On-line monitoring of photosynthetic activity based on pH data to assess microalgae cultivation

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Abstract

Microalgae performance of outdoor cultivation systems is influenced by environmental and operating dynamics. Monitoring and control systems are needed to maximise biomass productivity and nutrient recovery. The goal of this work was to corroborate that pH data could be used to monitor microalgae performance by means of data from an outdoor membrane photobioreactor (MPBR) plant. In this system, microalgae photosynthetic activity was favoured over other physical and biological processes, so that the pH data dynamics was theoretically related to the microalgae carbon uptake rate (CUR).

Short- and long-term continuous operations were tested to corroborate the relationship between the first derivate of pH data dynamics (pH´) and microalgae photosynthetic activity. Short-term operations showed a good correlation between gross pH´ values and MPBR performance. An indicator of the maximum daily average microalgae activity was assessed by a combination of on-line pH´ measurements obtained in the long-term
and a microalgae growth kinetic model. Both indicators contributed to the development of advanced real-time monitoring and control systems to optimise microalgae cultivation technology.

1. Introduction

Microalgae cultivation has been receiving increasing interest from the scientific community since it allows nutrient recovery, CO₂ biofixation and valorisation of the algal biomass produced (Guldhe et al., 2017). However, industrial microalgae cultivation plants are still scarce, mainly due to their low efficiency which increases operating costs (Acién et al., 2018). Improving the microalgae activity in photobioreactors (PBRs) and open ponds is likely to be a way of reducing these high costs (Salama et al., 2017). In this respect, some authors have evaluated indirect measurements to analyse the microalgae photosynthetic activity. By way of example, Perin et al. (2016) measured the chlorophyll fluorescence in vivo of *Nannochloropsis gaditana*. Romero-Villegas et al. (2018) used the maximum quantum yield (Fᵥ/Fₘ) to indirectly measure the photosynthetic activity; while Rossi et al. (2018) used standardised respirometric assays to evaluate microalgae and bacteria activity simultaneously. However, these off-line methods require a certain delay and cannot be monitored in real-time.

Microalgae activity can also be assessed by key performance indicators such as microalgae biomass productivity and nutrient recovery rates (González-Camejo et al., 2020a; Marazzi et al., 2019), for which suspended solids and nutrient concentrations must be measured. Although on-line probes and analysers can monitor ammonium, nitrate, and suspended solids concentrations, they usually have high capital and maintenance costs and are not always as reliable as expected (Havlik et al., 2013). For
this reason, they are often measured by time-consuming and expensive laboratory analyses (Foladori et al., 2018). Other parameters like pH, light and temperature are also highly related to microalgae growth (Abu-Ghosh et al., 2020; Robles et al., 2020). These variables are commonly measured by on-line sensors, which are reliable and involve lower costs (Ruano et al., 2009). Developing on-line monitoring strategies based on dynamic modelling of data obtained by cost-effective sensors would thus be of great interest. Some authors have made advances in this research field; for instance, Pawlowski et al. (2019) described a model-based control to regulate pH in raceway ponds; Robles et al. (2020) used pH and dissolved oxygen on-line sensors to describe the performance of a raceway pond during the start-up phase; De-Luca et al. (2018) proposed two optimisation approaches to prevent critical conditions caused by using inaccurate weather forecast; while Foladori et al. (2018) evaluated the nutrient removal of a microalgae-bacteria culture for lab-scale wastewater treatment by using pH, oxygen and oxidation-reduction potential sensors. However, long-term full-scale data to apply on-line monitoring strategies are still needed to make microalgae cultivation systems more efficient.

An approach based on pH data to on-line monitor microalgae photosynthetic activity in a membrane photobioreactor (MPBR) fed by sewage coming from an anaerobic membrane bioreactor (AnMBR) is proposed in this work. An indicator of instantaneous microalgae activity was obtained from these on-line pH measurements which could be used as an input for real-time short-term control. An indicator of the maximum microalgae activity was assessed by a combination of these on-line pH measurements and a microalgae growth kinetic model which provided the long-term monitoring and control of microalgae performance. These indicators would rapidly help to detect significant variations in the microalgae cultivation system.
2. Material and Methods

2.1. Membrane photobioreactor plant

The MPBR plant was operated outdoors in Valencia (Spain). Two methacrylate PBRs were connected to a membrane tank (MT) which allowed solids (SRT) and hydraulic retention time (HRT) to be decoupled. Two different systems were used: i) one containing 550-L PBRs (25-cm wide); and ii) another equipped with 230-L PBRs (10-cm wide).

