Modeling of photosynthesis and respiration rate for microalgae-bacteria consortia

Sánchez Zurano, A., Gómez Serrano, C., Acién-Fernández F.G., Fernández-Sevilla J.M., Molina-Grima E.

Chemical Engineering Department, University of Almeria, Ctra. Sacramento, s/n, 04120 Almería, Spain

Keywords: Microalgae-wastewater, bacteria, photosynthesis, respiration, heterotrophic, nitrification.

Abstract

In this paper, the influence of culture conditions (irradiance, temperature, pH, and dissolved oxygen) on the photosynthesis and the respiration rates of microalgae-bacteria consortia in wastewater treatment was analyzed. Specifically, some short photo-respirometric experiments, simulating outdoor raceway reactors, were performed to evaluate the response of microalgae, heterotrophic bacteria and nitrifying bacteria to variations in environmental parameters. Results demonstrate that irradiance is the most dominant variable to determine microalgae photosynthesis rates. However, reduction in microalgae activity was not observed at higher irradiance, ruling out the existence of photoinhibition phenomena. Related to heterotrophic and nitrifying bacteria, their activities were strongly affected by the influence of temperature and pH. Moreover, the effect of dissolved oxygen concentrations on microalgae and bacteria activities was studied, displaying a reduced photosynthetic rate at dissolved oxygen concentrations above 20 mg/l. Data have been used to develop an integrated model for each population (microalgae, heterotrophic bacteria and nitrifying bacteria) based on considering the simultaneous influence of irradiance, temperature, pH, and dissolved oxygen. The models fit the experimental results in the range of culture conditions tested, and they were validated using data obtained by the simultaneous modifications of the variables. These individual models serve as a basis for developing a global biologic microalgae-bacteria model for wastewater treatment to improve the optimal design and management of microalgae-based processes, especially outdoors, where the cultures are subject to variable daily culture conditions.

1. Introduction

Currently, the demand for clean water has become a worldwide requirement and impose an investment in water management about of €150 billion per year. As a result, conventional wastewater treatment processes offer a treatment composed of several stages based on physical, chemical and biological methods which provide satisfactory levels of nutrient removal (carbon, nitrogen, phosphorous, etc) (Cabanelas et al., 2013). However, these common systems require a high complexity and electrical power consumption. As a solution to beat the disadvantages associated to the commonly used wastewater treatment methods, the search for environmentally friendly alternatives has brought the interest in biological treatment using microalgae (Park and Craggs, 2010). Not only further reducing microalgae wastewater treatment the cost of the process but also it avoids the nutrients loss such as nitrates, ammonia and phosphates, which can be used for algae growth (Li et al., 2019). Microalgae wastewater treatment is performed by complex microalgae-bacteria consortia which vary as a function of the environmental and operational conditions (Acién et al., 2016). Despite the relevant role of microalgae and bacteria improving wastewater treatment efficiency was put into evident in late 1950s (Oswald et al., 1953), compared to conventional technologies, little is known about the internal functioning of microalgae-bacteria wastewater processes [6]. From a macro point of view, several authors have described this synergistic performance of microalgae-bacteria in wastewater. In the presence of light, microalgae perform photosynthesis in which reduce CO_2 and produce oxygen. The released oxygen is used by aerobic bacteria (heterotrophic bacteria) for the degradation of organic compounds present in wastewater. Concurrently, bacteria produce carbon dioxide through aerobic respiration, which is essential for photosynthesis (Muñoz et al., 2009; Petrini et al., 2018; Quijano et al., 2017; Zambrano et al., 2016). In addition to heterotrophic bacteria, the nitrifying bacteria, which perform the nitrification process, establish different interactions with microalgae. On the one side, it has been proposed that microalgae may stimulate nitrification process by increasing the dissolved oxygen by oxygenic photosynthesis and thereby stimulating NH4 oxidation. However, other authors have suggested that microalgae could suppressed nitrification activity by reducing N-NH4 availability (Risgaard-Petersen et al., 2004).

Therefore, within these systems occur multiple physical, chemical and biological processes which should be evaluated in detail. Within this context, it is mandatory to get

insight of the complex interaction between microalgae and bacteria, mathematical models being a powerful tool to predict the performance and to optimize the design of the microalgae-bacteria wastewater processes (Solimeno and García, 2017) . In this direction, for convectional wastewater technologies, different bacteria models have been developed and promoted by the International Water Association (IWA, former International Association on Water Pollution Research and Control) (Henze et al., 1999; Henze et al., 2015). On the other side, several microalgae mathematical models to understand microalgae growth have been validated. Firstly, multiple models which only taking account one factor to predict microalgae growth were developed (Eilers and Peeters, 1988; Grima et al., 1994). And gradually numerous microalgae models which consider more than one factor have been described (Costache et al., 2013b; Ippoliti et al., 2016). The most recent models have introduced different environmental and substrate factors which determine microalgae activity. However, very little research has focused on developing dynamic models to understand the interactions between microalgae and bacteria in wastewater treatment. Despite the first simple microalgae-bacteria model developed in 1983 (Buhr and Miller, 1983a), it is not until recently that interest has focused on developing microalgae-bacteria models which combine the overall biochemical processes involved in these systems and the simultaneous effects of environmental parameters on biomass growth, highlighting BIO_ALGAE model (Solimeno et al., 2017).

Traditionally, respirometric tecniques have been proposed as a rapid tool for bacteria characterization in activated sludge processes (Ellis et al., 1996). Currently, this technology has been extended to microalgae-bacteria cultures (Flores-Salgado et al., 2021; Rossi et al., 2018) . In microalgae-bacteria cultures, the use of respirometry allows one to determine the phototrophic activity by measuring the oxygen production rate (OPR) under light conditions and the oxygen uptake rate (OUR) in the dark. The methodology allows to assess heterotrophic and nitrifying activity in microalgal-bacterial consortia too (Petrini et al., 2020; Sánchez-Zurano et al., 2020). These measurements, which are based on oxygen production/consumption, are rapid and easily obtainable (Tang et al., 2014).

Given the need for progress in this area, in this paper, the influence of major environmental parameters (irradiance, temperature, pH and dissolved oxygen) was evaluated by determining the net microalgae photosynthesis rate and the respiration rates of both heterotrophic and nitrifying bacteria in wastewater processes using respirometric

tecniques. The results were used to develop individual models that allow the simulation of the net microalgae photosynthesis rate and respiration rates of the two bacteria populations (heterotrophic and nitrifying bacteria) under different culture conditions. The models were validated by experimental data compiled under different culture conditions tested. According to these results, the developed models are useful tools to optimize the design and operation control of photobioreactors developing an overall model which integrate microalgae and bacteria activities.

2. Materials and methods

2.1. Microorganisms and culture conditions

The *Scenedesmus almeriensis* strain was used as the control microorganism. Stock cultures were maintained photo-autotrophically in spherical flasks (1.0 L capacity) using Arnon medium (Allen and Arnon, 1955). The culture was continuously bubbled with an air–1 % CO_2 mixture to control the pH at 8.0. The culture temperature was set at 22°C, controlled by regulating the air temperature in the chamber. The culture was artificially illuminated in a 12:12 h L/D cycle using four Philips PL-32W/840/4p white-light lamps, providing an irradiance of 750 $\mu E/m^2$ s on the spherical 1.0 L flask surface.

For the experiments, this inoculum was transferred to laboratory-scale photobioreactors. Details of the reactor and culture medium used in each one are given below. The average composition of the wastewaters used is reported in *Table 1*.

2.2. Laboratory photobioreactors

Experiments were performed in four stirred-tank reactors made with polymethylmethacrylate (0.08 m in diameter, 0.2 m in height and with a 1 L capacity) operated in the laboratory but simulating outdoor raceway reactors. These reactors were filled with sewage taken directly after primary treatment from the wastewater treatment plant in Roquetas de Mar (Almería) and 20% of *Scenedesmus almeriensis* inoculum. First, they were operated in batch mode for 6 days to achieve a high biomass concentration, next it being operated in continuous mode, by replacing 20% of the culture volume daily with fresh wastewater. Once the reactors volume was renewed twice and the reactors concentration remained stable, it was considered that the reactors have reached steady state. To prevent the adverse effect of excessive dissolved oxygen accumulation, the dissolved oxygen was controlled below 200% saturation by supplying air on demand;

CO_2 was also injected on demand to control the pH at 8. Concerning illumination, the reactors were artificially illuminated using eight 28 W fluorescent tubes (Philips Daylight T5) on a simulated solar cycle. The maximum irradiance (PAR) inside the reactors in the absence of cells was 1000 μE m^{-2} s^{-1}, measured using an SQS-100 spherical quantum sensor (Walz GmbH, Effeltrich, Germany). The culture temperature was kept at 25 °C by controlling the temperature of the ambient air of the culture chamber in which the reactors were located.

