Year-long evaluation of microalgae production in wastewater using pilot-scale raceway photobioreactors: Assessment of biomass productivity and nutrient recovery capacity

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Abstract

The production of *Scenedesmus* sp. using wastewater was validated with pilot-scale raceway photobioreactors during a complete annual cycle in Almería (Spain). Three different dilution rates (0.1, 0.2, or 0.3 day^{-1}) were evaluated. Biomass productivity was significantly affected by season (temperature and solar radiation) achieving a maximum value of 25.1 g·m^{-2}·day^{-1} when operating at a dilution rate of 0.2 day^{-1} in summer. Up to 96% of the N-NH_4+ present in the media was either assimilated by microalgae to produce biomass, converted to N-NO_3^+ by the action of nitrifying bacteria, or desorbed (stripping). Maximum nitrogen removal rates reached 4286.6 mg·m^{-2}·day^{-1} in summer. In terms of P-PO_4^{3+}, up to 75% was removed, with removal rates ranging from 147.5 mg·m^{-2}·day^{-1} in winter to 227.2 mg·m^{-2}·day^{-1} in summer. Data reported herein was used to validate the ABACO model, which demonstrated to be robust enough to accurately predict biomass productivity in pilot-scale outdoor open raceways throughout the year (R^2=0.929; 0.05). The current study demonstrates the potential of raceway reactors and *Scenedesmus* sp. to recover nutrients from unprocessed wastewater with an exceptionally high content of N-NH_4^+ at pre-industrial scale.

Keywords: Bioremediation, microalgae, biomass, photobioreactor, water reuse, circular economy.

1. Introduction

Approximately 40% of the world's population will suffer severe water stress by 2030, caused by an increased demand for water plus contamination of water resources and lack of novel technologies to reclaimed used water [1]. Water is too precious to waste. Wastewater treatment and reuse is key to achieve a sustainable future. In the EU, Regulation (EU) 2020/741 known as the Water Reuse Regulation will apply from June 2023, and is expected to boost circular approaches to water reuse in agriculture. Wastewater treatment cannot be managed by a single technology mainly because of variability in scales, type and amount of contaminants, and regional conditions involved. Conventional wastewater treatment facilities focus on both, the mechanical removal of suspended solids and the reduction of biological oxygen demand by activated sludge [2]. However, the capacity of this strategy to remove nutrients and contaminants is limited and have restrictions because of their high-energy requirements and environmental impact, mainly due to the emission of greenhouse gases. Treated wastewater can still pollute water bodies if the removal capacity of the system is inefficient.

Microalgae have been proposed as an alternative to improve nutrient removal efficiencies of conventional processes. These valuable microorganisms are photosynthetic, which means that use an inexhaustive source of energy (light) and a chemical compound we need to get rid of (carbon dioxide) to produce oxygen and biomass. The microalgae-bacteria consortia that forms in microalgae-based wastewater treatment processes is especially interesting. The oxygen produced by microalgae is used by bacteria, naturally present in wastewater, to remove pollutants [3]. In turn, bacteria produce carbon dioxide, micronutrients, siderophores, and growth stimulants that promote microalgal growth [4,5]. The composition of the consortia depends on environmental and operational conditions but, if managed properly, over 95% of the produced biomass will be microalgae [6]. Using microalgae in wastewater treatment processes could bring an important additional value besides sustainability and improved nutrient recovery, as the produced algal biomass can be further transformed into useful agricultural products [7,8]. For example, different *Scenedesmus* strains have shown biostimulant effects in plants [9] and can be produced using free or low-cost waste streams [10].

Several microalgal strains were suggested as potential candidates for wastewater treatment and these include *Chlorella* [11], *Arthrospira* [12], *Nitzchia* [13], and the above-mentioned *Scenedesmus* [4]. The latter has been widely studied because of its high tolerance to adverse conditions [14] and its ability to produce and accumulate valuable compounds such as lutein [15]. To date, most of the studies reported in the literature were carried out using bench-scale photobioreactors [10,16–19]. A limited number of studies demonstrated the robustness of *Scenedesmus* strains outdoors in pilot-scale reactors. This strains was recently produced using wastewater at pilot-scale using thin-layer cascade bioreactors [4,20]. Thin-layer cascade bioreactors are highly productive in terms of biomass production [21]. However, they have the disadvantage that because of their low culture depth, they allow to process lower volumes of water per surface area when compared to other designs. Because of the potential to process larger volumes of water and because of their low cost and ease of operation, raceway designs are the preferred option for wastewater treatment. Raceways are generally built on compacted soil covered by polymers and allow to achieve biomass concentrations around 0.5 $g \cdot L^{-1}$ with productivities varying from 9 to 25 $g \cdot m^{-2} \cdot day^{-1}$ depending on environmental and operational conditions [22]. For example, the maximum biomass productivity achieved using a 7.2 m^2 raceway reactor was 22.9 $g \cdot m^{-2} \cdot day^{-1}$ [23]. Raceways can be located outdoors or inside greenhouses, which is a common practise in the south of Europe. Although greenhouses represent and additional capital cost, they are necessary for microalgal production in some regions [24] and allow a higher control of the process [25].

Nutrient recovery from wastewater using microalgae-bacteria consortia has been widely studied. Despite promising results, most of the studies carried out to date were conducted under controlled conditions at lab-scale or outdoors but using “small” reactors for a short period of time [1]. Up-scaling microalgae production has been a major drawback for microalgae biotechnology and the yields from real production systems are still not close to theoretical values [26]. Thus, the main goal of the current study was to optimise the operation of pilot-scale raceway bioreactors and to validate the potential of the microalga *Scenedesmus* sp. to remove nutrients from wastewater during one year. Large 11.8 m^3 raceway reactors were used and operated in semi-continuous mode. Fluctuations in culture and environmental conditions can influence microbial diversity and therefore, depuration efficiency [27]. The current study will also discuss the effect of environmental conditions namely temperature and solar radiation on the performance of the system. A secondary aim of the manuscript was to validate the ABACO model, a robust tool that can be used to predict biomass productivity as a function of environmental (solar radiation, temperature, pH, and dissolved oxygen concentration) and biological parameters (microalga growth rate, nutrient saturation coefficients, and nutrient inhibition coefficients, among others) [28]. The validation of models using data generated using large reactors located outdoors is of key importance as this allows to predict the suitability of models for predicting industrial processes.

2. Materials and methods

2.1 Selected microorganism

The strain selected was *Scenedesmus* sp. because it is a fast-growing and highly-productive strain that is particularly adapted to stressful and environmental conditions of the region. The microalga *Scenedesmus* sp. (CCAP 276/24) was obtained from the culture collection of the Department of Chemical Engineering of the University of Almería in Spain. The inocula were prepared using controlled 5 L photobioreactors at laboratory scale that were further up-scaled using 80 L pH-controlled bubble columns photobioreactors located outdoors as described previously [21]. The culture medium was prepared using 0.90 $g \cdot L^{-1}$ $NaNO_3$, 0.18 $g \cdot L^{-1}$ $MgSO_4$, 0.14 $g \cdot L^{-1}$ K_2PO_4, and 0.03 $g \cdot L^{-1}$ of Karentol® (Kenogard, Spain), which is a commercial solid mixture of micronutrients.