The oxygen concentration in each PBR was always above saturation due to their continuous aeration system and the microalgae activity. Pure CO$_2$ (99.9%) was introduced into this system when the pH was over a set value of 7.5 to maintain it within a controlled range (i.e. 7.0-7.5). Adding CO$_2$ also avoided carbon limitation and limited phosphorus precipitation and ammonia volatilisation (Iasimone et al., 2018).

White LED lamps (Unique IP65, 40w) supplied a continuous irradiance of 300 μmol·m$^{-2}·$s$^{-1}$ on the back surface of each PBR.

The following on-line sensors were used to monitor the outdoor MPBR plant: i) one pH-temperature sensor (pHDsc DPD1R1, Hach Lange) and ii) one dissolved oxygen (LDOsc LXV416.99.20001, Hach Lange) sensor in each PBR; and iii) one sensor to measure the photosynthetically active radiation (PAR) on the PBR surface (Apogee Quantum SQ-200). To maintain the accuracy of the pH and oxygen sensors, they were calibrated every two weeks. In addition, the buffer and the salt bridge were replaced once a year. To perform process control and data acquisition, the sensors were connected to a PLC controlled by a SCADA system, which was fully described by Viruela et al. (2018).
2.1.2. Wastewater medium and microalgae

Microalgae were originally collected from the walls of the clarifiers of an urban WWTP as explained in González-Camejo et al. (2020b). They were cultivated using the effluent of an AnMBR plant (Durán et al., 2020), which nutrient concentrations are shown in Table A.1.

Green microalgae *Chlorella* and *Scenedesmus* were the main microorganisms of the culture according to microscopic observations. Variations in the culture strain composition were not considered to involve significant changes in MPBR performance (González-Camejo et al., 2019; 2020a; Sutherland et al., 2020). The inoculum also contained heterotrophic and nitrifying bacteria in negligible concentrations.

2.1.3. Operating conditions

Short- and long-term MPBR performance were evaluated with the goal of correlating pH data with instantaneous and daily average microalgae photosynthetic activity, respectively. Short-term continuous operation was assessed in the 10-cm MPBR plant for six days (SRT = 4.5 d; HRT = 1.25 d). The MPBR plant was operated long-term continuously from June 2015 to November 2017. In the 25-cm-wide MPBR plant, the HRT varied from 1 to 3.5 d, while SRT changed from 4.5 to 9 d. In the 10-cm-wide MPBR plant, HRT and SRT from 1 to 1.5 d and 2 to 4.5 d, respectively (Table A.2).

All the periods shown in Table A.2 began with a start-up stage which is described in González-Camejo et al. (2019). Periods used for maintenance labours and start-up stages were not considered in this long-term evaluation.

2.2. Analytical methods

Standard Methods (APHA, 2012) was followed to measure ammonium (4500-NH3-G), phosphate (4500-P-F), nitrite (4500-NO2-B) and nitrate (4500-NO3-H) concentrations.
To this aim, Smartchem 200 (Westco Scientific Instruments) was used. Volatile suspended solids (VSS) concentration of the MPBR was analysed by method 2540-E (APHA, 2012). Optical density of 680 nm (OD) was obtained using a fluorometer (AquaPen-C AP-C 100). Six respirometric tests which followed the protocol of Rossi et al. (2018), were done in a period of two weeks to assess the microalgae and nitrifying bacteria activity simultaneously.

2.3. pH monitoring

The pH control (see Section 2.1) was turned off each day for 30 minutes at midnight while keeping the constant artificial light supplied by the lamps (Section 2.1). Due to variations in the equilibrium of inorganic carbon species (Foladori et al., 2018), during this non-pH-controlled periods a lineal increase in pH values was observed (Figure A.1). The first derivative of this pH data dynamics (pH’) was used as an on-line monitoring parameter and depended on several factors:

- Microalgae photosynthetic activity (main factor), which in turn depends on other factors such as light irradiance, biomass concentration and pigment content amongst others (Fernández et al., 2016; Wagner et al., 2018). Theoretically, the faster the metabolic activity of microalgae, the faster inorganic carbon is consumed and the higher the pH’ (Eze et al., 2018; Robles et al., 2020).