2.3. Measurement of photosynthesis and respiration rates

A photo-respirometer was used to obtain the microalgae net photosynthesis rate and the bacteria respiration rates for the biologic model. The protocol and methodology applied allow to distinguish between the metabolisms of the three main populations which appear in microalgae-bacteria wastewater treatment: the microalgae, the heterotrophic bacteria, and the nitrifying bacteria. The equipment operation is based on determining any variation in dissolved oxygen concentration in microalgae-bacteria culture samples under controlled conditions. An adequate protocol was applied to determine the microalgae net photosynthesis rate, the heterotrophic respiration rate and the nitrifying respiration rate. Firstly, samples of the microalgae-bacteria cultures should be taken and subjected to nutrient starvation (continuous light of 200 μEm-2 s-1 and an aeration rate of 0.2 v·v-1·min-1) during 24 hours to remove the organic matter and the ammonium present in the medium. Subsequently, the methodology consists on placing a sample of the culture inside the photo-respirometer and subjecting to four light–dark periods of 4 minutes each one during which the variation in dissolved oxygen under different conditions is measured. In the following section, the determination of each microbial metabolism is described, including the expected biological reactions affecting the dissolved oxygen concentration:

- For evaluating the microalgae net photosynthesis rate of each microalgae-bacteria culture, a sample of the culture is exposed to four light–dark cycles of 4 min each to measure and register the variation in dissolved oxygen. Air is provided through the diffuser to recover the 100% saturation between the dark and light cycles. The first minute of exposure (light and dark phases) was disregarded as it was adaptation time. During the light phases, the photosynthetic microalgae generated dissolved oxygen while this dissolved oxygen is consumed by the endogenous respiration during the dark periods. Therefore, the endogenous respiration activity

or the oxygen consumption rate (OCR) was obtained through linear regression analysis of DO data (negative trend) in the dark, while the oxygen production rate (OPR) was obtained through linear regression analysis of DO data (positive trend) in the light. Thus, the microalgae net photosynthesis rate was calculated as the difference between the slope of the OPR during the light period minus the slope of the OCR during the dark period.

- Subsequently, another sample of the culture was used to determine the heterotrophic respiration rate. For this purpose, 0.8 mL of sodium acetate (30g/L) was added to the sample and it was exposed to four light–dark cycles of 4 min each one. The respiration rate of the heterotrophic bacteria was calculated as the slope of the oxygen consumption with sodium acetate minus the slope of the oxygen consumption during the dark period in the endogenous culture.
- By following the same method, another sample was used to measure the nitrifying respiration rate of the culture. However, the nitrifying activity was determined using 0.8 mL of ammonium chloride (3g/L) instead of sodium acetate. The respiration rate of the nitrifying bacteria was calculated as the slope of the oxygen consumption with ammonium chloride minus the slope of the oxygen consumption during the dark period in the endogenous culture.

Finally, to correct the influence of oxygen desorption on the photo-respirometric measurements, the oxygen mass transfer coefficient (K_La) was calculated and included. This coefficient was measured in the system according to (Eqs (1)).

$$\frac{dC_{O2}}{dt} = K_L a\,(C_{02}^{*} - C_{O2}) \qquad \text{Equation 1}$$

Where dC_{O2}/dt is the oxygen accumulation expressed as the derivate of C_{O2} (mg/L) over time, K_La is the global oxygen mass transfer coefficient (h^{-1}), and C_{O2}^{*} is the oxygen saturation concentration in the liquid. The final value obtained was 1.08 h^{-1}. A further detailed description of the equipment, the experimental protocol and the metabolic rates calculations are described in (Sánchez-Zurano et al., 2020).

The protocol described below was applied to determine the microalgae net photosynthesis rate and bacteria respiration rates exposed to different irradiancies inside the glass chamber. Furthermore, experiments were performed also modifying the temperature by heating/cooling the samples. According to the pH, it was adjusted in the samples from the

laboratory photobioreactor by adding HCl or NaOH. Finally, experiments were performed modifying the dissolved oxygen by bubbling pure oxygen or pure nitrogen into the sample. For each measurement, a new sample of culture in steady state, coming from the laboratory photobioreactors described in section 2.2, was used, to avoid accumulation of effects.

2.4. Biomass concentration and analytical methods

The microalgae biomass concentration was measured by dry weight. It was used 100 mL aliquots of the culture filtered through a pre-dried 1 μm filter (Macherey-Nagel GmbH &Co.KG, Germany). Then, the filters were dried in an oven at 80ºC for 24 h. Standard official methods were used to analyse the composition of the wastewater samples and the water from the reactors. The phosphate was measured by visible spectrophotometry through the phospho-vanado-molybdate complex (Phosphate Standard for IC: 38364). The nitrate was quantified by measuring optical density at 220 nm and 275 nm (Nitrate Standard for IC: 74246). The ammonium was measured according to the Nessler method (Ammonium standard for IC: 59755). The Chemical Oxygen Demand (COD) was determined by spectrophotometric measurement using Hach-Lange kits (LCl-400).

2.5. Software and statistical analysis

The DaqFactory programme (Azeotech, USA) was used to gather the photosynthesis and respiration rate data. Data analysis was carried out using the Statgraphics Centurion XVI software package, in which non-linear regression was used to fit experimental data to the proposed models, and to determine the characteristic parameter values. These models were used to obtain simulations in Microsoft Excel. All the experiments were performed at least by triplicate to allow calculating the mean values and standard deviation that are shown.

3. Results and discussions

During the last decades, different types of mathematical models have been presented to describe the growth of microalgae and bacteria influenced by environmental variables (Béchet et al., 2013; Buhr and Miller, 1983b; Sah et al., 2011; Zambrano et al., 2016). As with all microalgae pure culture (Costache et al., 2013a; Ippoliti et al., 2016), under unlimited nutrient conditions, the most important factors for microalgae-bacteria wastewater processes are irradiance, temperature, pH and dissolved oxygen (Solimeno et al., 2017). To optimize the productivity of the microalgae-bacteria systems, the influence

of these factors should be analysed in the laboratory simulating outdoor environmental changes to develop models that can be integrated in outdoors photobioreactors. Therefore, to model the activity of both microalgae and bacteria cells to environmental conditions, samples from the lab-scale cultures were collected and used to determine the photosynthesis and respirations rates of the three main populations.

Regarding irradiance, the net photosynthesis rate was zero at zero irradiance and increased with irradiance to a maximum of 106 mgO2/$g_{biomass}$·h at an irradiance of 650 µE/m^2·s, then remained constant at higher irradiance. Photo-inhibition was not observed at high irradiance values, keeping the photosynthetic activity as was reported using the green microalga Chlorella vulgaris by (Yun and Park, 2003). The lack of photo-inhibition in cells activity is in breach of previous studies with pure *Scenedemus almeriensis* culture in which the photosynthesis rate increased with light availability up to values of 400 µE/m^2·s, remaining constant up to values of 1.000 µE/m^2·s, and finally decreased at higher irradiances (Costache et al., 2013a). Despite several light intensity models developed to describe microalgae photosynthesis and growth kinetics (Aiba, 1982; Eilers and Peeters, 1988), experimental data have been fitted to the Molina model (Eqs(2)) (Grima et al., 1994), in which the net photosynthesis rate is a function of specific maximum photosynthetic rate (PO_2,max), average irradiance (Iav), constant representing the affinity of algae to light (Ik) and a form parameter (n). By fitting experimental data to this equation, the characteristic parameter values were determined (PO_2,max= 113 mgO2/$g_{biomass}$·h , n=1.68, Ik=168 µE/m2·s), verifying that the model reproduces the behaviour of the measurements performed. Heterotrophic and nitrifying bacteria respiration remained constant along the irradiance values applied, that any light intensity model was used. As a result, heterotrophic bacteria respiration rate was 5.8 mgO2/$g_{biomass}$·h and nitrifying bacteria respiration rate was 4.4 mgO2/$g_{biomass}$·h (Figure 1A).

