbon supply. This gas flow rate must be calculated taking into consideration that the CO_2 is not supplied throughout the daylight period but mainly in the central hours of the day when the photosynthesis rate is maximal. Only by adequately considering these phenomena can the successful performance of microalgae cultures in raceway reactors be achieved.

4. Conclusions

Carbon losses in raceways are mainly determined by the CO_2 supply system used. Variations in the CO_2 molar fraction or the gas flow rates do not modify biomass productivity but do influence carbon losses in the system. Major carbon losses take place in the sump and from stripping in the channels and the paddlewheel; these losses

being greater the higher the CO_2 molar fraction or the gas flow rate used. However, reducing the CO_2 molar fraction in the gas, or the gas flow rate, is limited by the amount of carbon required for the culture, according to the biomass productivity.

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6. References

1. Acien, F., González-López, C.V., Fernández, J.M., Molina, E., 2012. Conversion of CO2 into biomass by microalgae: How realistic a contribution may it be to significant CO2 removal? Appl. Microbiol. Biotechnol. 96, 577-586.
2. Acién, F.G., Fernández, J.M., Molina, E., 2013. Photobioreactors for the production of microalgae, Rev Env Sci Biotechnol. 1-21.
3. Anjos, M., Fernandes, B.D., Vicente, A.A., Teixeira, J.A., Dragone, G., 2013. Optimization of CO2 bio-mitigation by *Chlorella vulgaris*, Bioresour. Technol. 139, 149-154.
4. Benemann, J., 2013. Microalgae for biofuels and animal feeds, Energies. 6, 5869-5886.
5. Benemann, J., 1997. CO2 mitigation with microalgae systems, Energy Convers. Manage. 38, S475-S479.
6. Benemann, J., Tillett, D.M., Weissman, J.C., 1987. Microalgae biotechnology, Trends Biotechnol. 5, 47-53.
7. Berenguel, M., Rodríguez, F., Acién, F.G., García, J.L., 2004. Model predictive control of pH in tubular photobioreactors, J Proc Control. 14, 377-387.
8. Brennan, L., Owende, P., 2010. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products, Renewable Sustainable Energy Rev. 14, 557-577.
9. Camacho, F., Acién, F.G., Sánchez, J.A., García, F., Molina, E., 1999. Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture, Biotech Bioeng. 62, 71-86.
10. Cheng, L., Zhang, L., Chen, H., Gao, C., 2006. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor, Sep Pur Technol. 50, 324-329.
11. Chiaramonti, D., Prussi, M., Casini, D., Tredici, M.R., Rodolfi, L., Bassi, N., Zittelli, G.C., Bondioli, P., 2013. Review of energy balance in raceway ponds for microalgae cultivation: Re-thinking a traditional system is possible, Appl. Energy. 102, 101-111.
12. Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol, Trends Biotechnol. 26, 126-131.