- CO₂ stripping, which is related to the mass transfer efficiency, which in turn depends on bubble size, air flow rate, culture height, and pH set-point. All these parameters remained constant during MPBR operations, except for the air flow rate, which varied with the PBR light path (Table A.2).

- Temperature, which affects CO₂ stripping due to variations in CO₂ solubility in water. This variation was considered negligible since CO₂ solubility in the MPBR
plant (temperatures around 20-30 °C) only varied in the range of 0.13-0.17% (Perry et al., 1997). The PBRs were also closed to the atmosphere, which reduces CO₂ stripping.

- CO₂ production by heterotrophic bacteria. This was considered negligible due to the low BOD₅ entering the MPBR (11-13 mgO₂·L⁻¹), which favoured microalgae autotrophic growth (Rossi et al., 2020). In fact, microalgae biomass concentration was found to be directly related to microalgae cell concentration in the evaluated system (González-Camejo et al., 2020a), suggesting that microalgae were the predominant organism in the culture.

- Nitrifying bacteria activity, which affects pH since nitrification reduces the culture alkalinity (Foladori et al., 2018). However, nitrification was not considered relevant during the experimental period as the sum of nitrite and nitrate concentrations, which can be used as an indirect measure of nitrification rate (González-Camejo et al., 2020c) remained at concentrations lower than 10 mgN·L⁻¹. This was also corroborated by six respirometric tests (according to the protocol of Rossi et al. (2018)), in which oxygen uptake rate (OUR) of the nitrifying bacteria only reached an average of 4.4% of the oxygen production rate (OPR) of microalgae (Figure A.2).

- CO₂ production by microalgae respiration. However, microalgae respiration rate is usually associated with microalgae activity (Rossi et al., 2018). Indeed, the respirometric tests showed that the OUR due to microalgae respiration accounted for an average of 11% of the net microalgae OPR (p-value < 0.05; R² = 0.672; n = 6). Microalgae respiration was therefore not considered to significantly influence pH variation dynamics through the experimental period.
Summarising, microalgae activity was considered as the only factor related to carbon concentration variations which had a significant influence on pH dynamics in the MPBR plant. pH’ was thus theoretically related to the microalgae carbon uptake rate (CUR) in the evaluated system (Eq. 1).

\[ \text{CUR} \approx - \alpha_1 \cdot \text{pH'} \]  
(Eq. 1)

Where CUR (mgC·L\(^{-1}·d^{-1}\)) is the carbon uptake rate, pH’ is the first derivative from pH data dynamics (pHunit·d\(^{-1}\)), and \(\alpha_1\) is a distributed factor.

pH’ could be therefore assessed as an on-line measurement of the daily average microalgae CUR, which is in turn related to microalgae photosynthetic activity. It is important to note that if microalgae CUR is assessed from pH data in other cultivation system where factors different than photosynthetic activity are neither negligible nor constant, an adjustment in the model would be required.

2.3.1. Short-term pH monitoring

During the short-term period, the culture pH varied freely (no CO\(_2\) addition, Section 2.1) for 10 minutes each hour of the day to measure pH’. These pH’ calculations were used as an on-line indicator of the instantaneous microalgae activity under the system’s specific operating and environmental conditions.

2.3.2. Microalgae growth kinetic model

An indicator of the daily maximum microalgae activity was assessed using a model based on the pH’ calculations and previous results on microalgae activity modelling (Robles et al, 2020). As already mentioned, pH variations were mainly related to microalgae CUR in the evaluated system. Since CUR is usually related to the average light irradiance (\(I_{av}\)) by a hyperbolic function (Fernández et al., 2016; Tripathi and
Kumar, 2017), Eq. (2) was used to determine pH´ as a function of $I_{av}$ when considering constant respiration conditions, no nutrient limitation and non-inhibiting dissolved oxygen and pH conditions:

$$pH´ = pH´_{max} \cdot I_i + \alpha_2 \quad (\text{Eq. 2})$$

Where $pH´_{max}$ is the maximum pH´ ($\text{pH}_{\text{unit} \cdot \text{d}^{-1}}$), $I_i$ (i=1:3) is a given function related to the average light irradiance ($I_{av}$), and $\alpha_2$ is a distributed factor.