2.2 Photobioreactor and operation conditions

The photobioreactors used for wastewater treatment and *Scenedesmus* sp. production were three raceways placed inside a greenhouse in the facilities of the Institute for Agricultural and Fisheries Research and Training (IFAPA) in Almería, Spain. All three raceway reactors were identical, which allowed to conduct the experiments in triplicate. The operating volume of the reactors was 11.8 m^3 and their surface 80 m^2. The reactors were inoculated with a 10% of their total volume with *Scenedesmus* sp. culture and the reactors were filled up until the desired culture depth (0.135 m) using wastewater from primary treatment. Experiments were conducted on semi-continuous mode at dilution rates of 0.1, 0.2, or 0.3 day^{-1} during the four seasons of the year, which allowed to assess different ranges of temperature and incident irradiance (Figure 1). All the studied dilution rates were assessed at least three times per season. The reactors were operated in semi-continuous mode until the volume of the reactor was replace at least twice. After this period, 90% of the culture was removed and the 10% remaining was used as the inoculum for the following replicate. The pH, temperature, and dissolved oxygen concentration of the culture were measured using 5083T and 5120 probes (Crison Instruments, Spain) connected to an MM44 control-transmitter unit (Crison Instruments, Spain) and Labview data acquisition software (National Instruments, US) providing complete monitoring of the facilities. The pH was controlled by on-demand injection of carbon dioxide and evaporation was compensated by daily addition of fresh water. The culture was harvested using an industrial SSD 6-06-007 GEA separator (GEA Westfalia Separator Group, Oelde, Germany).

2.3 Wastewater composition

The wastewater used was domestic wastewater collected from the University of Almería in Spain. Domestic wastewater includes blackwater (wastewater from toilets) and greywater (water used for washing, bathing and kitchen). Because of the location where the wastewater was generated, most of the wastewater consisted of blackwater: approximately 20,000 people with only a limited number of kitchens, showers, and washing machines. The wastewater used was not subjected to any conventional depuration treatment besides removal of solids and for these reasons, the nutrient and bacterial load of the inlets were exceptionally high when compared to conventional urban wastewater.

2.4 Analytical determinations

2.4.1 Culture parameters

Biomass concentration was calculated by dry weight by filtering 100 mL of culture through 1 µm filters followed by drying in an oven at 80 °C for 24 h. Biomass productivity was calculated as the product of biomass concentration and the dilution rate, which was wither 0.1, 0.2, or 0.3 day^{-1}. The chlorophyll fluorescence ratio (F_v/F_m) was checked daily with an AquaPen AP 100 fluorometer (Photon System Instruments, Czech Republic). Absorbance at 400-800 nm was daily measured using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Spain). The extinction coefficient (k_a) and the average irradiance inside the culture (I_{av}) were calculated as described previously [21] using the equations:

$$k_a = \frac{Abs}{C_b \cdot p}$$

where Abs is the above-mentioned absorbance, C_b is the biomass concentration (g·m^{-3}), and p is the cuvettes' light path (0.01 m), and

$$I_{av} = \frac{I_0}{k_a \cdot C_b \cdot p} \cdot (1 - e^{-k_a \cdot c_b \cdot p})$$

where I_0 is the irradiance at the surface of the culture (µE·m^{-2}·s^{-1}), k_a is the extinction coefficient (m^2·g^{-1}), C_b is the biomass concentration (g·m^{-3}), and p is the light path inside the reactor (m).

2.4.2 Nutrient removal

The concentration of ammonium, nitrates, and phosphates in the inlet and outlet of the raceways was measured using standard official methods approved by the Spanish Ministry of Agriculture as described previously [4]. Briefly, phosphorous and nitrates were measured spectrophotometrically through the phospho-vanado-molybdate complex and by measuring the absorbance at 220-s75 nm using Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Spain). The concentration of ammonium was determined using the Nessler reactive method.

2.5 Process modelling

Experimental results were compared to those predicted by the ABACO model [28] using MATLAB (Mathworks, MA, USA). This tool allows to simulate the dynamics of different components of the system and to predict daily biomass productivity as well as the approximate composition of the microalgae-bacteria consortia in terms of microalgae, heterotrophic bacteria, and nitrifying bacteria. The specific growth rate of the microalga (μ_{ALG}; day^{-1}) was calculated as a function of light availability inside the reactor and modified by different variables using the equation:

$$\mu_{ALG} = [\mu_{ALG}(I_{AV}) \cdot \overline{\mu_{ALG}}(T) \cdot \overline{\mu_{ALG}}(pH) \cdot \overline{\mu_{ALG}}(DO) \cdot \overline{\mu_{ALG}}(CO_2) \cdot \overline{\mu_{ALG}}(N) \cdot \overline{\mu_{ALG}}(P)] - m_{ALG}$$

where $\mu_{ALG}(I_{AV})$ is the specific growth rate as a function of the light availability inside the reactor (day^{-1}), and $\overline{\mu_{ALG}}(T)$, $\overline{\mu_{ALG}}(pH)$, $\overline{\mu_{ALG}}(DO)$, and $\overline{\mu_{ALG}}(CO_2)$ represent the influence of temperature, pH, dissolved oxygen concentration and carbon dioxide. The influence of nitrogen and phosphorus availability was also considered and are represented as $\overline{\mu_{ALG}}(N)$ and $\overline{\mu_{ALG}}(P)$ respectively.

Biomass productivity was calculated from the oxygen productivity that was obtained using the formula:

$$\begin{aligned} P_{O_2} = P_{O_2}ALG\big([I] \cdot \overline{[T]} \cdot \overline{[pH]} \cdot \overline{[DO]} \cdot \overline{[CO_2]} \cdot \overline{[N]} \cdot \overline{[P]}\big) - R_{O_2}HET \\ \cdot \big([I] \cdot \overline{[T]} \cdot \overline{[pH]} \cdot \overline{[DO]} \cdot \overline{[N]} \cdot \overline{[P]}\big) - R_{O_2}NIT \\ \cdot \big([I] \cdot \overline{[T]} \cdot \overline{[pH]} \cdot \overline{[DO]} \cdot \overline{[CO_2]} \cdot \overline{[N]} \cdot \overline{[P]}\big) \end{aligned}$$

where P_{O_2} is the oxygen productivity (g·m^{-2}·day^{-1}), $P_{O_2}ALG$ is the oxygen produced during photosynthesis by microalgae (g·m^{-2}·day^{-1}), $R_{O_2}HET$ is the oxygen consumed by heterotrophic bacteria during respiration (g·m^{-2}·day^{-1}), and $R_{O_2}NIT$ is the oxygen consumed by nitrifying bacteria during respiration (g·m^{-2}·day^{-1}). How the solar radiation, pH, dissolved oxygen concentration, concentration of carbon dioxide, nitrogen, and phosphorus affect the specific growth rate of the culture as well as oxygen productivity is describes in previous reports [28,29]. The processes was modelled in triplicate and compared against experimental data obtained when operating the reactors in semi-continuous mode with a dilution rate of 0.2 day^{-1}.

2.6 Statistical analysis

Data shown are the mean values of three independent determinations ± standard deviation (SD). Differences between determinations were analysed using analysis of variance with JMP 13 (SAS Institute Inc., USA). A Tukey pairwise comparison of the means was carried out to identify where sample differences occurred with a criterion of $p<0.05$. A bivariate Pearson's' correlation analysis was conducted to identify relationships between different variables.

3. Results and discussion

3.1 Effect of environmental conditions on microalgal cultures

Raceways are the most widespread bioreactors used for microalgae production mainly because of their flexibility, ease of operation and scale-up, low construction costs, and low energy consumption for mixing, which is in the order of 4 W·m^{-3} [30]. Besides their lower costs, raceways are especially interesting for wastewater treatment because of their lower surface-to-volume ratio (when compared to other more productive designs such as thin-layer cascade photobioreactors) which allows to process larger volumes of water per surface area [31]. When produced outdoors, microalgal growth depends largely on environmental conditions, namely temperature and solar radiation that, in turn, depend on the location of the reactor. Figure 1 shows the maximum, minimum, and average temperature and solar radiation inside the greenhouse where the raceways were located. Maximum and minimum values shown in Figure 1 and Figure 2 are the average of all the daily maximum and minimum values measured during the experiment. Measurements were taken every 1 s and are available at the database of the H2020 SABANA project at http://sabana.ual.es/. As expected, higher temperatures and solar radiations were measured in summer ($p<0.05$), reaching an average maximum daily temperature of 33.7 °C. Minimum temperature values of 8.2 °C were achieved in winter. Average temperature throughout the year varied from 14.2 to 26.1 °C.

