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***Limnospira indica* PCC8005 growth in photobioreactor: model and simulation of the ISS and ground experiments**

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Abstract

The Arthrospira-B experiment is the first experiment in space ever allowing the online measurements of both oxygen production rate and growth rate of *Limnospira indica* PCC8005 in batch photobioreactors running on-board ISS. Four bioreactors were integrated in the ISS Biolab facility. Each reactor was composed of two chambers (gas and liquid) separated by a PTFE membrane and was run in batch conditions. Oxygen production was monitored by online measurement of the total pressure increase in the gas chamber. The experiments are composed of several successive batch cultures for each reactor, performed in parallel on ISS and on ground. In this work, a model for the growth of the cyanobacterium *Limnospira indica* PCC8005 (also known as *Arthrospira* or spirulina) in these space membrane photobioreactors was proposed and the simulation results obtained are compared to the experimental results gathered in space and on ground.

The photobioreactor model was based on a light transfer limitation model, already used to describe and predict the growth and oxygen production in small to large scale ground photobioreactors. It was completed by a model for pH prediction in the liquid phase allowing assessment of the pH increase associated to the bicarbonate consumption for the biomass growth. A membrane gas-liquid transfer model is used to predict the gas pressure increase in the gas chamber. Substrate limitation is considered in the biological model.

A quite satisfactory fit was achieved between experimental and simulation results when a suitable mixing of the liquid phase was maintained. The data showed that microgravity has no first order effect on the oxygen production rate of *Limnospira indica* PCC8005 in a photobioreactor operating in space in zero gravity conditions.

Keywords:

MELISSA,

Limnospira indica PCC 8005,

Photobioreactors,

Model,

ISS Flight experiment,

Microgravity

1. Introduction

Long-term space missions (e.g., to Mars), including the establishment of a long-term, manned base, imply the development of a reliable life-support system including food supply and waste management. For far and long duration space missions, supplying all food, oxygen, and water from Earth will result in a tremendous weight and cost. Therefore, space life-support systems have to become increasingly regenerative. Food production can only be achieved by biological means, and the introduction of biological techniques opens a new set of solutions for other life-support requirements such as atmosphere, water, and waste management. For the last 30 years, the MELiSSA (Micro-Ecological Life-Support System Alternative) project, inspired by an aquatic ecosystem, has been set up to be a model for the study of regenerative life-support systems for long-term space missions (Hendrickx 2006; Lasseur et al. 2010; Walker and Granjou, 2017). MELiSSA has six major compartments (C1-C5) or subsystems, coupled together in a closed circulatory loop. Respectively, it involves anaerobic thermophilic bacteria (C1), photo-heterotrophic bacteria (C2) for waste degradation, nitrifying bacteria for ammonia and urine treatment (C3), photosynthetic cyanobacteria (C4a) and higher plants (C4b) for air revitalisation to supply the crew compartment (C5) with food. MELiSSA project is organized in five phases. Phase 1 is for basic research and development, phase 2 is focused on testing the processes in space conditions via space flight experiments, phase 3 concerns ground demonstrations of the MELiSSA loop and is mainly represented by the MELiSSA Pilot Plant (Godia et al., 2014), phase 4 is for technology transfer and phase 5 is for education and communication. Although the effects of space microgravity and its ground analogues on microorganisms have been studied for more than 50 years, conflicting and diverse results have been reported from different experiments, especially regarding microbial growth and secondary metabolism (Huang et al.,

2018). Microgravity has a significant impact on microorganisms in liquid culture (Walther et al., 1996; Kacena et al., 1999). Gravity affects a microbial (bioreactor) culture at two main scales: (i) it has a direct physical effect on the gas/liquid transfer, and more generally on fluid dynamics and mixing of bioreactor; and (ii) it affects the physiology and metabolism of microorganisms themselves. Several effects have been observed in microgravity (Hendrickx et al., 2005, Mastroleo et al., 2008, 2009), but the responses are depending on the growth media and growth conditions used. Therefore, it is still difficult to know if the effects observed are a direct consequence of the microgravity on the cells or a cellular response to other environmental changes due to microgravity (such as partial pressure gradients of dissolved gas, inhomogeneous compounds distribution in fluids, etc.). Moreover, in space flight conditions, the radiation effects must be added to microgravity effects being even more important than the microgravity effects (Mastroleo et al., 2009). Niederwieser et al. (2018^b) have reviewed the algal research in space, concluding that even though a large number of spaceflight experiments were conducted (mainly with *Chlorella sp.*), data are still quite limited, and no experiments have been successfully reported in-flight growth rate of algae. So, studies on the effects of microgravity in space on microbial growth and metabolism are still challenging both in terms of technology for bioreactor operation and control in microgravity and in terms of understanding specific cause-and-effect mechanisms of microbial responses to microgravity.