$$PO_2 = \frac{PO_{2,max} \cdot {I_{av}}^n}{{I_k}^n + {I_{av}}^n} \qquad \text{Equation 2}$$

Regarding microalgae respiration, it may be influenced by several factors such as temperature, oxygen tension, exogenous substrates, etc. The illumination level before the measurement also influences the microalgae respiration rates that are found to be higher when the microalgae algae exposed to high irradiance while lower respiration rates were

measured after weaker illumination conditions (Grobbelaar and Soeder, 1985). This tendency related with the light exposition was observed in this work too. The respiration rate was 3.4 mgO2/$g_{biomass}$·h at zero irradiance, increasing with irradiance up to 16.4 mgO2/$g_{biomass}$·h at an irradiance of 1000 µE/m^2·s, then remaining constant up to 2000 µE/m^2·s (Fig. 1B). Experimental data have been fitted to the hyperbolic model with no inhibition (Eqs(3)) and the characteristic parameter values were determined (RO_2 min= 3.4 mgO2/$g_{biomass}$·h , RO_2,max= 12.7 mgO2/$g_{biomass}$·h , n_r=1.4, Ik_res=134 µE/m2·s).

$$RO_2 = RO_2\,\min + \frac{RO_{2,max} \cdot {I_{av}}^{n\_r}}{{I_{k\_res}}^{n\_r} + {I_{av}}^{n\_r}} \qquad \text{Equation 2}$$

As in previous studies (Acién Fernández et al., 1999; Fernández et al., 1998), results have verified that under real conditions, the cultures are mainly photo-limited, the average irradiance being from 100 to 300 µE/m^2·s. The net photosynthesis rate was saturated at 500 µE/m^2·s, taking this value, 200 µE/m^2·s was selected as a constant light intensity to determine the influence of the other main environmental parameters, which could modulate the response of the net photosynthesis rate and the bacteria respiration rate to irradiance (such as temperature, pH and dissolved oxygen). These experiments will allow to determine the normalized net photosynthesis rate and bacteria respiration rates as a function of these culture conditions.

Consequently, experiments were performed by modifying the temperature of the cultures to calculate microalgae net photosynthesis rate and bacteria respiration rates over a wide range of temperatures. Temperature has been pointed as one of the main environmental conditions which determining the structure of the microbial community and the process performance (such as the nitrification activity) in wastewater treatment (Chen et al., 2017). Concerning the microalgae activity, the net photosynthesis rate was maximal at a temperature of 30 °C, lower than the optimum temperature of the *Scenedesmus almeriensis* reported by (Costache et al., 2013a). However, other authors showed the maximum specific growth rate of *Scenedesmus sp* at 25 °C and a wide activity range at temperatures from 10 to 30 °C (Xin et al., 2011). In this way, the microalgae activity decreased at high temperatures, with zero activity at extreme temperatures above 49 °C. According to these data, previews studies reported that *Scenedemus* did not grow at 42

°C (Westerhoff et al., 2010). Note that these experiments have been carried out at short periods of exposure, so that the photosynthetic response of the culture could be conditioned by varying the exposure time, even at moderate temperatures such as 35 ºC (Karemore et al., 2020).

Bacterial growth is quite dependent to the temperature. For them, as the temperature rises, enzyme reactions in the cell proceed at more fast rates, with rates approximately doubling with every 10 °C increase. However, above a certain temperature, growth slows and, if the temperature continues to increase, bacteria could die (Rajeshwari et al., 2000; Spellman, 1999). This effect has been observed in the experiments, in which the oxygen consumption rate of the heterotrophic bacteria increases at high values of temperature, showed their optimum activity at 36 °C and the higher rate of respiration was at 39.5 ºC. For nitrifying bacteria, temperature effects are more complex, because influence in nitrifiers viability and its activity rates (Hülsen et al., 2016). Traditionally, nitrification process in wastewater has been considered the most temperature-sensitive step among the microbial activities, due to the fact that nitrifying activity could decrease by 50 % with each temperature decrease of 10 °C (Wang and Li, 2015). Results showed the optimal temperature was 30 °C and a wide range of activity from 0 to 49 °C, appreciating a strong decrease in activity below 30 °C. Just like heterotrophic bacteria, nitrifying bacteria are especially sensitive to high temperature, the activity of them being zero at extreme temperatures (50-60 °C) (Henze et al., 2001) (Figure 2).

The influence of temperature in the normalized net photosynthesis rate and the normalized respiration rate for heterotrophic and nitrifying bacteria, was fitted to the cardinal model developed for bacteria (Rosso et al., 1993) and validated for microalgae (Bernard and Rémond, 2012) (Eqs(4)). The cardinal model is a simple equation which considers a maximum, a minimum and an optimal value, the values of the variable (for instance, temperature) only existing on the range between maximum and minimum tolerable values (Ippoliti et al., 2016). Currently, cardinal equations are well accepted in the microalgae-bacteria models because they are helpful to represent experimental data and make the models straightforward to understand (Rossi et al., 2020).

$$RO2(T) = \frac{(T - Tmax)(T - Tmin)2}{(Topt - Tmin)(((Topt - Tmin)(T - Topt)) - ((Topt - Tmax)(Topt + Tmin - 2T)))} \quad \text{Equation 4}$$

Concerning to the influence of pH on microalgae activity, the normalized net photosynthesis rate was considered fairly high at pH from 7.5 to 8.5 with an optimum pH above 8.5. At pH values lower than 7.0, the photosynthesis rate reduced slowly just like pH values higher than 9.0. Photosynthesis took place even at high pH values, but at pH 13, the photosynthesis rate was zero. It is important to note that experiments were performed by modifying the pH through the addition of sodium hydroxide or hydrochloric acid. However, because the system was not bubbled, no decarbonation took place although concentrations of different carbonate–bicarbonate–carbonic acid species modified as a function of pH (Costache et al., 2013a). The results agree well with the reported literature (Difusa et al., 2015; Gardner et al., 2011) where a pH trend ranging from 7.0 to 9.0 provided favourable condition for high growth rate of Scenedesmus.

Bacteria, just as happens with the microalgae, do not tolerate pH values below 4 or above 9,5. Despite most wastewater treatments work at a pH near to neutral, there are situations that involve excursions to high or low pH values. Certain bacterial strains are still capable of working at these pH values (acidophiles, neutrophiles and alkalinophiles) in wastewatater (Gerardi, 2006). The maximal heterotrophic bacteria respiration rate was at pH 8 and, it keeps up to pH 9, but their tolerance to low pH values were lower than microalgae. Under pH 6, heterotrophic bacteria did not show activity. Despite there is a wide range in the reported pH optima (pH 6.5 to 8.6) for nitrifying bacteria in the activated sludge process and there is general agreement that as the pH shifts to the acid range, the rate of nitrification decline (Cheremisinoff, 1997). The results showed an optimal pH higher, above 9.7, with tolerance to high values of pH (Figure 3). The observed influence of pH on net photosynthesis rate and bacteria respiration rate exhibit similar behaviour to that previously observed for temperature. Thus, cardinal model allowed to model the response of the net photosynthesis rate and bacteria respiration rate to pH (Eqs(5)).

$$RO2(pH) = \frac{(pH - pHmax)(pH - pHmin)2}{(pHopt - pHmin)(((pHopt - pHmin)(pH - pHopt)) - ((pHopt - pHmax)(pHopt + pHmin - 2pH)))} \quad \text{Equation 5}$$

The effect of dissolved oxygen on microalgae and bacteria activity was also studied (Figure 4). According to the microalgae activity, at low dissolved oxygen concentrations and at saturation concentration (9.0 mg/L), the net photosynthesis rate was maximal, decreasing until zero activity at 32 mg/L. Previous studies reported that oxygen levels above air saturation could inhibit photosynthesis in some microalgae. Also oxygen

accumulation could be the cause of photo-oxidation in microalgae culture (Molina Grima et al., 1999). These data were comparable with previews results using *Scenedesmus* cultures, in which the maximal dissolved oxygen concentration tolerable by the culture was 25 mg/L (225 %Sat.) (Barceló-Villalobos et al., 2019). However, other microalgae species have showed less tolerance under extreme dissolved oxygen concentrations. For instance, photosynthesis activity of some polar sea ice microalgae was restricted at dissolved oxygen concentration above 20 mg/L (McMinn et al., 2005), even so strains such as *Isochrysis galbana* reduce the photosynthesis rate to zero at 20 mg/L (Ippoliti et al., 2016). To model the microalgae's response to dissolved oxygen concentration, an equation considering the inhibition by product was used, previously reported (Costache et al., 2013a; Ippoliti et al., 2016) (Eqs(6)).