13. Chiu, S., Kao, C., Chen, C., Kuan, T., Ong, S., Lin, C., 2008. Reduction of CO2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor, Bioresour. Technol. 99, 3389-3396.
14. Costache, T.A., Acien, F., Morales, M.M., Fernández-Sevilla, J.M., Stamatin, I., Molina, E., 2013. Comprehensive model of microalgae photosynthesis rate as a function of culture conditions in photobioreactors, Appl. Microbiol. Biotechnol. 97, 7627-7637.
15. Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production, J. Appl. Phycol. 24, 329-337.
16. de Godos, I., Mendoza, J.L., Acién, F.G., Molina, E., Banks, C.J., Heaven, S., Rogalla, F., 2014. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases, Bioresour. Technol. 153, 307-314.
17. Fulke, A.B., Mudliar, S.N., Yadav, R., Shekh, A., Srinivasan, N., Ramanan, R., Krishnamurthi, K., Devi, S., Chakrabarti, T., 2010. Bio-mitigation of CO2, calcite formation and simultaneous biodiesel precursors production using *Chlorella* sp. Bioresour. Technol. 101, 8473-8476.
18. González-López, C.V., Acién, F.G., Fernández-Sevilla, J.M., Sánchez, J.F., Molina, E., 2012. Development of a process for efficient use of CO2 from flue gases in the production of photosynthetic microorganisms, Biotechnol. Bioeng. 109(7), 1637-1650.
19. Grobbelaar, J.U., 2013. Inorganic Algal Nutrition, Handb. of Microalgal Cult. : Appl. Phycology and Biotechnol. 123-133.
20. Hase, R., Oikawa, H., Sasao, C., Morita, M., Watanabe, Y., 2000. Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai City, J Biosci Bioeng. 89, 157-163.
21. Ketheesan, B., Nirmalakhandan, N., 2012. Feasibility of microalgal cultivation in a pilot-scale airlift-driven raceway reactor, Bioresour. Technol. 108, 196-202.
22. Ketheesan, B., Nirmalakhandan, N., 2013. Modeling microalgal growth in an airlift-driven raceway reactor, Bioresour. Technol. 136, 689-696.
23. Koller, M., Muhr, A., Braunegg, G., 2014. Microalgae as versatile cellular factories for valued products, Algal Research. 6, 52-63.
24. Laws, E.A., Berning, J.L., 1991. A study of the energetics and economics of microalgal mass culture with the marine chlorophyte *Tetraselmis suecica*: Implications for use of power plant stack gases, Biotechnol. Bioeng. 37, 936-947.
25. Li, S., Luo, S., Guo, R., 2013. Efficiency of CO2 fixation by microalgae in a closed raceway pond, Bioresour. Technol. 136, 267-272.
26. Mendoza, J.L., Granados, M.R., de Godos, I., Acién, F.G., Molina, E., Banks, C., Heaven, S., 2013a. Fluid-dynamic characterization of real-scale raceway reactors for microalgae production, Biomass Bioenergy. 54, 267-275.
27. Mendoza, J.L., Granados, M.R., de Godos, I., Acién, F.G., Molina, E., Heaven, S., Banks, C.J., 2013b. Oxygen transfer and evolution in microalgal culture in open raceways, Bioresour. Technol. 137, 188-195.
28. Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: A review, Water Res. 40, 2799-2815.
29. Oswald, W.J., 1991. Introduction to advanced integrated wastewater ponding systems, Water Sci. Technol. 24, 1-7.

30. Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011. Wastewater treatment high rate algal ponds for biofuel production, Bioresour. Technol. 102, 35-42.
31. Pawlowski, A., Fernández, I., Guzmán, J.L., Berenguel, M., Acién, F.G., Normey-Rico, J.E., 2014. Event-based predictive control of pH in tubular photobioreactors, Comp Chem Eng. 65, 28-39.
32. Posten, C., 2009. Design principles of photo-bioreactors for cultivation of microalgae, Eng Life Sci. 9, 165-177.
33. Putt, R., Singh, M., Chinnasamy, S., Das, K.C., 2011. An efficient system for carbonation of high-rate algae pond water to enhance CO2 mass transfer, Bioresour. Technol. 102, 3240-3245.
34. Ramanan, R., Kannan, K., Deshkar, A., Yadav, R., Chakrabarti, T., 2010. Enhanced algal CO2 sequestration through calcite deposition by *Chlorella* sp. and *Spirulina platensis* in a mini-raceway pond, Bioresour. Technol. 101, 2616-2622.
35. Richmond, A., Lichtenberg, E., Stahl, B., Vonshak, A., 1990. Quantitative assessment of the major limitations on productivity of Spirulina platensis in open raceways, J. Appl. Phycol. 2, 195-206.
36. Sompech, K., Chisti, Y., Srinophakun, T., 2012. Design of raceway ponds for producing microalgae, Biofuels. 3, 387-397.
37. Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae, J Biosci Bioeng. 101, 87-96.
38. Stengel E., Soeder C.J. 1978. Method and arrangement for optimally supplying autotrophic organisms with CO2 nutrient. Patent US 4084346 A.
39. Vonshak, A., Guy, R., 1992. Photoadaptation, photoinhibition and productivity in the blue-green alga *Spirulina platensis* grown outdoors, Plant, Cell and Environment. 13, 613-616.
40. Weissman, J.C., Goebel, R.P., Benemann, J.R., 1988. Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. Biotechnol Bioeng. 31, 336-344.

7. Table and figure captions

Table 1.- Variation in pH values (maximum and minimum), and CO_2 injection times (min) as a function of the CO_2 molar fraction in the gas flow, for experiments performed at a gas flow of 100 $l \cdot min^{-1}$, during winter and summer time.

Table 2.- Variation in pH values (maximum and minimum), and CO_2 injection times (min) as a function of gas flow, for experiments performed at CO_2 molar fractions in the gas flow of 6% and 14%, during winter and summer time.