It is important to monitor environmental conditions as these have a striking effect on the conditions of the culture, namely temperature, dissolved oxygen concentration, and average irradiance inside the culture. In terms of culture temperature, values were in line with those measured inside the greenhouse where the reactors were located. Maximum and minimum culture temperature values were an average of 1.9 and 1.7 °C higher than the temperature inside the greenhouse ($p<0.05$) suggesting a slight but significant overheating of the culture. Culture temperature was higher in spring and summer, reaching an average maximum value of 34.0 ± 0.9 °C (Figure 2). This value is relatively high and could be lethal for some strains, demonstrating the importance of selecting a robust microalga such as the *Scenedesmus* strain used in the current study. The selected strain can withstand up to 48 °C [14]. It is important to highlight that this was the maximum daily temperature reached during the experiment and was only maintained during a short period of time – average culture temperature in summer was 28.0 ± 1.2 °C.

Maximum oxygen saturation values ranged between 150 and 200% and were achieved during summer and autumn ($p<0.05$). Higher dissolved oxygen concentrations were caused by a higher biomass productivity and therefore to a higher production of oxygen through photosynthesis. Controlling dissolved oxygen is of key importance as previous reports demonstrated that higher dissolved oxygen concentrations led to limited microalgal growth, although this effect was observed in thin-layer cascade reactors that are more productive than raceways [21].

Photosynthetic efficiency depends largely on light availability inside the culture, which is the most important factor when producing any photosynthetic organism. The average irradiance inside the culture reached a maximum value of 230.3 ± 16.3 $\mu E \cdot m^{-2} \cdot s^{-1}$ in summer ($p<0.05$). No differences were observed in the average irradiance inside the culture in spring and autumn, while lower values were obtained in winter because of a lower solar radiation ($p<0.05$). Overall, values were lower than those calculated when producing the same strain in the same location using freshwater [21], probably caused by a slight turbidity of primary wastewater.

The pH of the culture could also play an important role affecting the performance of the system. However, in the current study the pH of the culture was continuously online monitored and controlled at 8.0 ± 1.0 by on-demand injection of carbon dioxide. Results reported herein were comparable to those described previously by our research group when producing the same microalga in the same reactors but using freshwater instead of wastewater [21]. This means that the effect of environmental conditions on the pH, temperature, and dissolved oxygen concentration of the cultures is independent on the type of water used, demonstrating the huge potential of microalgae to process wastewater and achieve high biomass productivities. Moreover, results demonstrate the robustness of the strain used and of the process which was not affected, in terms of biomass productivity, by the type of water used.

3.2 Biomass productivity

Besides environmental conditions, the most relevant operational conditions in open large-scale raceways are the dilution rate and culture depth. The latter was kept constant at 0.135 m, which is the optimum height of these three reactors to increase light availability and the performance of the cells while ensuring adequate circulation of the culture through the reactor. The dilution rate represents the amount of culture that is daily harvested and substituted with fresh culture medium, in this case wastewater. To optimise biomass productivity, it is important to operate using the dilution rate that allows to maximise light and nutrient availability. In the current study, three different dilution rates (0.1-0.3 day^{-1}) were studied. The effect of operating at different dilution rates throughout the year is represented in Figure 3. Briefly, operating at a dilution rate of 0.2 day^{-1} led to higher biomass concentration and productivity in all four seasons, especially in summer ($p<0.05$). Higher biomass productivity in summer can be attributed to higher light availability and more appropriate temperatures for *Scenedesmus* sp. Results were in line with those reported previously demonstrating that the optimum dilution rate for raceway reactors in the south of Spain is 0.2 day^{-1} [21]. Similar *Scenedesmus* sp. productivity values (24 $g \cdot m^{-2} \cdot day^{-1}$) were reported previously at pilot-scale using a 32 m^2 raceway reactor and centrate from anaerobic digestion as the sole nutrient source [32]. Results were also comparable to those obtained outdoors using a 7.2 m^2 raceway reactor (22.9 $g \cdot m^{-2} \cdot day^{-1}$) [23].

Values of chlorophyll fluorescence, shown in Figure 3, represent a non-invasive measurement of photosystem II activity [33]. The optimal F_v/F_m value for any microalgal strain is 0.6-0.7, and lower values indicate that the culture is subjected to certain stress condition (for example, excess of light, lack of nutrients, presence of toxins or heavy metals, or inadequate pH or temperature). In the current study, F_v/F_m values varied between 0.55 and 0.70 and were especially higher when operating at a dilution rate of 0.2 day^{-1} ($p<0.05$). This can be attributed to higher light and nutrient availability when operating using this conditions and correlates well with the observed higher values of biomass productivity. Because of the low oxygen saturation values of the culture, effectiveness of the pH control, and temperatures achieved, lower F_v/F_m values could be caused by the composition of the wastewater that was especially rich in N-NH_4^+. Indeed, ammonia demonstrated toxic effects on *Scenedesmus* sp. previously [34]. Overall, the F_v/F_m values reported herein suggest that when the reactors were operated at 0.2 day^{-1}, microalgae were not subjected to stress conditions and that the environmental conditions of the region as well as the temperature, pH, dissolved oxygen concentration, and light availability of the reactors were suitable to produce *Scenedesmus* sp. at pre-industrial scale throughout the year.

3.3 Nutrient removal

The major goal of the current study, besides microalgae production, was to recover nutrients from primary wastewater and to allow its safe disposal or reutilisation. The composition of wastewaters varies with sources and has been summarised previously [17]. In the current study, the composition of the wastewater used was not constant and was especially rich in N-NH_4^+ and P-PO_4^{3-}, both easily assimilated by microalgae to produce biomass. Not only N-NH_4^+ but also N-NO_3^+ contribute to the total nitrogen content of wastewater. Results shown in Figure 4 are those obtained when operating at a dilution rate of 0.2 day^{-1}, as these conditions led to higher biomass productivity. Wastewater treatment using *Scenedesmus* sp. led to a significant decrease in the content of N-NH_4^+, which varied from 168.3 ± 12.5 to 210.6 ± 15.2 $mg \cdot L^{-1}$ in the inlet to 8.8 ± 1.5 to 18.6 ± 3.6 $mg \cdot L^{-1}$ in the outlet representing an 89.9-95.5% removal (Figure 4). Results suggest that almost all the N-NH_4+ present in the media was assimilated by microalgae to produce biomass. However, the content of N-NO_3^+ was higher in the outlet than in the inlet ($p<0.05$), suggesting that part of the N-NH_4^+ eliminated in the reactor was converted to N-NO_3^+ by the action of nitrifying bacteria. Nitrification is the biological oxidation of ammonia to nitrite followed by the oxidation of nitrite to nitrate by ammonia-oxidising and nitrite-oxidising bacteria respectively [35]. Nitrification had an important effect on total nitrogen removal, probably caused by the low light utilisation of current raceway designs when compared to other reactor designs [36], which facilitates the growth of non-photosynthetic microorganisms. A recent study concluded that high starting N-NH_4^+ loads promote the activity of ammonia oxidising bacteria and therefore, the formation of nitrites [37]. Total inorganic nitrogen removals varied between 18.3 ± 2.5 $mg \cdot L^{-1} \cdot day^{-1}$ in winter to 29.1 ± 3.7 $mg \cdot L^{-1} \cdot day^{-1}$ in summer, representing a removal of approximately 50 and 80% respectively. Results were in line with those reported for centrate treatment using the marine microalga *Nannochloropsis gaditana* [38]. Removal values reported herein were higher than those obtained in a previous report where a consortium of *Chlorella* sp. and *Scenedesmus* sp. was used to process anaerobically digested piggery effluent [39]. In that study, the N-NH_4^+ removal rate was around 10 $mg \cdot L^{-1} \cdot day^{-1}$. It is likely that the lower biomass productivity of the 1500 L raceway reactor used in that study (4.2 $g \cdot m^{-2} \cdot day^{-1}$) was the reason for the lower nitrogen removal. Indeed, thin-layer cascade reactors that are highly productive allow higher areal nitrogen removal rates – but allow to process less wastewater per surface area [20]. Higher removal efficiencies (85-