$$RO2(DO_2) = 1 - \left(\frac{DO_2}{DO_{2,max}}\right)^m \qquad \text{Equation 6}$$

It is mandatory to determine bacteria activity under different dissolved oxygen concentration because of the competitive interaction of heterotrophs and nitrifies for dissolved oxygen is well known for years (Furumai and Rittmann, 1992). Heterotrophic bacteria support a wide range of dissolved oxygen values, being able to live at very low values (<0.9 mg / L) and their activity increases with increasing dissolved oxygen, fitting experimental data to Monod equation (Eqs(7)).

$$RO_2\,(DO_2) = \frac{DO_2}{DO_2 + Ks} \qquad \text{Equation 7}$$

Concerning the effect of dissolved oxygen on nitrification in wastewater treatment, one of the earliest works to quantify nitrification in wastewater treatment processes was that performed by A. L. Downing and Associates at the Water Pollution Lab at Stevenage (Downing and Scragg, 1958). They reported that the respiration rate in activated sludge plants fall off when the DO concentration fell below 0.3 mg/L , while several years after this initial work, other authors reported that nitrifying growth is inhibited even so at dissolved oxygen levels below 2.0 mg/L (Abbassi et al., 2000). Also it has been described that the rate of nitrification in wastewater treatment increases as the DO concentration is increased to 7 or 8 mg/L (Stenstrom and Poduska, 1980). According to high levels of dissolved oxygen for nitrification, respirometric experiments have been shown that high

oxygen concentrations are initially inhibitory but acclimatisation occurs after several days at high oxygen levels according to previous authors (Charley et al., 1980). The findings of this study showed an optimal range of dissolved oxygen between 5 and 13 mg/L, decreasing slowly at high values of dissolved oxygen. Furthermore, nitrifying bacteria were capable to tolerate low levels of oxygen, showing activity at 0,9 mg/L. From the nitrifying bacteria respiration rate variation with the dissolved oxygen concentration, an Andrews equation (Andrews et al., 1968), similar to that used by heterotrophic bacteria but taking into account the inhibition by product has been applied (Eqs(8)).

$$RO_2\,(DO_2) = \frac{DO_2}{(DO_2 + Ks)(1 + \frac{DO_2}{Ki})} \qquad \text{Equation 8}$$

According to these results, the characteristic parameter values were determined (Table 2) and the microalgae net photosynthesis and bacteria respiration rates could be modelled by combining these equations to obtain a general equation representing the overall behaviour based on the observed patterns. Thus, Eqs. (9), (10) and (11) allow to model the microalgae net photosynthesis rate, heterotrophic bacteria respiration rate and nitrifying bacteria respiration rate as a function of the culture conditions (irradiance, temperature, pH and dissolved oxygen) to which the cells are exposed.

$$PO2_{ALG} = PO2(I) \cdot \overline{PO2(T)} \cdot \overline{PO2(pH)} \cdot \overline{PO2(DO2)} - RO2(I) \qquad \text{Equation 9}$$

$$RO2_{Het} = RO2(I) \cdot \overline{PO2(T)} \cdot \overline{PO2(pH)} \cdot \overline{PO2(DO2)} \qquad \text{Equation 10}$$

$$RO2_{Nit} = RO2(I) \cdot \overline{PO2(T)} \cdot \overline{PO2(pH)} \cdot \overline{PO2(DO2)} \qquad \text{Equation 11}$$

In order to validate the proposed models, some experiments were performed modifying the culture conditions studied (irradiance, temperature, pH and DO2). With these experimental data and the simulated data from the developed models and the characteristic parameter values, it was possible to determine a correlation between them. Figure 5 showed that the microalgae model fitted the experimental data of the net photosynthesis rate with a correlation coefficient of 0.87. The models were validated for heterotrophic and nitrifying bacteria activity too. Using the experimental data changing

the values of the environmental parameters and the simulated data from the model, was possible correlating them. The bacteria models fitted the experimental data of the heterotrophic and nitrifying respiration rate with a correlation coefficient of 0.73 and 0.64, respectively. The results obtained demonstrated that the models fitted experimental values determined indoors and allow the identification of the characteristic parameter values for the microalgae-bacteria consortia in wastewater.

4. Conclusions

Environmental parameters (Irradiance, temperature, pH and dissolved oxygen) are significant variables determining the performance of microalgae-bacteria consortia in wastewater treatment. In this work, it has been developed and validated for first time, a microalgae-bacteria model based on the photosynthesis and respiration rates for microalgae wastewater processes. Next steps are aimed at validating the proposed models in outdoors conditions using industrial-scale raceway photobioreactors. This implementation will allow to design and management microalgae wastewater processes improving the productivity and reducing the cost of the systems.

5. Acknowledgements

This research was funded by the SABANA project (grant # 727874) of the European Union's Horizon 2020 Research and Innovation Programme, by the PURASOL project CTQ2017-84006-C3-3-R (Ministerio de Economía y Competitividad, Gobierno de España) as well as being supported by the Marine Microalgal Biotechnology group (BIO 173) in the Chemical Engineering Department, University of Almeria, Spain, Fundación Cajamar and the Spanish Ministry of Education through the National FPU Programme (grant number FPU16/05996).

6. Declarations

There are no potential financial or other interests that could be perceived to influence the outcomes of the research. No conflicts, informed consent, human or animal rights applicable. All authors confirmed the manuscript authorship and agreed to submit it for peer review.

7. Author contributions

Ana Sánchez Zurano: Methodology, Investigation, Formal análisis, Writing-Original Draft. Cintia Gómez Serrano: Conceptualization, Data curation, Resources. Francisco Gabriel Acién Fernández: Supervision, Writing-Reviewing and Funding acquisition. José María Fernández Sevilla: Formal analysis, Software, Supervision. Emilio Molina Grima: Writing- Reviewing and Editing, Project administration, Funding acquisition.

8. References

Abbassi B, Dullstein S, Räbiger N. 2000. Minimization of excess sludge production by increase of oxygen concentration in activated sludge flocs; experimental and theoretical approach. *Water Res.* **34**:139–146.

Acién FG, Gómez-Serrano C, Morales-Amaral MM, Fernández-Sevilla JM, Molina-Grima E. 2016. Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? *Appl. Microbiol. Biotechnol.* **100**:9013–9022.

Acién Fernández FG, García Camacho F, Chisti Y. 1999. Photobioreactors: light regime, mass transfer, and scaleup. In: Osinga, R, Tramper, J, Burgess, JG, Wijffels, RH, editors. *Prog. Ind. Microbiol.* Elsevier. Marine Bioprocess Engineering, Vol. 35, pp. 231–247. http://www.sciencedirect.com/science/article/pii/S0079635299801180.

Aiba S. 1982. Growth kinetics of photosynthetic microorganisms. *Adv. Biochem. Eng.* http://agris.fao.org/agris-search/search.do?recordID=US201302006198.

Allen MB, Arnon DI. 1955. Studies on Nitrogen-fixing Blue-green Algae. *Physiol. Plant.* **8**:653–660.

Andrews JF, Andrew JF, Andrews JF. 1968. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. https://www.scienceopen.com/document?vid=fc14eadf-b579-4c0d-b963-59f78be53c00.

Barceló-Villalobos M, Serrano CG, Zurano AS, García LA, Maldonado SE, Peña J, Fernández FGA. 2019. Variations of culture parameters in a pilot-scale thin-layer reactor and their influence on the performance of Scenedesmus almeriensis culture. *Bioresour. Technol. Rep.* **6**:190–197.

Béchet Q, Shilton A, Guieysse B. 2013. Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation. *Biotechnol. Adv.* **31**:1648–1663.

Bernard O, Rémond B. 2012. Validation of a simple model accounting for light and temperature effect on microalgal growth. *Bioresour. Technol.* **123**:520–527.

Buhr HO, Miller SB. 1983a. A dynamic model of the high-rate algal-bacterial wastewater treatment pond. *Water Res.* **17**:29–37.

Cabanelas ITD, Ruiz J, Arbib Z, Chinalia FA, Garrido-Pérez C, Rogalla F, Nascimento IA, Perales JA. 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. *Bioresour. Technol.* **131**:429–436.

