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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, CO_2 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 (F_v/F_m) 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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).
- CO_2 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 CO_2 stripping due to variations in CO_2 solubility in water. This variation was considered negligible since CO_2 solubility in the MPBR

plant (temperatures around 20-30 ºC) only varied in the range of 0.13-0.17% (Perry et al., 1997). The PBRs were also closed to the atmosphere, which reduces CO_2 stripping.

- CO_2 production by heterotrophic bacteria. This was considered negligible due to the low BOD_5 entering the MPBR (11-13 $mgO_2 \cdot L^{-1}$), 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 oncentrations, which can be used as an indirect measure of nitrification rate (González-Camejo et al., 2020c) remained at concentrations lower than 10 $mgN \cdot L^{-1}$. 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).
- CO_2 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; $R^2 = 0.672$; 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).

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

Where CUR ($mgC \cdot L^{-1} \cdot d^{-1}$) is the carbon uptake rate, pH´ is the first derivative from pH data dynamics ($pHunit \cdot d^{-1}$), and α_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:

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

Where pH'_{max} is the maximum pH´ (pHunit·d^{-1}), I_i (i=1:3) is a given function related to the average light irradiance (I_{av}), and α_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} (µmol·m^{-2}·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).

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

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

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

where PAR (µmol·m^{-2}·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 µmol·m^{-2}·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).

224 2.3.3. Normalised pH´ and microalgae performance indicators

225 Based on the above-mentioned kinetics, the relationships between factors derived from
226 pH' and performance indicators derived from biomass productivity (BP), N-recovery
227 rate (NRR) and P-recovery rate (PRR) were assessed. For this, pH´, BP, NRR and PRR
228 were normalised by either one or two factors related to microalgae activity: i.e. I_i (I_1, I_2
229 or I_3), PAR, solar PAR (sPAR), OD or VSS. All the normalised parameters used for the
230 long-term data evaluation are shown in Table A.3.

231

232 *2.4. Statistical analysis*

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

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

240

241 **3. Results and discussion**

242 *3.1. Short-term pH data*

243 The main results from this short-term period are shown in Table 1. pH´ generally
244 increased during daytime hours due to the rising solar PAR, usually reaching the
245 maximum daily values around noon (Figure 1). An exception to the habitual behaviour
246 was observed on day 2, when pH´ was higher in the early morning (low solar PAR) than
247 at midday (maximum solar PAR) (Figure 1). This was probably due to the reduced
248 microalgae activity from day 1 to day 2, when both biomass productivity and NRR

249 significantly fell (Table 1). It should be noted that pH´ values in darkness were quite
250 high: 15-40 pHunits·d^{-1} (Figure 1) for two reasons: i) the PBRs were lit by an additional
251 source of artificial light (Section 2.1), and ii) carbon absorption of microalgae takes
252 place in the photosynthesis dark reactions, i.e. there is no need for light irradiance to
253 modify the pH (Manhaeghe et al., 2019).
254 The highest pH´ values, i.e. 35-45 pHunit·d^{-1} (Figure 1) occurred at the beginning of the
255 short-term operations. Since the short-term period was preceded by a start-up phase
256 (González-Camejo et al., 2019), microalgae were expected to be more active at this
257 point. The MPBR plant thus showed the highest NRR (26.3 mgN·L^{-1}·d^{-1}) and biomass
258 productivity (284 mgVSS·L^{-1}·d^{-1}) on day 1.
259 However, from midday of day 2 until the beginning of day 5 (hour 110), pH´ remained
260 at low values in the range of 17-23 pHunit·d^{-1} (Figure 1). This trend was corroborated
261 by reduced biomass productivity from 170 to 139 mgVSS·L^{-1}·d^{-1} in days 2-5; while
262 NRR fell from 22.9 to 16.4 mgN·L^{-1}·d^{-1} in the same period. Later, pH´ rose again, but
263 not as much as at the beginning, i.e. values of 25-33 pHunit·d^{-1} during hours 110-140
264 (Figure 1). In this case microalgae performance slightly increased: from 16.4 mgN·L^{-1}·d^{-1}
265 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
266 mgVSS·L^{-1}·d^{-1} for biomass productivity in the same period. NRR and biomass
267 productivity therefore seemed to be directly related to gross pH´ values (and hence to
268 gross CUR) in the short term, showing a good correlation, i.e. R^2 of 0.895 and 0.820 (n
269 = 4) for NRR and BP, respectively. Gross pH´ can thus be a good indicator of punctual
270 microalgae photosynthetic activity in this system. pH´ would hence allow on-line
271 monitoring of microalgae performance at any time of the cultivation process.
272 Regarding PRR, it followed a different trend than biomass productivity and NRR (Table
273 1). It is possible that phosphorus luxury uptake (Solovchenko et al., 2019) and/or