98%) were observed when processing domestic wastewater using the microalga *Chlorella variabilis* TH03 and 3.7 m^2 raceway reactors [40]. However, in that study, the concentration of nitrogen in the inlets was lower (40-70 $mg \cdot L^{-1}$) and therefore the productivity of the reactors ranged within 11 and 15 $g \cdot m^{-2} \cdot day^{-1}$. Nitrogen removal vas positively correlated to biomass productivity (R^2=0.864; 0.05) and average irradiance inside the culture (R^2=0.903; 0.05) which demonstrates that part of the nitrogen removed from the water was used for the production of biomass. Indeed, approximately 20-30% of the inorganic nitrogen present in the wastewater was used for biomass production (Figure 5). Results shown in Figure 5 also demonstrated that a large percentage of the nitrogen ($N\text{-}NH_4^+$) that entered the reactor was “lost” by stripping (desorption). These results were in line with those reported in a different study conducted in the same region using thin-layer cascade reactors [20].

In terms of phosphorous removal, values ranged from 147.5 ± 16.2 $mg \cdot m^{-2} \cdot day^{-1}$ in winter to 227.2 ± 27.7 $mg \cdot m^{-2} \cdot day^{-1}$ in summer (Figure 4). Approximately 50-75% of the $P\text{-}PO_4^{3-}$ that entered the reactors was effectively removed by the microalgae-bacteria consortia. These values were lower than those reported in a previous report that demonstrated total phosphorus removal values higher than 90% [41]. Similar results were obtained in a different study with total phosphorus removal values greater than 80% [42]. In these study, the waste streams were processed using different microalgal strains (*Chlorella* sp.) and laboratory-scale photobioreactors operated using artificial and controlled illumination. When processing wastewater in outdoor conditions, the performance of the system is subjected to environmental conditions and therefore the control of the process becomes a challenge. Recently, total phosphorus removal values close to 80% were reported when producing *Scenedesmus* sp. in 7.2 m^2 raceway reactors [38]. In that study, the microalga utilised was *Nannochloropsis gaditana* and the biomass productivity of the reactor was around 30 $g \cdot m^{-2} \cdot day^{-1}$. In the current study, $P\text{-}PO_4^{3-}$ removal was positively correlated to average irradiance (R^2=0.941; 0.05) and average temperature of the culture (R^2=0.739; 0.05) and was therefore highly influenced by season. A positive correlation was also found between $P\text{-}PO_4^{3-}$ removal and biomass productivity (R^2=0.955; 0.05) suggesting that the P-PO_4^{3-} removed from the wastewater was used for the production of biomass. Indeed, a mass balance conducted to the reactor demonstrated that most of the phosphorous that entered the reactor was either used for biomass production (50-80%) or left unused (20-50%) – Figure 5. No phosphorous precipitation was observed, demonstrating the good pH control of the system. Removal of both nitrogen and phosphorous from wastewater is of key importance as both of them are the main nutrients of concern in eutrophication and are limiting factors in most growth scenarios [43].

3.4 Process up-scaling and simulation

A secondary aim of the current paper was to validate the ABACO model previously described by our research group [28]. The validation of models using data generated under outdoor environmental conditions and pre-commercial scale is of key importance as data generated in the lab is not always representative of real industrial processes. The ABACO model was developed considering the main microalgal and bacterial processes that simultaneously occur in microalgae-based wastewater treatment processes. Light availability is the most important factor affecting microalgal growth [44], while the rest of environmental parameters (temperature, pH, and dissolved oxygen) and nutritional parameters (CO_2, N-NH_4^+, N-NO_3^- , P-PO_4^{3-}) provide a normalized effect in the model (0-1) and modify the productivity calculated based on the light incidence [28]. The heterotrophic growth is modelled as the product of maximum growth rate and switching functions for environmental parameters such as temperature, pH, and dissolved oxygen along with the biodegradable soluble organic matter, ammonium nitrogen, and phosphate phosphorous. The growth of nitrifying bacteria, which perform the oxidation of ammonium to nitrate, is determined by environmental parameters and the nutrients present in the culture such as CO_2, N-NH_4^+, and P-PO_4^{3-}. All these commonly measured variables (irradiance, dissolved oxygen, pH, and temperature) in the raceways were used as inputs for the model, in addition to the concentration of major components and nutrients involved into the biological process. The seasonal biomass productivity estimated using the ABACO model is shown in Figure 6. Although the model was developed using indoor photobioreactors, it demonstrated to be robust enough to accurately predict biomass productivity in pilot-scale outdoor open raceways. Both experimental and predicted biomass productivities were in good agreement (R^2=0.929; 0.05).

As highlighted before, domestic wastewater includes blackwater (wastewater from toilets) and greywater (water used for washing, bathing, and kitchen). Wastewater used in the current study was that generated at the University of Almeria, most of it blackwater because of a very limited number of sinks, showers, washing machines, etc. when compared to a city. The nitrogen and phosphorous content of the wastewater used in the current study doubled that of conventional primary wastewater, with nitrogen and phosphorous contents of approximately 80 and 12 $mg \cdot L^{-1}$ in Almeria [4]. For this reason, the concentration of these compounds in the outlets exceeded the maximum discharge limits of Spanish regulations set at 1-2 $mg \cdot L^{-1}$ of phosphorous and 10-15 of $mg \cdot L^{-1}$ of nitrogen [45]. Up-scaling the results to a theoretical 10,000 m^2 raceway reactor the process would allow the removal of 10.6 and 0.5 tonnes of nitrogen and phosphorous per year and produce 56.5 tonnes of valuable biomass. *Scenedesmus* strains are also interesting sources for valuable biomolecules such a lutein, which could be used for food applications [46]. However, as the biomass obtained in the current study was produced using wastewater, it cannot be used as human food. The produced biomass of *Scenedesmus* sp. could be used as a feed additive [47] or as a biostimulant in agriculture [9] – agricultural field trials are ongoing. Using a 10,000 m^2 raceway reactor would also allow to process 81.0 m^3 of wastewater. These data were calculated considering 300 days of production and 35 days dedicated to up-scaling, cleaning, and other operations.

Overall, results suggested that *Scenedesmus* sp. could be used as a pre-treatment in the processing of wastewater with very high nitrogen and phosphorous contents. This strategy could be used as the unique wastewater treatment in wastewater with a lower nitrogen and phosphorous content. To improve the nutrient removal efficiency, the outlet of the reactors could be recirculated into the reactor, or membranes could be used to separate the hydraulic retention time from the cellular retention time, thus maximising nutrient recoveries. Studies on the use of membranes attached to raceway reactors and water recirculation are ongoing.