Eq. (2) is only valid under non-nutrient-limiting conditions. For this, pH´ values obtained under limiting nitrogen concentrations; i.e. under 10 mgN-L$^{-1}$ (González-Camejo et al., 2019), were discarded.

Three different normalising factors related to $I_{av}$ ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (I$_1$, I$_2$, and I$_3$) were tested. I$_1$ (Eq. 3) is analogous to the duty cycle (Robles et al., 2020), which is defined as the proportional time which microalgae receive light (Fernández-Sevilla et al., 2018). I$_2$ (Eq. 4) corresponds to a Monod-type factor in which $I_{av}$ is analogous to the substrate and PAR serve as “semisaturation constant” (Martínez et al., 2019). Lastly, I$_3$ is modified Monod-type factor obtained from Fernández et al. (2016) (Eq. 5).

$$I_1 = \frac{I_{av}}{\text{PAR}} \quad (\text{Eq. 3})$$

$$I_2 = \frac{I_{av}}{I_{av} + \text{PAR}} \quad (\text{Eq. 4})$$

$$I_3 = \frac{I_{av}^n}{k_i e^{m \cdot I_{av} + I_{av}^n}} \quad (\text{Eq. 5})$$

where PAR ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) corresponds to the total photosynthetically active radiation received by the PBR (i.e. solar light and irradiance from LED lamps), while $n$ (1.045), $m$ (0.0021) and $k_i$ (174 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) are form parameters reported by Fernández et al. (2016). The $I_{av}$ was calculated with the equations reported by González-Camejo et al. (2020a).
2.3.3. Normalised pH’ and microalgae performance indicators

Based on the above-mentioned kinetics, the relationships between factors derived from pH’ and performance indicators derived from biomass productivity (BP), N-recovery rate (NRR) and P-recovery rate (PRR) were assessed. For this, pH’, BP, NRR and PRR were normalised by either one or two factors related to microalgae activity: i.e. I₁ (I₁, I₂ or I₃), PAR, solar PAR (sPAR), OD or VSS. All the normalised parameters used for the long-term data evaluation are shown in Table A.3.

2.4. Statistical analysis

To assess the long-term data (n = 170), the Partial Least Squares (PLS) algorithm was applied. pH’ and its normalised parameters were used as predictors (X), while MPBR performance indicators and their normalised parameters were selected as responses (Y) (Table A.3). PLS was carried out by using the mix Omics library through R-software (version 3.2.3).

Long-term data were scaled to unit variance (and mean-centred) to equalise the weight of the variables in the PLS models (González-Camejo et al., 2020a).

3. Results and discussion

3.1. Short-term pH data

The main results from this short-term period are shown in Table 1. pH’ generally increased during daytime hours due to the rising solar PAR, usually reaching the maximum daily values around noon (Figure 1). An exception to the habitual behaviour was observed on day 2, when pH’ was higher in the early morning (low solar PAR) than at midday (maximum solar PAR) (Figure 1). This was probably due to the reduced microalgae activity from day 1 to day 2, when both biomass productivity and NRR
significantly fell (Table 1). It should be noted that pH´ values in darkness were quite high: 15-40 pHunits·d⁻¹ (Figure 1) for two reasons: i) the PBRs were lit by an additional source of artificial light (Section 2.1), and ii) carbon absorption of microalgae takes place in the photosynthesis dark reactions, i.e. there is no need for light irradiance to modify the pH (Manhaeghe et al., 2019).

The highest pH´ values, i.e. 35-45 pHunit·d⁻¹ (Figure 1) occurred at the beginning of the short-term operations. Since the short-term period was preceded by a start-up phase (González-Camejo et al., 2019), microalgae were expected to be more active at this point. The MPBR plant thus showed the highest NRR (26.3 mgN·L⁻¹·d⁻¹) and biomass productivity (284 mgVSS·L⁻¹·d⁻¹) on day 1.