Charley R, Hooper D, Mclee A. 1980. Nitrification kinetics in activated sludge at various temperatures and dissolved oxygen concentrations. *Water Res.* **14**:1387–1396.

Chen Y, Lan S, Wang L, Dong S, Zhou H, Tan Z, Li X. 2017. A review: Driving factors and regulation strategies of microbial community structure and dynamics in wastewater treatment systems. *Chemosphere* **174**:173–182.

Cheremisinoff NP. 1997. Nitrification and denitrification in the activated sludge process. In: . *Biotechnol. Waste Wastewater Treat.* Elsevier, pp. 151–188. http://linkinghub.elsevier.com/retrieve/pii/B9780815514091500066.

Costache TA, Acién Fernández FG, Morales MM, Fernández-Sevilla JM, Stamatin I, Molina E. 2013a. Comprehensive model of microalgae photosynthesis rate as a function of culture conditions in photobioreactors. *Appl. Microbiol. Biotechnol.* **97**:7627–7637.

Difusa A, Talukdar J, Kalita MC, Mohanty K, Goud VV. 2015. Effect of light intensity and pH condition on the growth, biomass and lipid content of microalgae Scenedesmus species. *Biofuels* **6**:37–44.

Downing AL, Scragg LJ. 1958. The Effect of Synthetic Detergents on the Rate of Aeration in Diffused-Air Activated Sludge Plants. *Water Amp Waste Treat. J.* **7**:102–7.

Eilers PHC, Peeters JCH. 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol. Model.* **42**:199–215.

Ellis TG, Barbeau DS, Smets BF, Grady CPL. 1996. Respirometric technique for determination of extant kinetic parameters describing biodegradation. *Water Environ. Res.* **68**:917–926.

Fernández FGA, Camacho FG, Pérez JAS, Sevilla JMF, Grima EM. 1998. Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: Effects of dilution rate, tube diameter, and solar irradiance. *Biotechnol. Bioeng.* **58**:605–616.

Flores-Salgado G, Thalasso F, Buitrón G, Vital-Jácome M, Quijano G. 2021. Kinetic characterization of microalgal-bacterial systems: Contributions of microalgae and heterotrophic bacteria to the oxygen balance in wastewater treatment. *Biochem. Eng. J.* **165**:107819.

Furumai H, Rittmann BE. 1992. Advanced Modeling of Mixed Populations of Heterotrophs and Nitrifiers Considering the Formation and Exchange of Soluble Microbial Products. *Water Sci. Technol.* **26**:493–502.

Gardner R, Peters P, Peyton B, Cooksey KE. 2011. Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of the chlorophyta. *J. Appl. Phycol.* **23**:1005–1016.

Gerardi MH. 2006. Wastewater Bacteria. John Wiley & Sons 268 p.

Grima EM, Camacho FG, Pérez JAS, Sevilla JMF, Fernández FGA, Gómez AC. 1994. A mathematical model of microalgal growth in light-limited chemostat culture. *J. Chem. Technol. Biotechnol.* **61**:167–173.

Grobbelaar JU, Soeder CJ. 1985. Respiration losses in planktonic green algae cultivated in raceway ponds. *J. Plankton Res.* **7**:497–506.

Henze M, Gujer W, Mino T, van Loosedrecht M. 2015. Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. *Water Intell. Online* **5**:9781780402369–9781780402369.

Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC, Marais G v. R, Van Loosdrecht MCM. 1999. Activated sludge model No.2D, ASM2D. *Water Sci. Technol.* **39**. Modelling and microbiology of activated sludge processes:165–182.

Henze M, Harremoes P, Jansen J la C, Arvin E. 2001. Wastewater Treatment: Biological and Chemical Processes. Springer Science & Business Media 436 p.

Hülsen T, Barry EM, Lu Y, Puyol D, Batstone DJ. 2016. Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. *Water Res.* **100**:537–545.

Ippoliti D, Gómez C, del Mar Morales-Amaral M, Pistocchi R, Fernández-Sevilla JM, Acién FG. 2016. Modeling of photosynthesis and respiration rate for Isochrysis galbana (T-Iso) and its influence on the production of this strain. *Bioresour. Technol.* **203**:71–79.

Karemore A, Yuan Y, Porubsky W, Chance R. 2020. Biomass and pigment production for Arthrospira platensis via semi-continuous cultivation in photobioreactors: Temperature effects. *Biotechnol. Bioeng.* **117**:3081–3093.

Li K, Liu Q, Fang F, Luo R, Lu Q, Zhou W, Huo S, Cheng P, Liu J, Addy M, Chen P, Chen D, Ruan R. 2019. Microalgae-based wastewater treatment for nutrients recovery: A review. *Bioresour. Technol.* **291**:121934.

McMinn A, Pankowski A, Delfatti T. 2005. EFFECT OF HYPEROXIA ON THE GROWTH AND PHOTOSYNTHESIS OF POLAR SEA ICE MICROALGAE1. *J. Phycol.* **41**:732–741.

Molina Grima E, Fernández FGA, García Camacho F, Chisti Y. 1999. Photobioreactors: light regime, mass transfer, and scaleup. *J. Biotechnol.* **70**. Biotechnological Aspects of Marine Sponges:231–247.

Muñoz R, Köllner C, Guieysse B. 2009. Biofilm photobioreactors for the treatment of industrial wastewaters. *J. Hazard. Mater.* **161**:29–34.

Oswald WJ, Gotaas HB, Ludwig HF, Lynch V. 1953. Algae Symbiosis in Oxidation Ponds: III. Photosynthetic Oxygenation. *Sew. Ind. Wastes* **25**:692–705.

Park JBK, Craggs RJ. 2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* **61**:633–639.

Petrini S, Foladori P, Andreottola G. 2018. Laboratory-scale investigation on the role of microalgae towards a sustainable treatment of real municipal wastewater. *Water Sci. Technol.* **78**:1726–1732.

Petrini S, Foladori P, Donati L, Andreottola G. 2020. Comprehensive respirometric approach to assess photosynthetic, heterotrophic and nitrifying activity in microalgal-bacterial consortia treating real municipal wastewater. *Biochem. Eng. J.* **161**:107697.

Quijano G, Arcila JS, Buitrón G. 2017. Microalgal-bacterial aggregates: Applications and perspectives for wastewater treatment. *Biotechnol. Adv.* **35**:772–781.

Rajeshwari KV, Balakrishnan M, Kansal A, Lata K, Kishore VVN. 2000. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renew. Sustain. Energy Rev.* **4**:135–156.

Risgaard-Petersen N, Nicolaisen MH, Revsbech NP, Lomstein BA. 2004. Competition between ammonia-oxidizing bacteria and benthic microalgae. *Appl. Environ. Microbiol.* **70**:5528–5537.

Rossi S, Bellucci M, Marazzi F, Mezzanotte V, Ficara E. 2018. Activity assessment of microalgal-bacterial consortia based on respirometric tests. *Water Sci. Technol.* **78**:207–215.

Rossi S, Casagli F, Mantovani M, Mezzanotte V, Ficara E. 2020. Selection of photosynthesis and respiration models to assess the effect of environmental conditions on mixed microalgae consortia grown on wastewater. *Bioresour. Technol.* **305**:122995.

Rosso L, Lobry JR, Flandrois JP. 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *J. Theor. Biol.* **162**:447–463.

Sah L, Rousseau DPL, Hooijmans CM, Lens PNL. 2011. 3D model for a secondary facultative pond. *Ecol. Model.* **222**:1592–1603.

Sánchez-Zurano A, Gómez-Serrano C, Acién-Fernández FG, Fernández-Sevilla JM, Molina-Grima E. 2020. A novel photo-respirometry method to characterize consortia in microalgae-related wastewater treatment processes. *Algal Res.* **47**:101858.

Solimeno A, García J. 2017. Microalgae-bacteria models evolution: From microalgae steady-state to integrated microalgae-bacteria wastewater treatment models – A comparative review. *Sci. Total Environ.* **607–608**:1136–1150.

Solimeno A, Parker L, Lundquist T, García J. 2017. Integral microalgae-bacteria model (BIO_ALGAE): Application to wastewater high rate algal ponds. *Sci. Total Environ.* **601–602**:646–657.

Spellman FR. 1999. Microbiology for Water and Wastewater Operators (Revised Reprint). CRC Press 228 p.