274 phosphorus precipitation would have had a significant influence on this short-term
275 assessment. In fact, in this period hydroxyapatite (HAP) and octacalcium phosphate
276 (OCP) were oversaturated (Table A.4 and A.5), which made them likely to precipitate.
277 Phosphorus uptake was therefore not directly related to the photosynthetic activity in
278 the short term.
279
280 *3.2. Long-term validation of pH data*
281 3.2.1. MPBR performance
282 The MPBR plant functionally operated under variable ambient and operating conditions
283 (see Table A.2) for 310 days in the 25-cm MPBR plant and 225 days in the 10-cm
284 MPBR plant. To assess the performance of each MPBR plant, NRR, PRR and biomass
285 productivity were used. In the 25-cm MPBR plant, nutrient recovery rates (Figure 2a)
286 varied in the range of 4-15 $mgN \cdot L^{-1} \cdot d^{-1}$ and 0.2-2 $mgP \cdot L^{-1} \cdot d^{-1}$, while productivity
287 (Figure 2b) was around 40-115 $mgVSS \cdot L^{-1} \cdot d^{-1}$. In the 10-cm MPBR plant, those
288 parameters rose to 10-35 $mgN \cdot L^{-1} \cdot d^{-1}$, 0.8-5 $mgP \cdot L^{-1} \cdot d^{-1}$ and 110-300 $mgVSS \cdot L^{-1} \cdot d^{-1}$,
289 respectively (Figure 2c;2d). The 10-cm plant thus showed significantly better results
290 than the 25-cm plant. Further information about differences in MPBR performance of
291 these systems can be found in González-Camejo et al. (2020c).
292 It must be highlighted that the theoretical correlation between the evolution of pH´ and
293 performance indicators during continuous operation is hardly observed in Figure 2,
294 probably because there were other factors that could have affected it. For this reason,
295 the correlation between pH´ measurements with the performance indicators needs to be
296 corroborated by statistical analyses such as PLS (see Sections 3.2.2 and 3.3).
297

298 3.2.2. Screening and classification of pH data

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

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

314 ▪ Parameters normalised by I_1 and I_2 gave similar results, obtaining a slight better
315 correlation with I_2. In this respect, de Farias Silva et al. (2020) reported that the
316 Monod model can be applied when no more than a limiting substrate is used. In this
317 study, only light was considered a limiting factor (see Section 2.3.2). I_2 was thus
318 selected for further assessment while I_1 was discarded.

319 ▪ As the PBRs were supplied with constant artificial light, PAR and sPAR presented
320 similar variability. For this, only the parameters normalised by PAR were
321 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 I_i, 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 pHunit·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.

345 3.2.3. pH data evaluation and validation

346 According to the data screening explained in Section 3.2.2, pH´, pH´:OD, pH´:PAR,
347 pH´:I_2, pH´:I_3, pH´:PAR:OD, pH´:I_2:OD and pH´:I_3:OD were used as predictors (X) in
348 the following PLS analyses, while analogous normalised parameters related to NRR,
349 PRR and biomass productivity were used in the Y-axis. Two additional PLS analyses
350 were carried out: one for data from the 25-cm plant (n = 88) and another for the 10-cm
351 plant (n = 82). For the 10-cm plant, three PCs accounted for 98.7% of the cumulative
352 explained variance for PC1 (45.4%), PC2 (30.4%) and PC3 (22.9%). For the 25-cm
353 plant, three PCs attained 99.1% of the cumulative explained variance, in which PC1,
354 PC2 and PC3 accounted for 65.2%, 24.2% and 9.7%, respectively.