However, from midday of day 2 until the beginning of day 5 (hour 110), pH´ remained at low values in the range of 17-23 pHunit·d⁻¹ (Figure 1). This trend was corroborated by reduced biomass productivity from 170 to 139 mgVSS·L⁻¹·d⁻¹ in days 2-5; while NRR fell from 22.9 to 16.4 mgN·L⁻¹·d⁻¹ in the same period. Later, pH´ rose again, but not as much as at the beginning, i.e. values of 25-33 pHunit·d⁻¹ during hours 110-140 (Figure 1). In this case microalgae performance slightly increased: from 16.4 mgN·L⁻¹·d⁻¹ in day 5 to 18.1 mgN·L⁻¹·d⁻¹ in day 6 for NRR and from 139 mgVSS·L⁻¹·d⁻¹ to 148 mgVSS·L⁻¹·d⁻¹ for biomass productivity in the same period. NRR and biomass productivity therefore seemed to be directly related to gross pH´ values (and hence to gross CUR) in the short term, showing a good correlation, i.e. $R^2$ of 0.895 and 0.820 (n = 4) for NRR and BP, respectively. Gross pH´ can thus be a good indicator of punctual microalgae photosynthetic activity in this system. pH´ would hence allow on-line monitoring of microalgae performance at any time of the cultivation process.

Regarding PRR, it followed a different trend than biomass productivity and NRR (Table 1). It is possible that phosphorus luxury uptake (Solovchenko et al., 2019) and/or...
phosphorus precipitation would have had a significant influence on this short-term assessment. In fact, in this period hydroxyapatite (HAP) and octacalcium phosphate (OCP) were oversaturated (Table A.4 and A.5), which made them likely to precipitate. Phosphorus uptake was therefore not directly related to the photosynthetic activity in the short term.

3.2. Long-term validation of pH data

3.2.1. MPBR performance

The MPBR plant functionally operated under variable ambient and operating conditions (see Table A.2) for 310 days in the 25-cm MPBR plant and 225 days in the 10-cm MPBR plant. To assess the performance of each MPBR plant, NRR, PRR and biomass productivity were used. In the 25-cm MPBR plant, nutrient recovery rates (Figure 2a) varied in the range of 4-15 mgN·L⁻¹·d⁻¹ and 0.2-2 mgP·L⁻¹·d⁻¹, while productivity (Figure 2b) was around 40-115 mgVSS·L⁻¹·d⁻¹. In the 10-cm MPBR plant, those parameters rose to 10-35 mgN·L⁻¹·d⁻¹, 0.8-5 mgP·L⁻¹·d⁻¹ and 110-300 mgVSS·L⁻¹·d⁻¹, respectively (Figure 2c;2d). The 10-cm plant thus showed significantly better results than the 25-cm plant. Further information about differences in MPBR performance of these systems can be found in González-Camejo et al. (2020c).

It must be highlighted that the theoretical correlation between the evolution of pH´ and performance indicators during continuous operation is hardly observed in Figure 2, probably because there were other factors that could have affected it. For this reason, the correlation between pH´ measurements with the performance indicators needs to be corroborated by statistical analyses such as PLS (see Sections 3.2.2 and 3.3).
3.2.2. Screening and classification of pH data

A preliminary PLS analysis was performed to corroborate the use of pH´ as on-line microalgae CUR measurement as predicted in Eq. (1). The pH´ values and pH´ normalised by light and/or biomass concentration were used as predictors. The analogous normalised parameters of NRR, PRR and BP were employed as responses (Table A.3). This preliminary PLS analysis (data not shown) allowed for the screening of the following variables:

- Parameters normalised by OD and VSS were closely related in all cases, which agrees with previous results that reported high correlation between these parameters (González-Camejo et al., 2020a; 2020c). The parameters normalised by VSS were thus discarded and those normalised by OD were selected for further evaluation. OD was the preferred option since it is related to the chlorophyll content of microalgae (González-Camejo et al., 2020a; Markou et al., 2017). However, VSS considers other microorganisms’ biomass, not only microalgae (Di Caprio, 2020). In addition, OD can be monitored on-line (Havlík et al., 2013; Lucker et al., 2014) but VSS is usually obtained off-line (APHA, 2012).

- Parameters normalised by I₁ and I₂ gave similar results, obtaining a slight better correlation with I₂. In this respect, de Farias Silva et al. (2020) reported that the Monod model can be applied when no more than a limiting substrate is used. In this study, only light was considered a limiting factor (see Section 2.3.2). I₂ was thus selected for further assessment while I₁ was discarded.