Stenstrom MK, Poduska RA. 1980. The effect of dissolved oxygen concentration on nitrification. *Water Res.* **14**:643–649.

Tang T, Fadaei H, Hu Z. 2014. Rapid evaluation of algal and cyanobacterial activities through specific oxygen production rate measurement. *Ecol. Eng.* **73**:439–445.

Wang L, Li T. 2015. Effects of seasonal temperature variation on nitrification, anammox process, and bacteria involved in a pilot-scale constructed wetland. *Environ. Sci. Pollut. Res. Int.* **22**:3774–3783.

Westerhoff P, Hu Q, Esparza-Soto M, Vermaas W. 2010. Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors. *Environ. Technol.* **31**:523–532.

Xin L, Hong-ying H, Yu-ping Z. 2011. Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. *Bioresour. Technol.* **102**:3098–3102.

Yun Y-S, Park JM. 2003. Kinetic modeling of the light-dependent photosynthetic activity of the green microalga Chlorella vulgaris. *Biotechnol. Bioeng.* **83**:303–311.

Zambrano J, Krustok I, Nehrenheim E, Carlsson B. 2016. A simple model for algae-bacteria interaction in photo-bioreactors. *Algal Res.* **19**:155–161.

9. Figures and Tables

Table 1. Composition of the waters used as effluent in the cultivation system.

Parameters	Primary domestic wastewater	Arnon Medium
pH	7.6±0.2	7.8±0.2
Conductivity, mS/cm^{-1}	1.8±0.3	2.3±0.2
Turbidity, FTU	16.9±0.5	0.0±0.0
SST, g/L	0.4±0.1	0.6±0.0
COD, mg/L	511.0±5.3	16.0±1.2
Sulphate, mg/L	98.1±6.4	6.3±0.8
Nitrogen-Nitrate, mg/L	23.2±1.7	139.9±3.1
Chloride, mg/L	411.6±23.5	78.9±2.1
Sodium, mg/L	222.5±12.1	276.1±7.9
Potassium, mg/L	8.6±1.6	325.1±6.3
Calcium, mg/L	30.1±0.2	364.9±5.5
Magnesium, mg/L	54.1±14.1	12.2±0.6
Phosphorus-Phosphate, mg/L	15.8±0.9	41.1±4.3
Nitrogen-Ammonium, mg/L	137.6±6.2	0.0±0.2
Iron, mg/L	0.19±0.01	5.0±0.3
Copper, mg/L	0.09±0.07	0.02±0.0
Manganese, mg/L	0.03±0.01	0.5±0.02
Zinc, mg/L	0.10±0.08	0.06±0.01
Boron, mg/L	0.35±0.09	0.4±0.03

Values correspond to the mean ± SD

Tabla 2. Values for the proposed model's parameter characteristics.

Microalgae net photosynthesis rate			**Heterotrophic respiration rate**			**Nitrifying respiration rate**		
Parameter	**Value**	**Units**	**Parameter**	**Value**	**Units**	**Parameter**	**Value**	**Units**
PO_2max	113	$mgO_2/g_{biomass}\cdot h$	RO_2max	5.4	$mgO_2/g_{biomass}\cdot h$	RO_2max	4.4	$mgO_2/g_{biomass}\cdot h$
Ik	168	$\mu E/m_2\cdot s$	Tmin	9	°C	Tmin	0	°C
n	1.7		Tmax	47	°C	Tmax	49	°C
Tmin	3.4	°C	Topt	36	°C	Topt	33.6	°C
Tmax	49	°C	pHmin	6		pHmin	2	
Topt	30	°C	pHmax	12		pHmax	13.4	
pHmin	1.8		pHopt	9		pHopt	9	
pHmax	12.9		$K_{S,DO2}$	1.98	mgO_2/L	$K_{S,DO2}$	1.08	mgO_2/L
pHopt	8.5					$K_{I,DO2}$	104.9	mgN/L
DO_2max	32	mgO_2/L						
m	4.15							
RO_2max	12.7	$mgO2/g_{biomass}\cdot h$						
RO_2min	3.4	$mgO2/g_{biomass}\cdot h$						
Ik_res	134	$\mu E/m^2\cdot s$						
n_r	1.4							

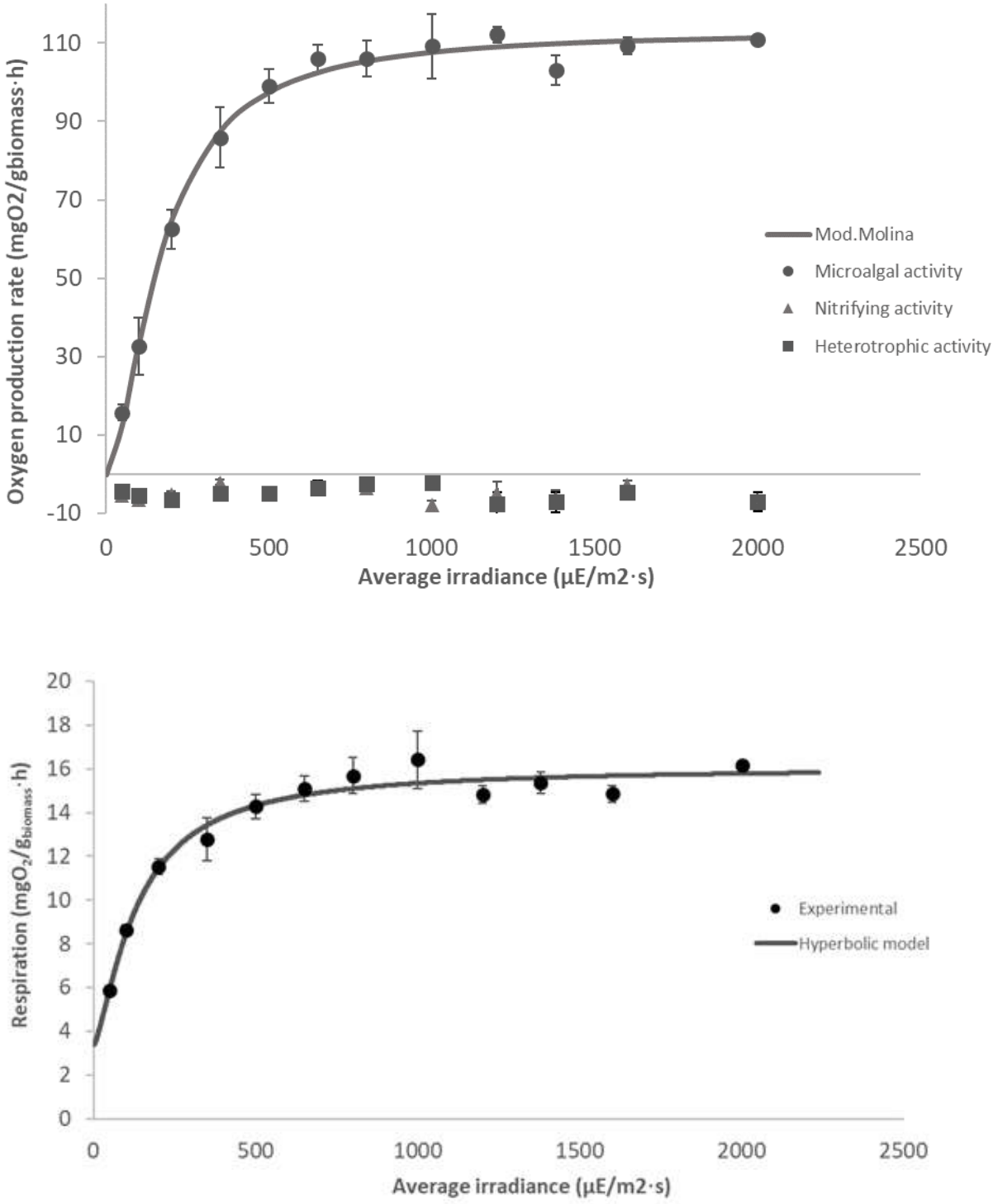


Figure 1. Influence of average irradiance in the culture on the oxygen production rate of microalgae, nitrifying bacteria and heterotrophic bacteria at 24 °C (A). Influence of average irradiance on the respiration rate of microalgae at 24 °C (B). Lines correspond to fit the proposed models (Eqs. (2,3)). Values correspond to the mean ± SD (n = 3).