In the past decade several MELiSSA-phase 2 spaceflights microbial experiments were carried out: MESSAGE1 (2002), MESSAGE2 (2003), MOBILISATSIA (2004), BASE-A (2006), BASE-B/C/D (2008) (Lasseur et al., 2010). These experiments were focused on gathering knowledge about the bacteria used in the MELiSSA loop or in closed life-support systems at the global level, including study of genomic, transcriptomic, and proteomic data to know more about their

response to spaceflight conditions with special attention to cosmic radiation, and microgravity. MELiSSA-phase 2 is also focusing on demonstration of technology, concept and validation of mathematical models via projects such as BIORAT (a very simplified ecosystem reduced to gas exchange between a photo-bioreactor and a consumer compartment) or ArtEMISS (*Arthrospira sp.* gene Expression and mathematical Modelling on cultures grown in the International Space Station).

Arthrospira-B was the spaceflight experiment on ISS of the ArtEMISS project with the objective to determine the effect of space conditions, including reduced gravity and increased radiation, on *Limnospira indica* PCC8005 bacterial morphology, physiology, and metabolism, and how this influences the bioprocesses in the photobioreactor. The cyanobacteria *Limnospira indica* PCC8005 (also known as *Arthrospira* or spirulina) is a candidate for use in spacecraft biological life support systems, for carbon dioxide (CO₂) and nitrate (NO₃⁻) removal, as well as oxygen (O₂) and edible biomass production. The strain was selected for compartment C4a of the MELiSSA Biological Life Support System (BLSS). To ensure the reliability of such a BLSS it is necessary to characterize the response of *Limnospira indica* PCC8005 to *in situ* spaceflight conditions. The concept and demonstration of the space compatible membrane photobioreactor was first developed in early 2000 (Cogne et al, 2003^{a, b}; Cogne et al., 2005). The ground demonstrator was designed in order to match two constraints: (i) to be adaptable to altered gravity conditions, ensuring gas / liquid exchanges through a membrane without any gas bubble exchange; (ii) to permit remote online monitoring of oxygen production rates by pressure increase measurement, enabling a direct assessment of both metabolic rates and biomass growth and allowing much more sensitive bioprocess analysis than classical end-of-point results. It must be outlined that Arthrospira-B is the first space flight experiment targeting a direct and on-line measurement of rates

(oxygen production rate) offering a new and insightful investigation on time derivatives variables instead of integral variables such as biomass concentration permitting the assessment of microgravity effects onto the behaviour of cellular metabolism.

Arthrospira-B experiment was launched on December 15th 2017 on board the SpaceX CRS-13 / Falcon 9 rocket for a 5 weeks experiment operated within the Biolab facility system of the Columbus module in ISS. This experiment was the first of its kind, having an active microbial bioreactor activated and running in space, with on-line remote monitoring of the bioprocess. Although it is only a small scale and short duration pilot test, it is the first step for the development of future bio-based life support systems to enable sustainable human presence in space. Experimental device and samples collected during the spaceflight were recovered from the ISS for further analysis (genomic / transcriptomic proteomic / metabolomic analysis) four months after the launch (SpaceX CRS-14, May 5th 2018).

The present paper presents the model that was used to monitor and predict the outcome of the bioprocess. The experimental total pressure data recorded online during the spaceflight experiment are also discussed, and their analysis versus (i) the same experiment performed in ground condition, and (ii) the simulation results using the predictive metabolic model of the behaviour of the cyanobacteria in the photobioreactor are presented. The analysis of biological, biochemical and biomolecular results collected during the experiment will be presented in a further paper.