Table 3.- Variation in carbon efficiency in the sump, carbon efficiency to biomass, and carbon losses from stripping in the channels and paddlewheel with the season of the year, CO_2 molar fraction in the gas inlet to the sump, and the gas flow rate at which the gas was supplied. Data from the variance analysis of the experimental values calculated from carbon mass balance.

Figure 1.- Schematic of the raceway reactor showing the the position of the pH probes used during the experiments. (1 before paddlewheel, 2 before sump, 3 after sump, 4 end of the right channel).

Figure 2.- Influence of the culture conditions tested on the biomass productivity of semicontinuous cultures of *Scenedesmus* sp. at 0.15 day^{-1} in the pilot-scale raceway reactor. Data obtained using a gas flow rate of 100 $L \cdot min^{-1}$ and CO_2 molar fraction of 10% in flue gas.

Figure 3.- Mass flow rate of streamed carbon for each of the experimental conditions tested during winter time. Data from semicontinuous cultures of *Scenedesmus* sp. grown in a pilot-scale raceway reactor at 0.15 day^{-1} with the on-demand injection of CO_2-air mixtures for pH control. A) Different CO_2 percentage at constant gas flow rate of 100 $l \cdot min^{-1}$, B) Different gas flow rates with constant CO_2 molar fraction of 6%, C) Different gas flow rates with constant CO_2 molar fraction of 14%.

Figure 4.- Mass flow rate of streamed carbon for each of the experimental conditions tested during the summer time. Data from semicontinuous cultures of *Scenedesmus* sp. grown in a pilot-scale raceway reactor at 0.15 day^{-1} with on-demand injection of CO_2-air mixtures for pH control. A) Different CO_2 percentage at constant gas flow rate of 100 $l \cdot min^{-1}$, B) Different gas flow rates with constant CO_2 molar fraction of 6%, C) Different gas flow rates with constant CO_2 molar fraction of 14%.

Figure 5.- Comparison between maximal carbon inlet and maximal carbon uptake to produce biomass, in the winter and summer time, along with the CO_2 molar fraction in the gas for experiments performed at a gas flow rate of 100 $l \cdot min^{-1}$.

Figure 6.- Schematic of the raceway reactor showing the position of the pH probes used during the experiments (1 before paddlewheel, 2 before sump, 3 after sump, 4 end of the right channel).