- As the PBRs were supplied with constant artificial light, PAR and sPAR presented similar variability. For this, only the parameters normalised by PAR were considered for further evaluation.
After this screening, a single PLS model was created using all the data (n = 170) from both MPBR plants (10-cm and 25-cm plants). Three principal components (PCs) accounted for the cumulative explained variance of 90.8%, which were from PC1 (37.2%), PC2 (35.0%) and PC3 (18.6%). Figure 3a and 3b show that pH´ is significantly correlated to MPBR performance in terms of NRR, PRR and BP, since these indicators are close together in the plot. Gross pH´ was thus confirmed as a valid parameter to monitor MPBR performance. It should be noted that the pH´ parameters normalised by Iᵢ, PAR and OD also showed a good correlation with their associated normalised performance indicators. The PLS results thus corroborate pH´ being a good parameter for on-line monitoring the long-term MPBR operation under variable environmental and operating conditions.

It should be noted that two discernible groups of data were found in both the X and Y blocks (Figure 3c and 3d) from both plants: 25-cm (samples 1-88, blue numbers) and 10-cm MPBR plant (samples 88-170, orange numbers). These results confirmed their different performance regarding the parameters analysed in the model. Indeed, Figure 2 shows different pH´ ranges for both MPBR systems, i.e. 4-18 pHun it·d⁻¹ and 8-25 pHunit·d⁻¹ for 25-cm and 10-cm MPBR plant, respectively. Apart from the different microalgae performance obtained in both systems (as previously reported in González-Camejo et al. (2020c)), these differences in pH´ values could also have been influenced by the different air flow rate supplied to the PBRs (Table A.2). For this, analysing the data obtained from each plant separately would better assess the potential of pH´ data for monitoring their performance.
According to the data screening explained in Section 3.2.2, pH′, pH′:OD, pH′:PAR, pH′:I$_2$, pH′:I$_3$, pH′:PAR:OD, pH′:I$_2$:OD and pH′:I$_3$:OD were used as predictors (X) in the following PLS analyses, while analogous normalised parameters related to NRR, PRR and biomass productivity were used in the Y-axis. Two additional PLS analyses were carried out: one for data from the 25-cm plant (n = 88) and another for the 10-cm plant (n = 82). For the 10-cm plant, three PCs accounted for 98.7% of the cumulative explained variance for PC1 (45.4%), PC2 (30.4%) and PC3 (22.9%). For the 25-cm plant, three PCs attained 99.1% of the cumulative explained variance, in which PC1, PC2 and PC3 accounted for 65.2%, 24.2% and 9.7%, respectively. As can be seen in Figure 4, in both plants normalised pH′ parameters showed better correlation with their normalised performance indicators than the non-normalised parameters as they are generally closer in the plots. These results therefore suggest that normalising pH′, BP, NRR and PRR to monitor maximum daily average microalgae activity can provide more reliable results to evaluate these microalgae cultivation systems than non-normalised factors. It should be remembered that the correlation between the normalised pH′ predictors and normalised PRR responses was usually less significant than the correlation with the normalised NRR and biomass productivity responses (Figure 4) probably influenced by phosphorus uptake being dependent on the intracellular phosphorus concentration (Solovchenko et al., 2019) and the possibility of phosphorus precipitation by means of HAP and OCP (Table A.4 and A.5) (not considered in this study). The PLS model for the 10-cm MPBR plant showed in general closer correlations between normalised pH′ and performance indicators than the 25-cm plant (Figure 4). It must be considered that there were some experimental periods operated at long SRT
the latter plant during which grazers and other organisms proliferated (González-Camejo et al., 2019). This varied the relationship between OD and VSS (Figure A.3) and could probably have had an influence on the relationship among the parameters evaluated.

It should also be noted that the closest correlations were obtained with the parameters normalised by $I_2$ or $I_3$ (which depend on $I_{av}$), although this correlation was similar to those between parameters normalised by $I_2$ and OD or by $I_3$ and OD (Figure 4). On the other hand, the parameters normalised by PAR displayed less significant correlations in both plants (Figure 4). This was probably due to light attenuation within the culture.

Light transmittance is exponentially reduced along the PBR mainly due to the light absorbed by the photosynthetic microalgae pigments (Wagner et al., 2018). Consequently, the same PAR on the PBR surface can supply significantly different $I_{av}$ values according to the culture characteristics (González-Camejo et al., 2020c; Romero-Villegas et al., 2018). $I_{av}$ thus appears as a relevant factor in the model. On the other hand, normalising by OD showed a good correlation between the parameters analysed but did not improve the correlation between parameters in comparison to $I_2$ and $I_3$. This was probably because OD is closely related to $I_{av}$ (Barbera et al., 2020).