4. Conclusions

Wastewater was processed during one complete year demonstrating the robustness of microalgae based wastewater treatment processes. Results reported herein demonstrate that the microalga *Scenedesmus* sp. can be produced at pilot-scale using primary wastewater as the sole nutrient source. Biomass productivity was comparable to that obtained when operating using freshwater and commercial fertilisers and nutrients, which would allow to produce biomass at a lower price. Most of the nitrogen and phosphorous present in the wastewater was assimilated by both microalgae and bacteria to produce biomass, although some of the ammonia was used by nitrifying bacteria to produce nitrates. Moreover, the data generated in the current study was used to validate the ABACO model, which demonstrated its potential for being used to simulate biomass productivity in pilot-scale raceways. Up-scaling microalgal processes is necessary to validate the potential of these valuable microorganisms to being used at industrial scale. The current study was carried out using 11.8 m^3 raceway reactors. Future studies will demonstrate the economic benefit of producing microalgae using wastewater and assess the potential utilisation of the produced biomass as a source for extracts with biostimulant properties in agriculture.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study

CRediT autor statement

Ainoa Morillas-España: Investigation, Writing – original draft; **Tomas Lafarga:** Formal analysis, supervision, Writing – review & editing; **Ana Sánchez-Zurano:** Formal analysis; **Francisco Gabriel Acién-Fernández:** Supervision, Funding acquisition; **Enrique Rodríguez-Miranda:** Formal analysis; **Cintia Gómez-Serrano:** Investigation, Formal analysis; **Cynthia Victoria González-López:** Supervision, Funding acquisition.

Figure legends

Figure 1. Seasonal variation of (A) solar radiation and (B) temperature inside the greenhouse. Average values are the average of all the measurements taken per day. Maximum and minimum values are the maximum and minimum value recorded every day. Results shown are the average of all the average, maximum and minimum daily measurements taken during the assays ± SD.

Figure 2. Seasonal variation of (A) temperature and (B) oxygen saturation in the culture. Average values are the average of all the measurements taken per day. Maximum and minimum values are the maximum and minimum value recorded every day. Results shown are the average of all the average, maximum and minimum daily measurements taken during the assays ± SD.

Figure 3. Effect of season and dilution rate on (A) biomass concentration, (B) biomass productivity and (C) chlorophyll fluorescence. Values shown are the average of three independent determinations ± SD.

Figure 4. Inlet and outlet concentration of (A) ammonia, (B) nitrates, and (C) phosphates and (D) nitrogen and (E) phosphorous removal. Values shown are the average of three independent determinations ± SD.

Figure 5. Mass balance of (A) nitrogen and (B) phosphorous when the reactors were operated with a dilution rate of 0.2 day^{-1}. Calculations were made assuming a nitrogen and phosphorus content of the biomass of 10 and 1%, respectively. Values shown are the average of three independent determinations ± SD.

Figure 6. Average productivity simulated using the ABACO model. Values shown are the average of three independent determinations/simulations ± SD.

Figure 1

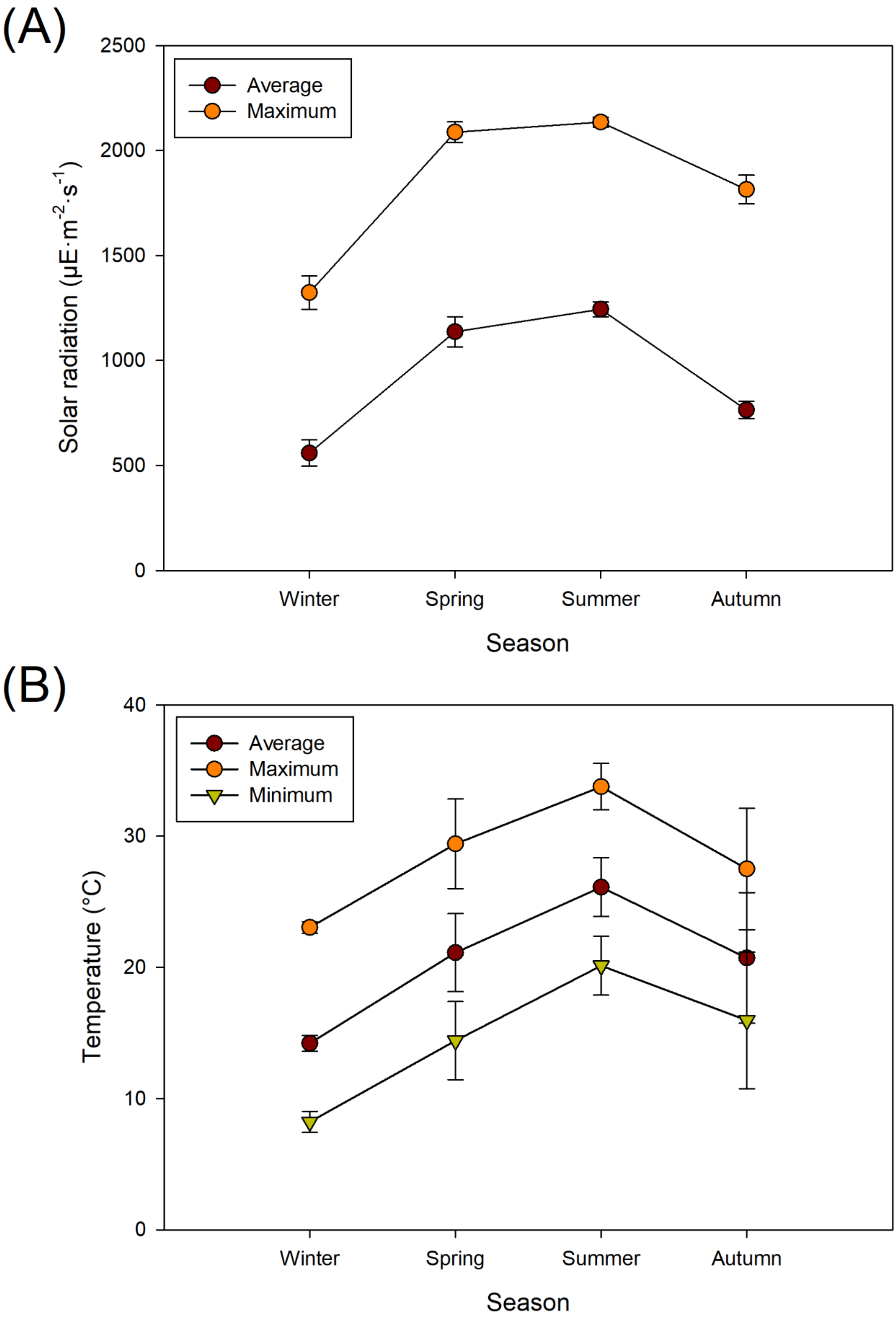

(A)
Average
Maximum
Solar radiation (μE·m^{-2}·s^{-1})
2500
2000
1500
1000
500
0
Winter
Spring
Summer
Autumn
Season
(B)
Average
Maximum
Minimum
Temperature (°C)
40
30
20
10
0
Winter
Spring
Summer
Autumn
Season