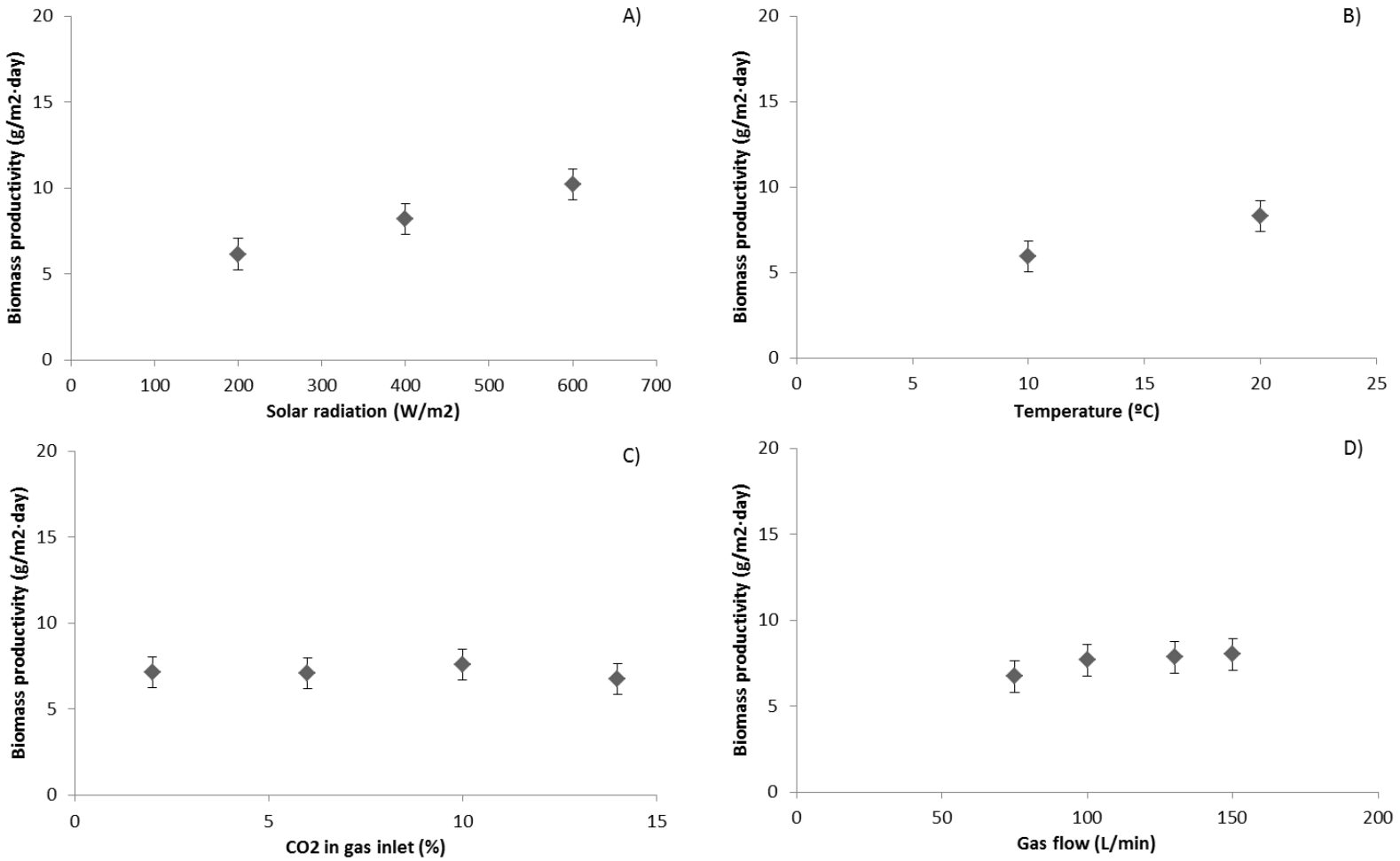

Figure 7.- Influence of the culture conditions tested on the biomass productivity of semicontinuous cultures of *Scenedesmus* sp. at 0.15 day^{-1} in the pilot-scale raceway reactor. Data obtained using a gas flow rate of 100 $L \cdot min^{-1}$ and CO_2 molar fraction of 10% in flue gas.