355 As can be seen in Figure 4, in both plants normalised pH´ parameters showed better
356 correlation with their normalised performance indicators than the non-normalised
357 parameters as they are generally closer in the plots. These results therefore suggest that
358 normalising pH´, BP, NRR and PRR to monitor maximum daily average microalgae
359 activity can provide more reliable results to evaluate these microalgae cultivation
360 systems than non-normalised factors. It should be remembered that the correlation
361 between the normalised pH´ predictors and normalised PRR responses was usually less
362 significant than the correlation with the normalised NRR and biomass productivity
363 responses (Figure 4) probably influenced by phosphorus uptake being dependent on the
364 intracellular phosphorus concentration (Solovchenko et al., 2019) and the possibility of
365 phosphorus precipitation by means of HAP and OCP (Table A.4 and A.5) (not
366 considered in this study).

367 The PLS model for the 10-cm MPBR plant showed in general closer correlations
368 between normalised pH´ and performance indicators than the 25-cm plant (Figure 4). It
369 must be considered that there were some experimental periods operated at long SRT in

370 the latter plant during which grazers and other organisms proliferated (González-
371 Camejo et al., 2019). This varied the relationship between OD and VSS (Figure A.3)
372 and could probably have had an influence on the relationship among the parameters
373 evaluated.
374 It should also be noted that the closest correlations were obtained with the parameters
375 normalised by I_2 or I_3 (which depend on I_{av}), although this correlation was similar to
376 those between parameters normalised by I_2 and OD or by I_3 and OD (Figure 4). On the
377 other hand, the parameters normalised by PAR displayed less significant correlations in
378 both plants (Figure 4). This was probably due to light attenuation within the culture.
379 Light transmittance is exponentially reduced along the PBR mainly due to the light
380 absorbed by the photosynthetic microalgae pigments (Wagner et al., 2018).
381 Consequently, the same PAR on the PBR surface can supply significantly different I_{av}
382 values according to the culture characteristics (González-Camejo et al., 2020c; Romero-
383 Villegas et al., 2018). I_{av} thus appears as a relevant factor in the model. On the other
384 hand, normalising by OD showed a good correlation between the parameters analysed
385 but did not improve the correlation between parameters in comparison to I_2 and I_3. This
386 was probably because OD is closely related to I_{av} (Barbera et al., 2020).
387 The parameters normalised by I_2 showed a slightly better correlation than those
388 normalised by I_3 in the 10-cm plant (Figure 4a and 4b). However, the correlation
389 between parameters normalised by I_2 in the 25-cm plant was quite similar to the
390 correlation between parameters normalised by I_3 (Figure 4c and 4d). It should be
391 considered that the I_3 factor was obtained from a dynamic model used for raceway
392 reactors (Fernández et al., 2016) which depths are usually around 15-45 cm (Arbib et
393 al., 2017) unlike flat-panel PBRs, which usually present light paths of around 1-10 cm
394 (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.

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

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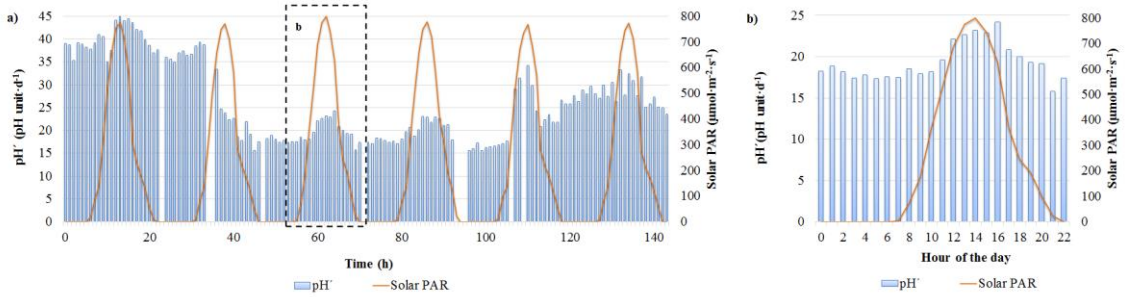


573

574 *Figure 1. Evolution of the first derivate of pH dynamics (pH´) and solar PAR during: a)*