Figure 2

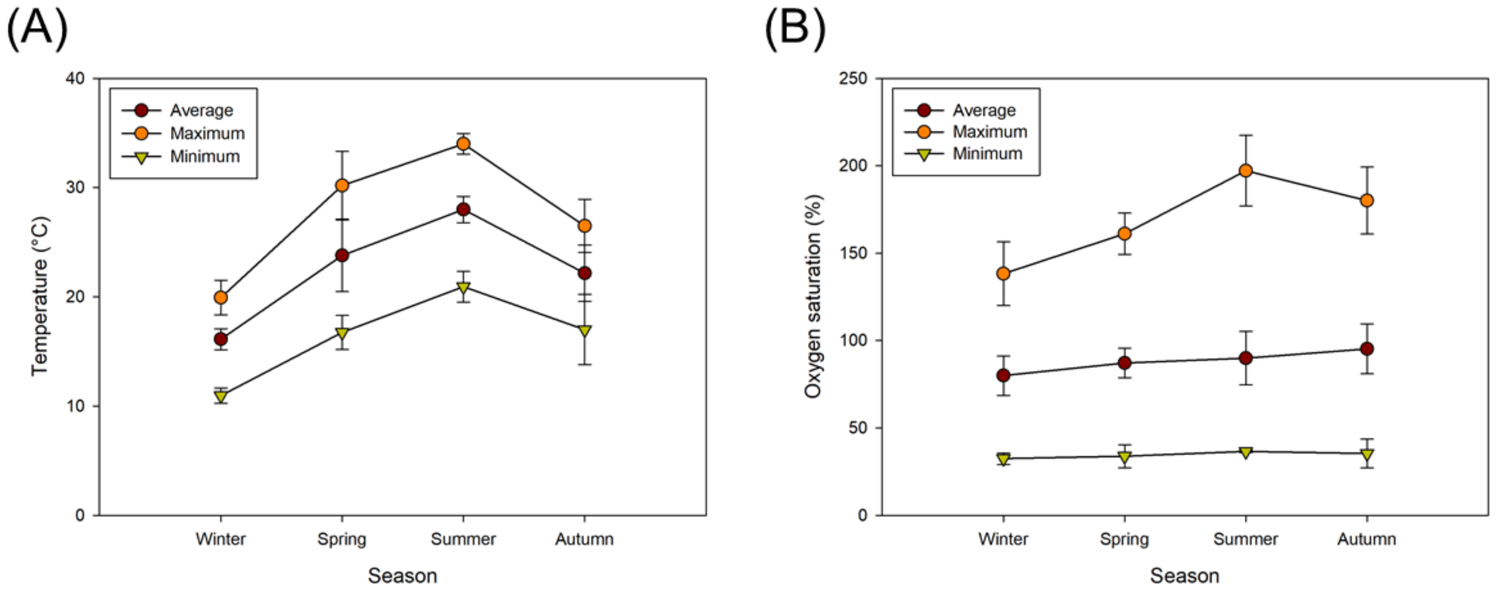

(A)
Average
Maximum
Minimum
Temperature (°C)
0
10
20
30
40
Winter
Spring
Summer
Autumn
Season
(B)
Average
Maximum
Minimum
Oxygen saturation (%)
0
50
100
150
200
250
Winter
Spring
Summer
Autumn
Season

Figure 3

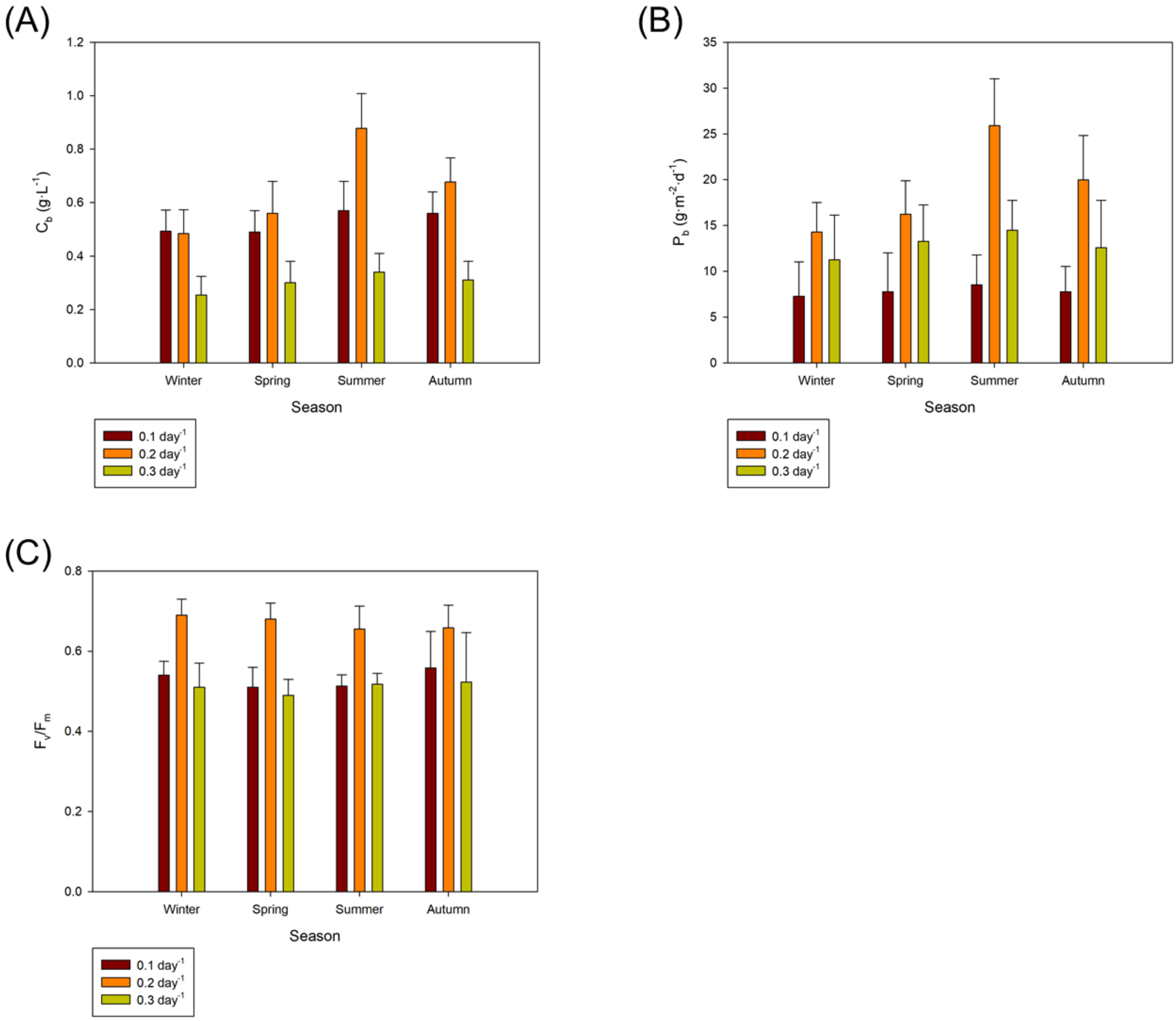

(A)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C_b (g·L^{-1})
Winter
Spring
Summer
Autumn
Season
0.1 day^{-1}
0.2 day^{-1}
0.3 day^{-1}
(B)
35
30
25
20
15
10
5
0
P_b (g·m^{-2}·d^{-1})
Winter
Spring
Summer
Autumn
Season
0.1 day^{-1}
0.2 day^{-1}
0.3 day^{-1}
(C)
0.8
0.6
0.4
0.2
0.0
F_v/F_m
Winter
Spring
Summer
Autumn
Season
0.1 day^{-1}
0.2 day^{-1}
0.3 day^{-1}