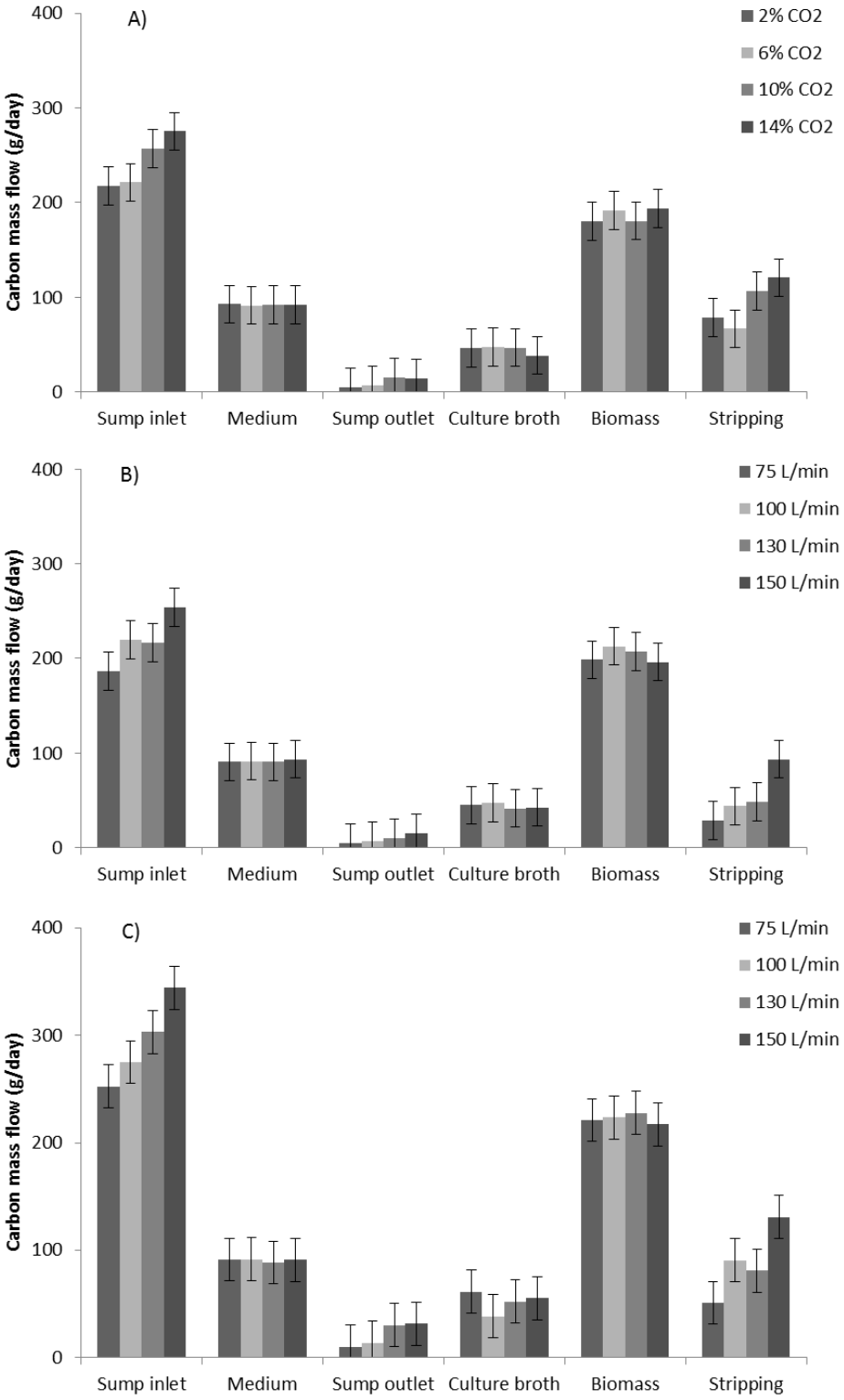

Figure 8.- Mass flow rate of streamed carbon for each of the experimental conditions tested during winter time. Data from semicontinuous cultures of *Scenedesmus* sp. grown in a pilot-scale raceway reactor at 0.15 day^{-1} with the on-demand injection of CO_2-air mixtures for pH control. A) Different CO_2 percentage at constant gas flow rate of 100 $l \cdot min^{-1}$, B) Different gas flow rates with constant CO_2 molar fraction of 6%, C) Different gas flow rates with constant CO_2 molar fraction of 14%.