The parameters normalised by $I_2$ showed a slightly better correlation than those normalised by $I_3$ in the 10-cm plant (Figure 4a and 4b). However, the correlation between parameters normalised by $I_2$ in the 25-cm plant was quite similar to the correlation between parameters normalised by $I_3$ (Figure 4c and 4d). It should be considered that the $I_3$ factor was obtained from a dynamic model used for raceway reactors (Fernández et al., 2016) which depths are usually around 15-45 cm (Arbib et al., 2017) unlike flat-panel PBRs, which usually present light paths of around 1-10 cm (Slegers et al., 2011). This model was thus likely to fit the 25-cm plant better than the
10-cm plant. To sum up, the results obtained in this study suggest that pH´ values can be used to monitor the maximum carbon assimilation capacity of microalgae in continuous long-term MPBR operations.

4. Conclusions

pH data were used to on-line monitor microalgae photosynthetic activity in an MPBR system. Short-term operations showed a relationship between on-line pH´ values and MPBR performance in terms of NRR and BP. Gross pH´ measurements were therefore identified as indicators of the microalgae photosynthetic activity dynamics throughout the day. Long-term operations showed a relationship between on-line pH´ measurements and microalgae performance indicators (i.e. BP, NRR and PRR), all of them normalised by considering a microalgae growth kinetic model. pH´ was therefore also identified as an indicator of daily maximum microalgae activity. This pH´ parameter could hence be used in advanced real-time monitoring and control strategies for MPBR optimisation.

Acknowledgments

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E-supplementary data can be found in on-line version of the manuscript.
References


Figure 1. Evolution of the first derivative of pH dynamics (pH') and solar PAR during: a) 6 days of continuous short-term operation; b) day 3.
Figure 2. Evolution during continuous operation of the 25-cm MPBR plant of: a) pH’, NRR and PRR; b) pH’ and biomass productivity; evolution during continuous operation of the 10-cm MPBR plant of: c) pH’, NRR and PRR; d) pH’ and biomass productivity.
Figure 3: Results of the PLS analysis (n = 170). Correlation circle plots from the integration of the selected predictors (pH’ and normalised predictors); and responses (NRR, PRR, BP and their normalised parameters): a) PC-1 and PC-2; b) PC-1 and PC-3; score plot of the preliminary PLS model: c) Predictors (X) and d) Responses (Y). Blue numbers (1-88): 25-cm MPBR plant; Orange numbers (89-170) 10-cm MPBR plant.
Figure 4. PLS analyses. Correlation circle plots from the integration of the selected predictors (pH’ and normalise predictors); and responses (NRR, PRR, BP and their normalised parameters): a and b) 10-cm MPBR plant (n = 82); c and d) 25-cm MPBR plant (n = 88).
Table 1. Mean values of the short-term operation of the MPBR plant

<table>
<thead>
<tr>
<th>Day</th>
<th>Solar PAR (µmol·m⁻²·s⁻¹)</th>
<th>pH' (pH unit·d⁻¹)</th>
<th>BP (mg VSS·L⁻¹·d⁻¹)</th>
<th>NRR (mg N·L⁻¹·d⁻¹)</th>
<th>PRR (mg P·L⁻¹·d⁻¹)</th>
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<tr>
<td>1</td>
<td>227 ± 279</td>
<td>39.8 ± 2.9</td>
<td>284</td>
<td>26.3</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>237 ± 278</td>
<td>39.9 ± 8.7</td>
<td>170</td>
<td>22.9</td>
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</tr>
<tr>
<td>3</td>
<td>214 ± 294</td>
<td>29.0 ± 2.3</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4</td>
<td>238 ± 283</td>
<td>19.3 ± 2.2</td>
<td>-</td>
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</tr>
<tr>
<td>5</td>
<td>232 ± 276</td>
<td>19.6 ± 2.8</td>
<td>138</td>
<td>16.4</td>
<td>3.3</td>
</tr>
<tr>
<td>6</td>
<td>223 ± 278</td>
<td>21.7 ± 2.5</td>
<td>148</td>
<td>18.1</td>
<td>2.9</td>
</tr>
</tbody>
</table>

*PAR: photosynthetically active radiation; pH': first derivative from pH data dynamics; BP: biomass productivity; NRR: nitrogen recovery rate; PRR: phosphorus recovery rate.*