Figure 4

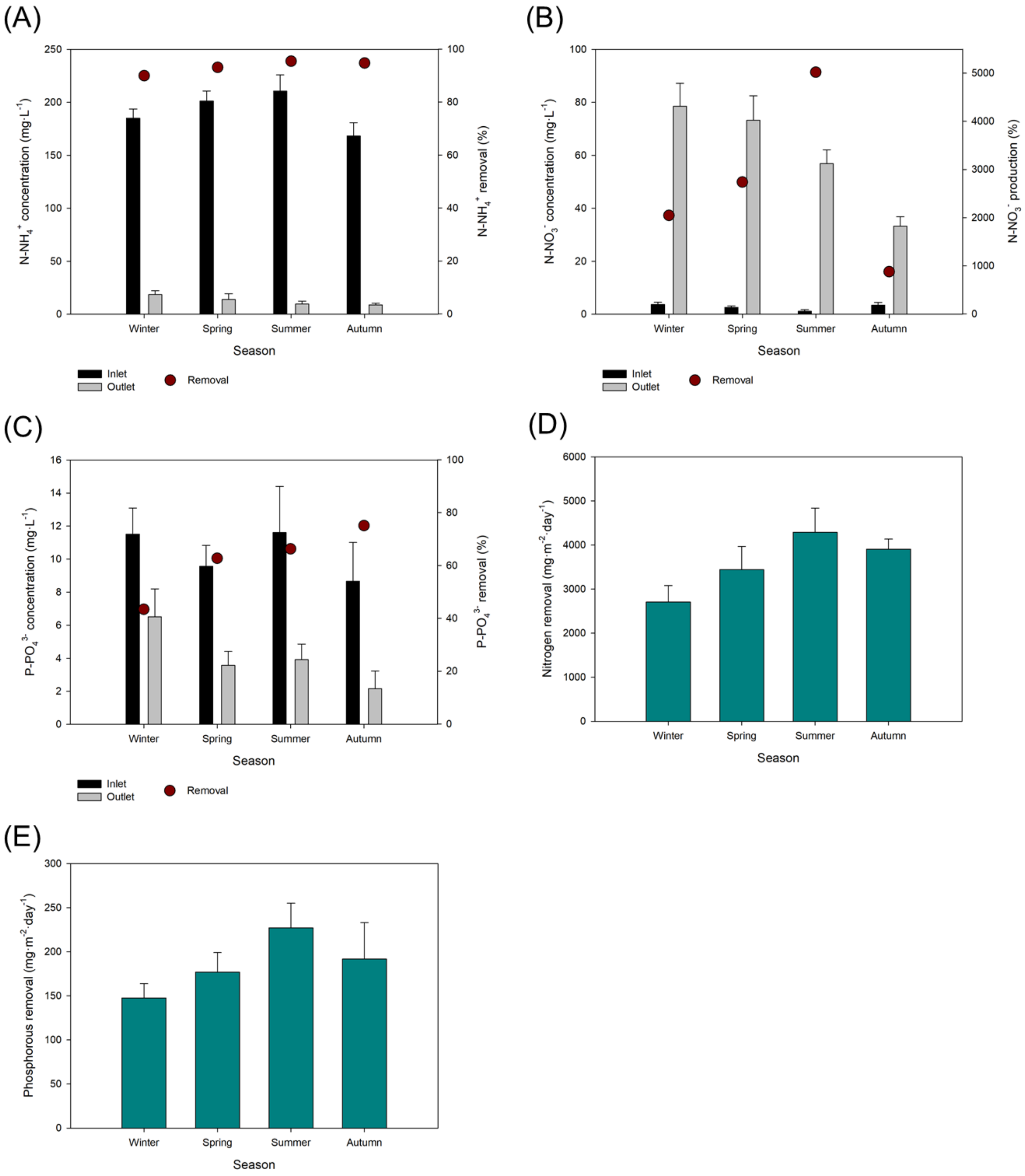
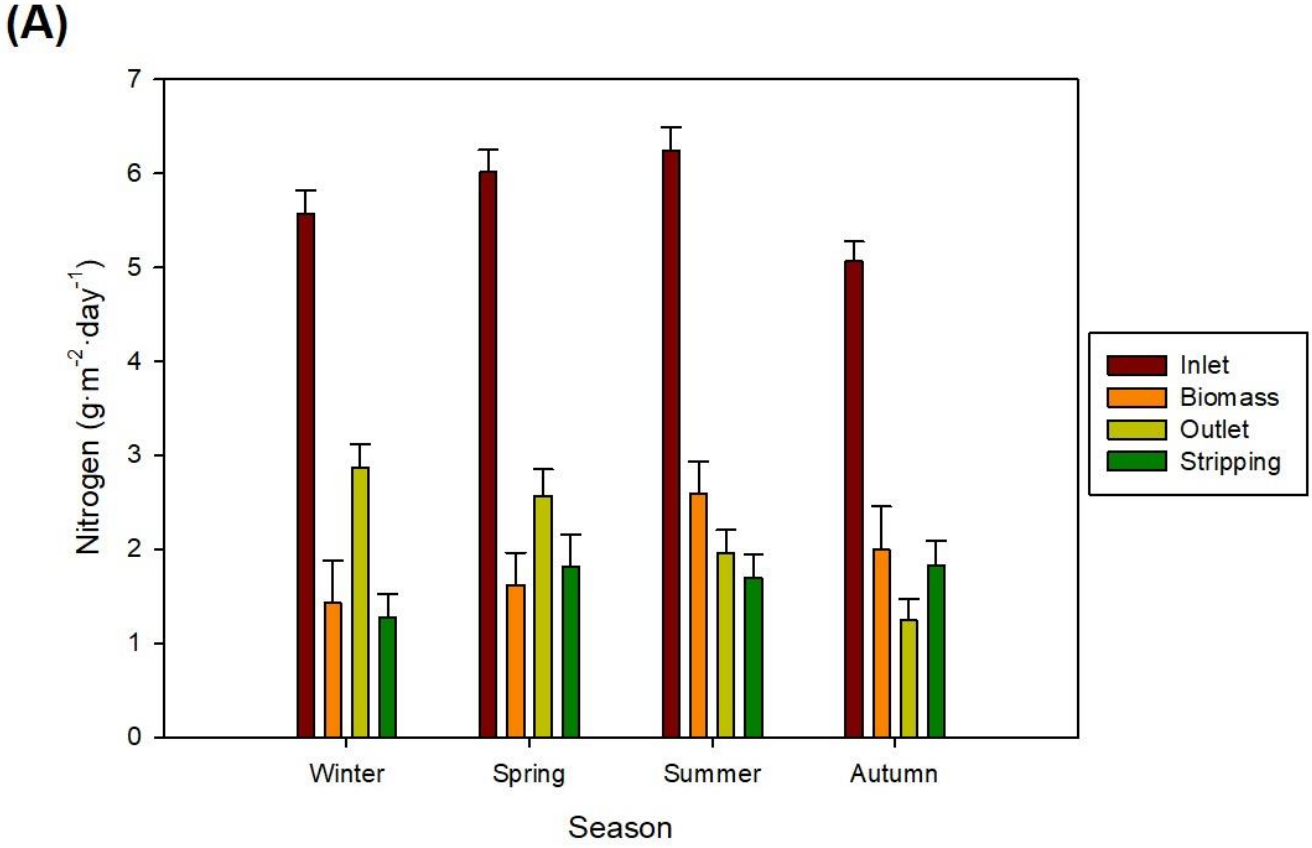

(A)
N-NH4+ concentration (mg·L-1)
N-NH4+ removal (%)
250
200
150
100
50
0
100
80
60
40
20
0
Winter
Spring
Summer
Autumn
Season
Inlet
Outlet
Removal
(B)
N-NO3- concentration (mg·L-1)
N-NO3- production (%)
100
80
60
40
20
0
5000
4000
3000
2000
1000
0
Winter
Spring
Summer
Autumn
Season
Inlet
Outlet
Removal
(C)
P-PO43- concentration (mg·L-1)
P-PO43- removal (%)
16
14
12
10
8
6
4
2
0
100
80
60
40
20
0
Winter
Spring
Summer
Autumn
Season
Inlet
Outlet
Removal
(D)
Nitrogen removal (mg·m-2·day-1)
6000
5000
4000
3000
2000
1000
0
Winter
Spring
Summer
Autumn
Season
(E)
Phosphorous removal (mg·m-2·day-1)
300
250
200
150
100
50
0
Winter
Spring
Summer
Autumn
Season

Figure 5


(A)
7
6
5
4
3
2
1
0
Nitrogen (g·m^{-2}·day^{-1})
Winter
Spring
Summer
Autumn
Season
Inlet
Biomass
Outlet
Stripping