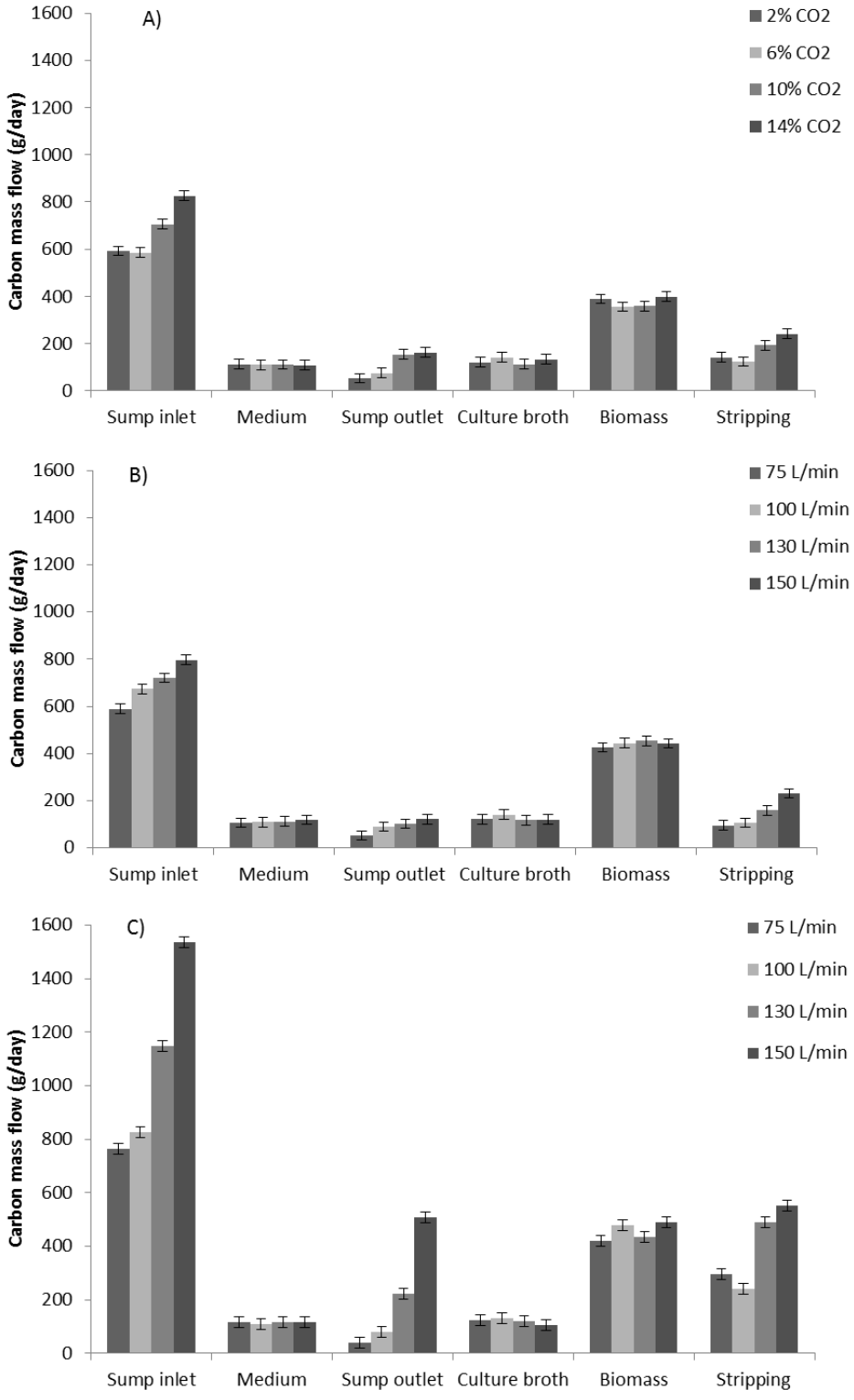

Figure 9.- Mass flow rate of streamed carbon for each of the experimental conditions tested during the summer time. Data from semicontinuous cultures of *Scenedesmus* sp. grown in a pilot-scale raceway reactor at 0.15 day^{-1} with on-demand injection of CO_2-air mixtures for pH control. A) Different CO_2 percentage at constant gas flow rate of 100 $l \cdot min^{-1}$, B) Different gas flow rates with constant CO_2 molar fraction of 6%, C) Different gas flow rates with constant CO_2 molar fraction of 14%.

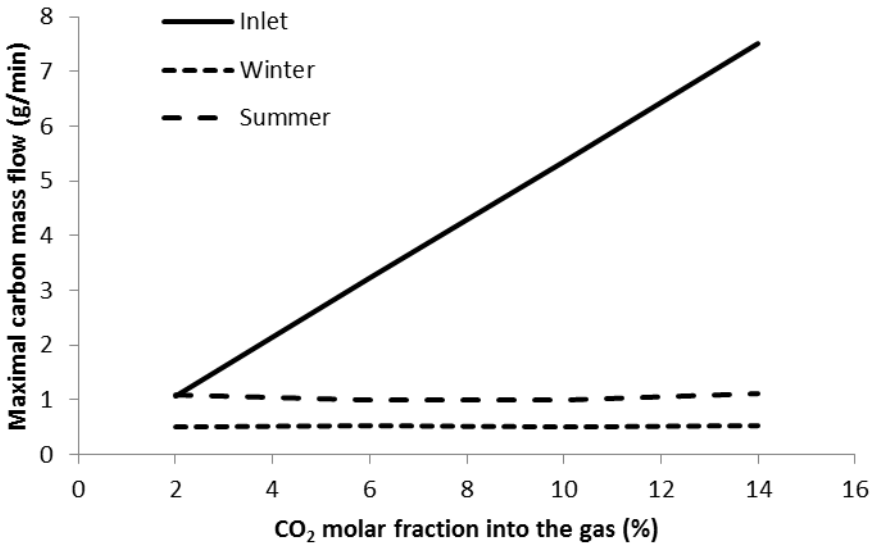

Figure 10.- Comparison between maximal carbon inlet and maximal carbon uptake to produce biomass, in the winter and summer time, along with the CO_2 molar fraction in the gas for experiments performed at a gas flow rate of 100 $l \cdot min^{-1}$.