575 *6 days of continuous short-term operation; b) day 3.*

576

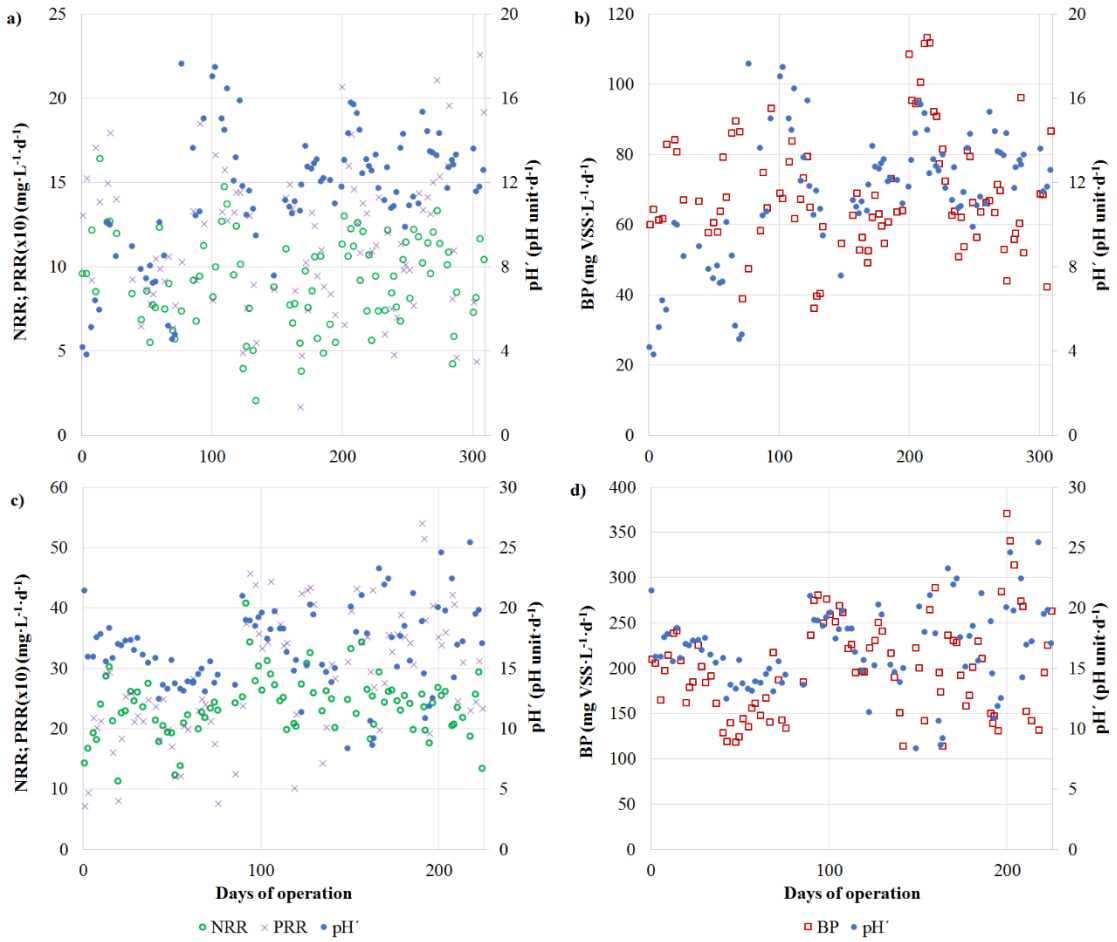
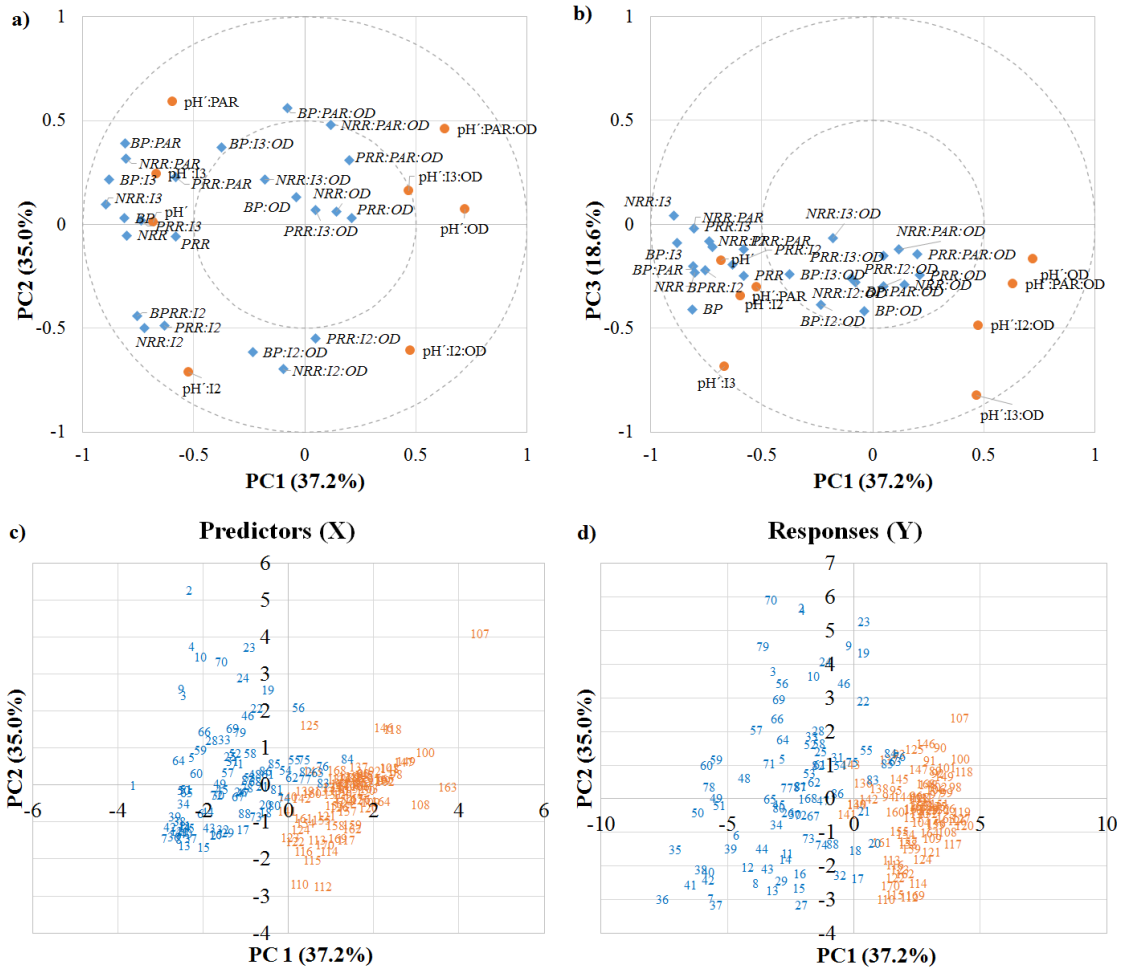