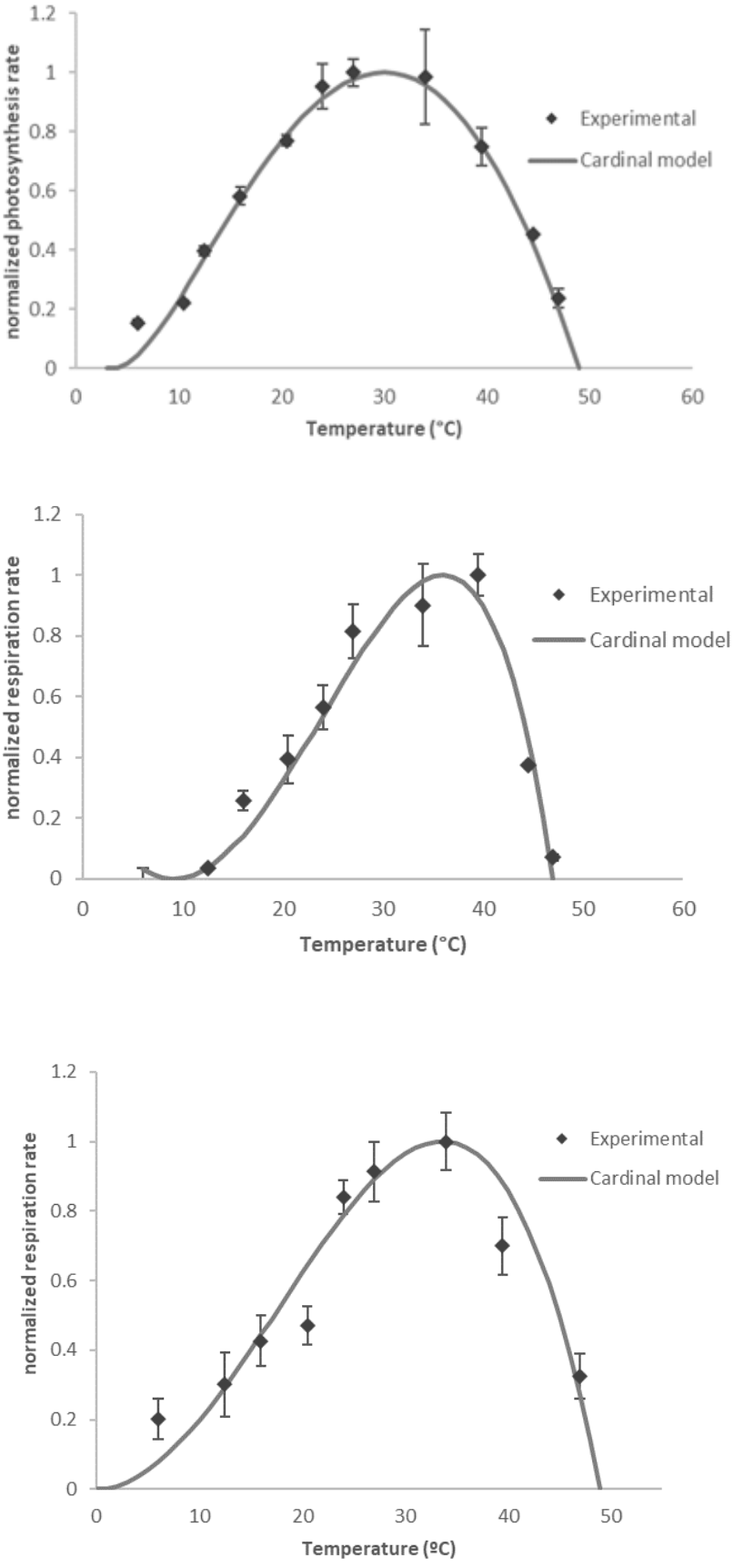
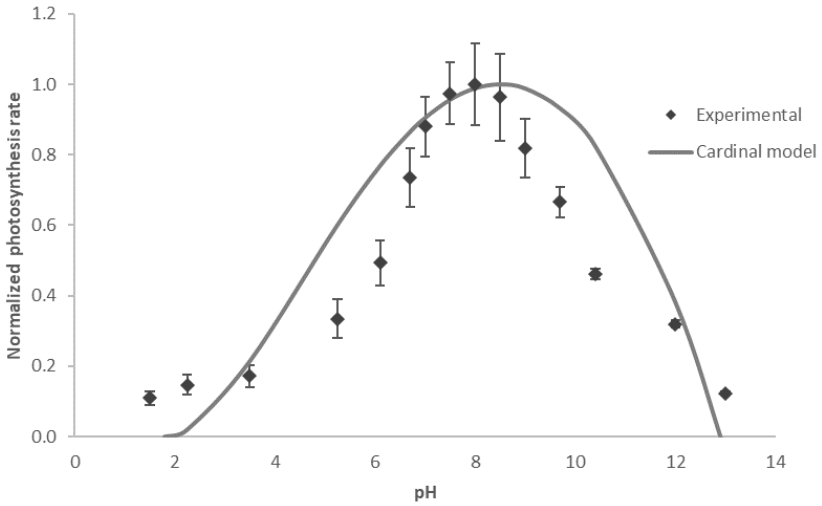
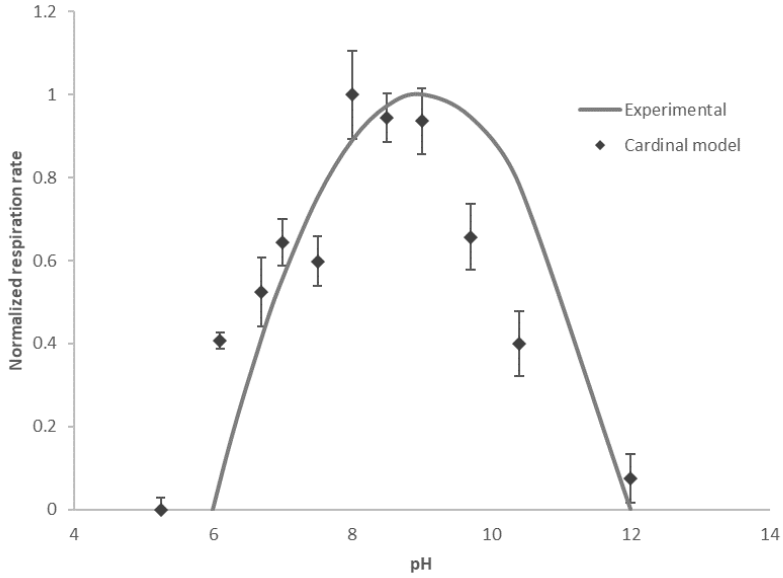


Figure 2. Influence of temperature on the normalized photosynthesis rate of microalgae (A), normalized heterotrophic bacteria respiration rate (B) and nitrifying bacteria respiration rate (C). Lines correspond to fit the proposed models (Eqs. (4)). Values correspond to the mean ± SD (n = 3).





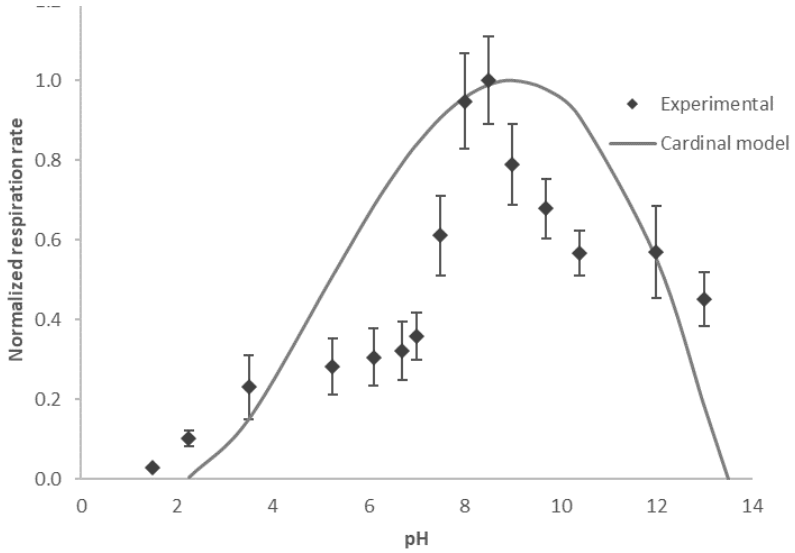


Figure 3. Influence of pH on the normalized photosynthesis rate of microalgae (A), normalized heterotrophic bacteria respiration rate (B) and normalized nitrifying bacteria respiration rate (C). Lines correspond to fit the proposed models (Eqs. (5)). Values correspond to the mean ± SD (n = 3).