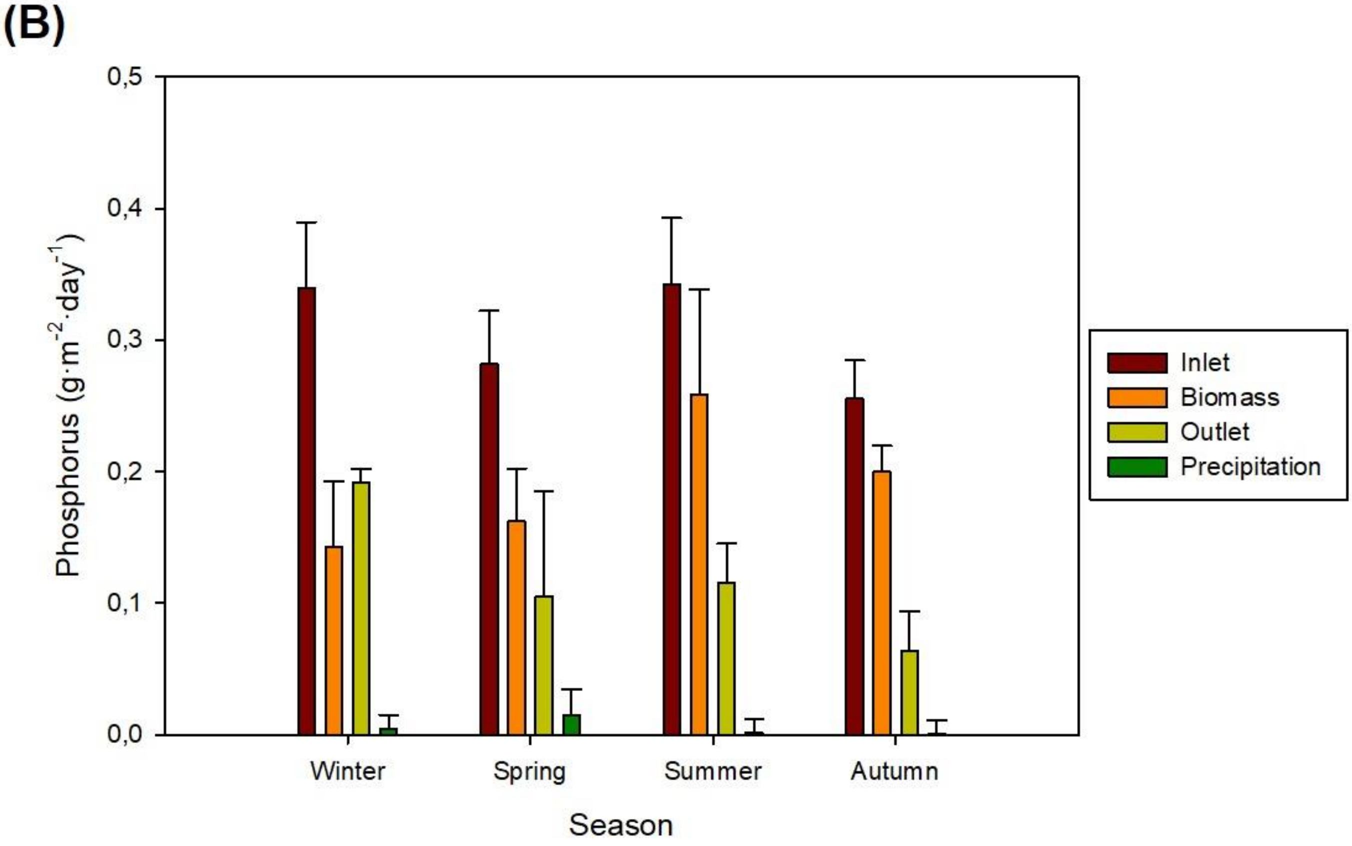
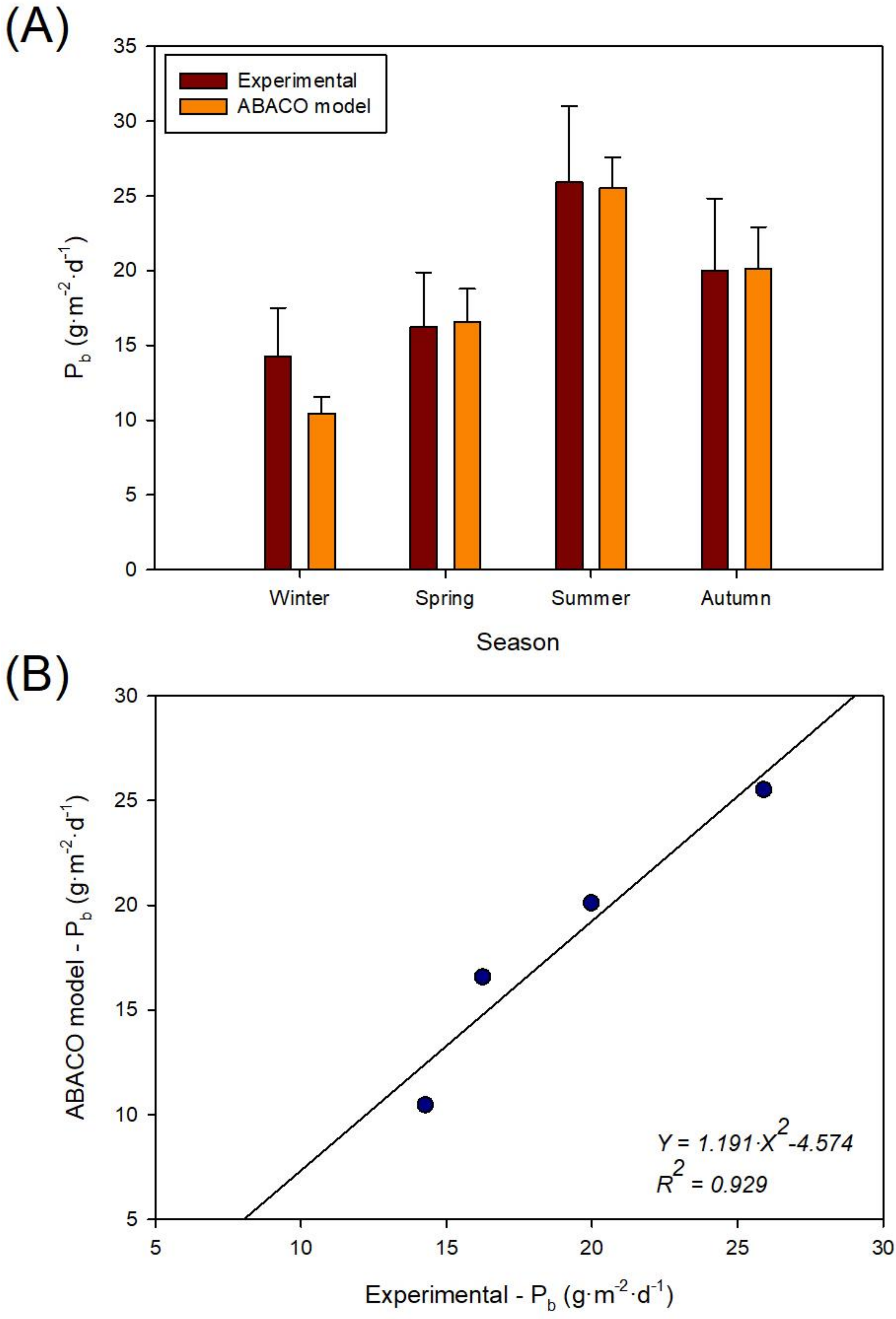

(B)
0,5
0,4
0,3
0,2
0,1
0,0
Phosphorus (g·m^{-2}·day^{-1})
Winter
Spring
Summer
Autumn
Season
Inlet
Biomass
Outlet
Precipitation

Figure 6


(A)
Experimental
ABACO model
P_b ($g \cdot m^{-2} \cdot d^{-1}$)
0
5
10
15
20
25
30
35
Winter
Spring
Summer
Autumn
Season
(B)
ABACO model - P_b ($g \cdot m^{-2} \cdot d^{-1}$)
Experimental - P_b ($g \cdot m^{-2} \cdot d^{-1}$)
5
10
15
20
25
30
$Y = 1.191 \cdot X^2 - 4.574$
$R^2 = 0.929$

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