Figure 2. Evolution during continuous operation of the 25-cm MPBR plant of: a) pH´, NRR and PRR; b) pH´ and biomass productivity; evolution during continuous operation of the 10-cm MPBR plant of: c) pH´, NRR and PRR; d) pH´ and biomass productivity.



582

583 *Figure 3: Results of the PLS analysis (n = 170). Correlation circle plots from the*

584 *integration of the selected predictors (pH´ and normalised predictors); and responses*

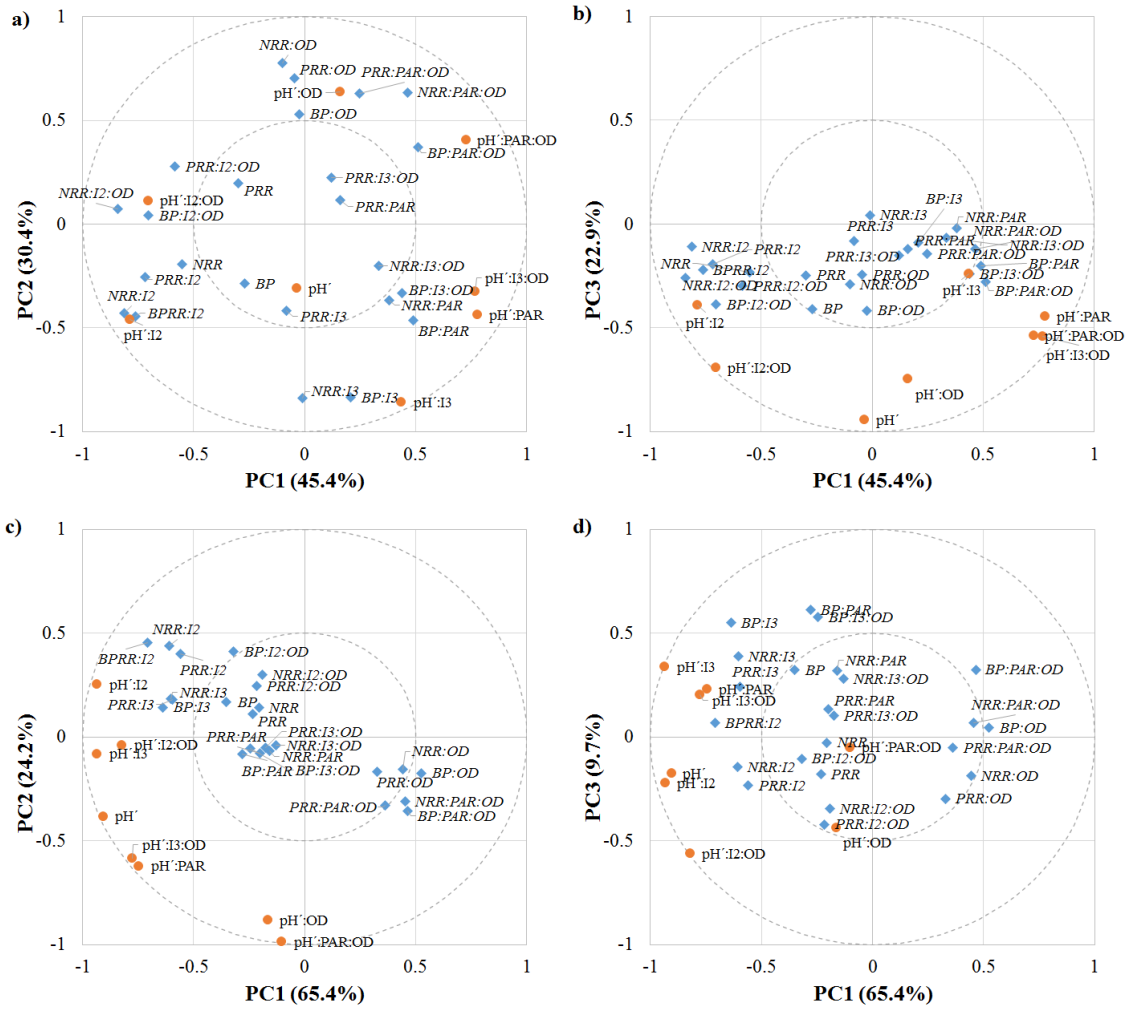
585 *(NRR, PRR, BP and their normalised parameters): a) PC-1 and PC-2; b) PC-1 and PC-*

586 *3; score plot of the preliminary PLS model: c) Predictors (X) and d) Responses (Y).*

587 *Blue numbers (1-88): 25-cm MPBR plant; Orange numbers (89-170) 10-cm MPBR*

588 *plant.*

589



590

591 *Figure 4. PLS analyses. Correlation circle plots from the integration of the selected*
592 *predictors (pH´ and normalise predictors); and responses (NRR, PRR, BP and their*
593 *normalised parameters): a and b) 10-cm MPBR plant (n = 82); c and d) 25-cm MPBR*
594 *plant (n = 88).*

595

Table 1. Mean values of the short-term operation of the MPBR plant

Day	Solar PAR ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	pH´ ($pH\ unit \cdot d^{-1}$)	BP ($mg\ VSS \cdot L^{-1} \cdot d^{-1}$)	NRR ($mg\ N \cdot L^{-1} \cdot d^{-1}$)	PRR ($mg\ P \cdot L^{-1} \cdot d^{-1}$)
1	227 ± 279	39.8 ± 2.9	284	26.3	2.0
2	237 ± 278	39.9 ± 8.7	170	22.9	2.5
3	214 ± 294	29.0 ± 2.3	-	-	-
4	238 ± 283	19.3 ± 2.2	-	-	-
5	232 ± 276	19.6 ± 2.8	138	16.4	3.3
6	223 ± 278	21.7 ± 2.5	148	18.1	2.9

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