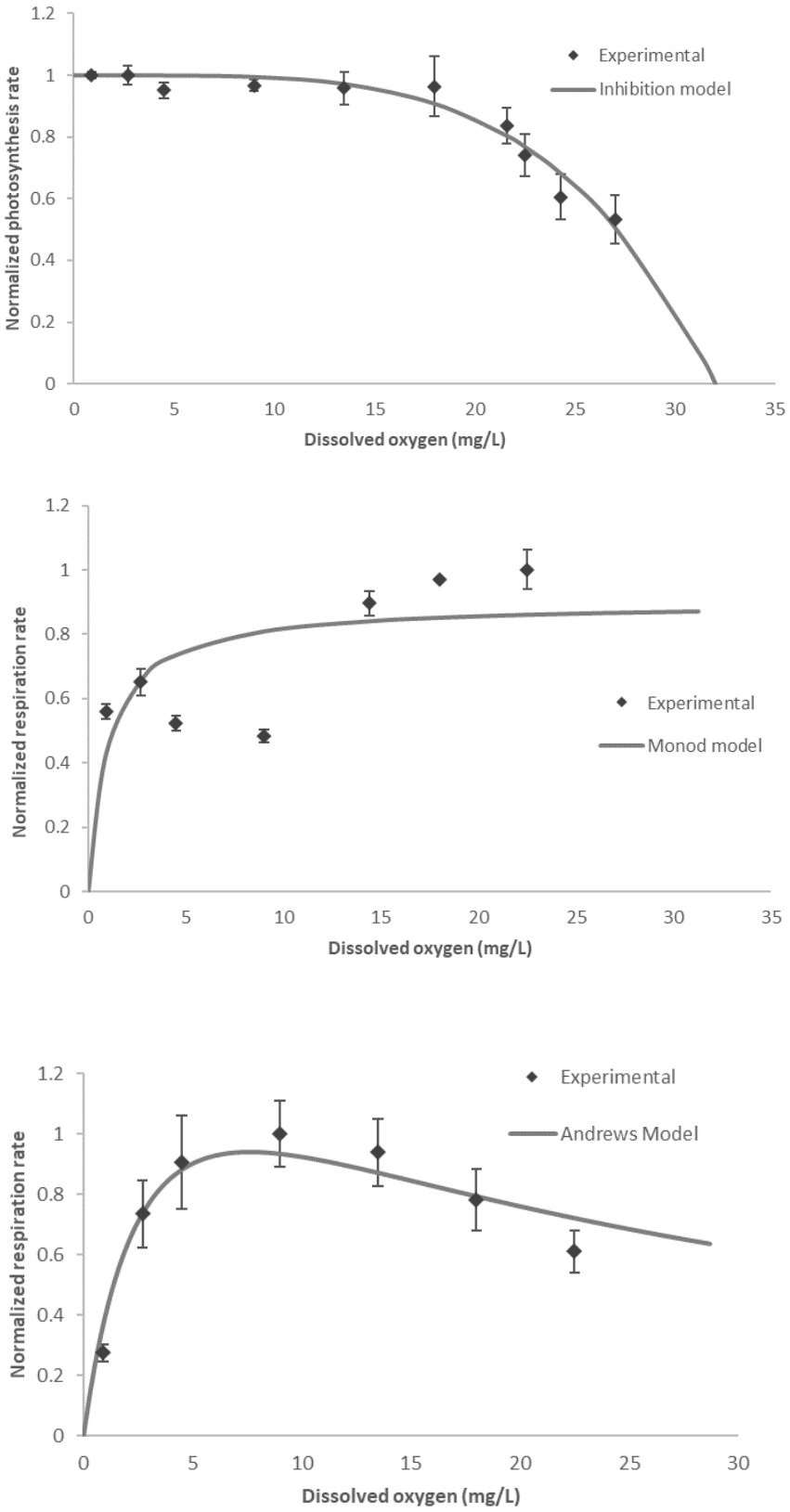


Figure 4. Influence of dissolved oxygen on the normalized photosynthesis rate of microalgae (A), normalized heterotrophic bacteria respiration rate (B) and normalized nitrifying bacteria respiration rate (C). Lines correspond to fit the proposed models (Eqs. (6-8)). Values correspond to the mean ± SD (n = 3).

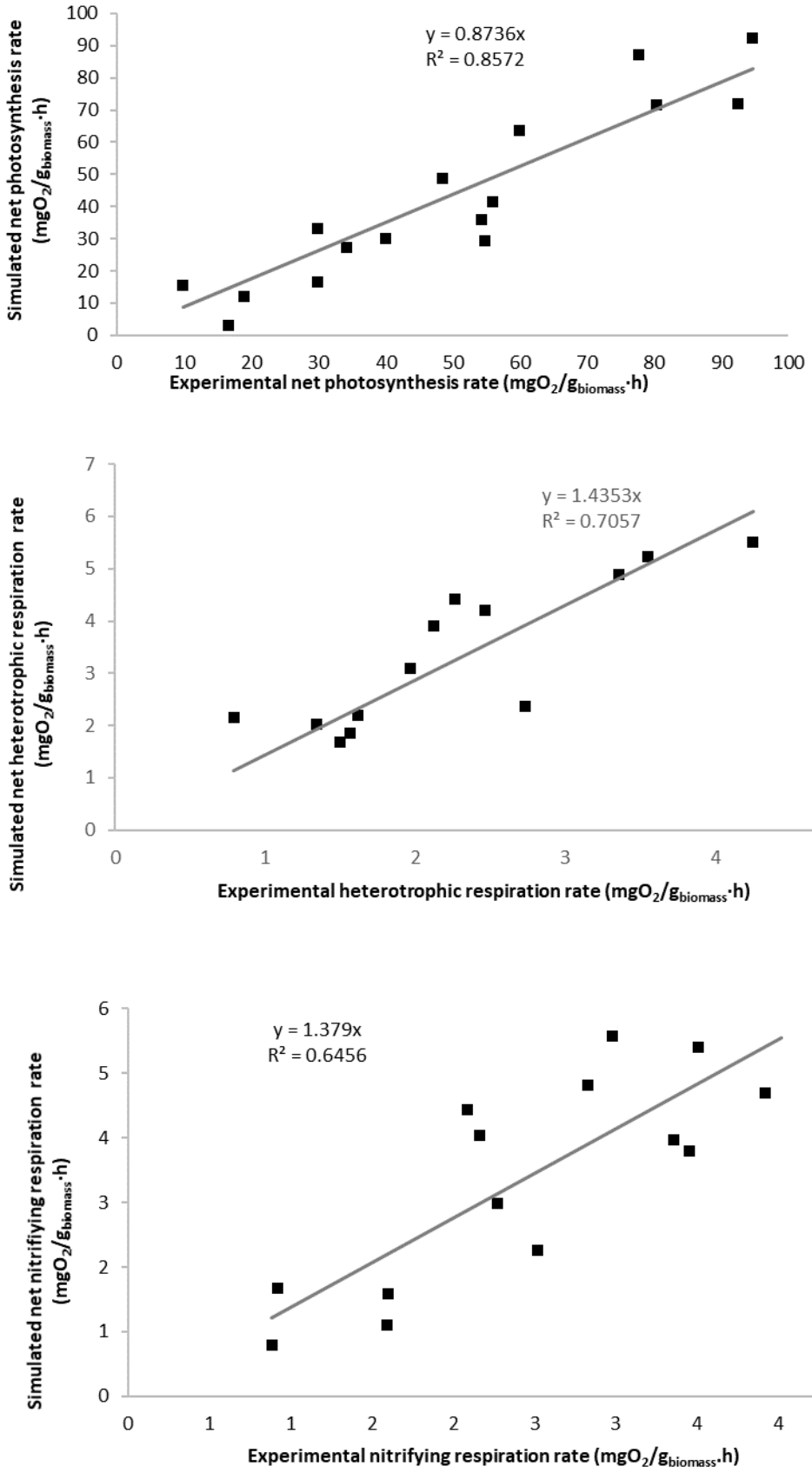


Figure 5. Correlation between the experimental and simulated values of (A) the net photosynthesis rate, (B) the heterotrophic bacteria respiration rate and (C) the nitrifying bacteria respiration rate.