2. Materials and methods

2.1 Strain and culture medium

The strain used is *Limnospira indica* PCC 8005, a gram-negative photosynthetic cyanobacterium, from the “Pasteur Culture Collection” (PCC), which was cultivated and maintained in axenic conditions. Recently, taxonomic classification of cyanobacteria has been revised and a new genus, *Limnospira*, and species *indica* has been established in place of the former *Arthrospira sp.* PCC 8005 (Nowicka-Krawczyk et al., 2019). The culture medium is the Zarrouk medium (Zarrouk, 1966) modified by Cogne et al. (2003^c), adjusted to pH 9.5 with a carbonate buffer: K_2HPO_4 0.5 g.L⁻¹, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.08 g.L⁻¹, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.11 g.L⁻¹, NaHCO_3 10.5 g.L⁻¹, NaNO_3 2.5 g.L⁻¹, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.03 g.L⁻¹, Na_2CO_3 7.6 g.L⁻¹, EDTA 0.08 g.L⁻¹, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g.L⁻¹, CaCl_2 0.03 g.L⁻¹, K_2SO_4 1 g.L⁻¹, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.23 g.L⁻¹, NaCl 1 g.L⁻¹. The bioreactors were sterilized and aseptically assembled before launch. All the inoculums (for space and ground experiments) came from the same preculture medium in order to prevent any physiological state difference. Cells (5mL at ~1.3g/L) were maintained in a separate compartment in the dark and at 4°C during launch. For inoculation, this small volume is injected in the culture chamber containing the growth medium and considering a 10-fold dilution. The culture was then run inside the reactor hermetically sealed from the outside atmosphere and all nutrients for growth were provided via the liquid Zarrouk medium, including dissolved CO_2 and bicarbonate HCO_3^- as CO_2 source.

2.2 Photobioreactor and hardware

Four reactors on ground and four reactors on-board ISS were used in parallel to test the behaviour of four independent bacterial cultures (4 independent biological replicates). It was important to assess the reproducibility of the results in independent bacterial cultures, as cultures originating initially from the same inoculum, can nevertheless evolve differently over multiple generations. Each reactor was placed in a separate Integrated Experimental

Container (IEC) (size 110x140x150mm, volume 2.2 L, mass 4.7 kg) designed and built by QinetiQ Space NV (Belgium). On-board ISS, the IEC was installed and operated within the Biolab facility in the European Columbus laboratory, providing IEC with power, atmosphere ventilation, as well temperature control via the Biolab thermal control unit. The two first reactors were installed on rotor A of Biolab on position A1 and A4 respectively while the two others were installed on rotor B on position B1 and B4. The two Biolab rotors had a separate atmosphere ventilation system but reactors on the same rotor share the same ventilation system. The IEC hardware (Figure 1) contained the following assemblies: the experiments container, the electronics assembly and the experimental unique equipment (EUE) (culture chamber with membrane and mixing unit, pump unit and liquid loop with 3-way valves, optical measurement unit, Zarrouk medium reservoir, sample reservoir and RNAlater[®] fixative reservoir for sample fixation).

The culture chamber (Figure 2) is the photobioreactor part of the EUE and contained: the liquid compartment for the growth of the cyanobacteria in a light transparent inflatable culture bag (51 mL); a gas permeable membrane (hydrophobic PTFE membrane of 57 ± 1 micron thickness; Sartorius-Sedim 11807 pore-size 0.2 micron); a magnetic stirrer sitting on top of the membrane and kept in place by the stirrer bar holder; a gas compartment (24 mL); a polycarbonate transparent windows and a LED PCB for illumination of the liquid compartment (36 LEDs delivering a continuous light intensity ranging between 20 and 50 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ in the photosynthetic active range of 400-700nm; NICHIA NESL064AT). A cartridge solenoid valve (Staiger VA 204-715) vented the gas compartment inside the IEC, which was connected to the Biolab ventilation system (or ambient air for ground

experiments). Venting occurred every 24h and/or if the pressure that is measured by the pressure sensor Kulite XTL-193-190 has risen above the threshold of 1.25 bar. During each venting cycle, air is flushed several seconds to ensure that air in gas chamber is replaced by ambient air. Temperature of the liquid chamber was recorded (44007RC Precision Epoxy NTC Thermistor) and was controlled with Biolab thermal control unit to obtain a temperature of $33^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in the bioreactor during the operational phase of the experiment.

The Optical Measurement Unit (OMU) is composed of a cell density measurement instrument (a spectrometer measuring light absorbance at 790 nm, 632 nm and 468 nm) and a photosynthetic activity measurement instrument (a miniaturized pulsed amplitude modulated (PAM) fluorometry measurement instrument) (Gademan Instrument/Heinz Walz GmbH). This OMU was placed on the external liquid loop ($V_{LL}=9\text{mL}$), in which continuous biomass circulation was achieved by a peristaltic pump at an average flow rate of $1\text{mL}\cdot\text{min}^{-1}$. Each 12h, the circulation was stopped during 20 minutes to perform optical density and fluorimetry measurements on cells adapted to dark conditions.

2.3 Arthrospira-B experiment, timeline and data acquisition

The inoculum culture was dormant stored in the dark at 4°C in spent medium, in the liquid loop of the bioreactor in the IEC, during upload and on-board ISS storage; and reactivated by moving and mixing it with fresh Zarrouk medium in the culture chamber at the start of the bioreactor experiment in the Biolab facility. Cultures were initiated at a fixed cell (OD 790nm = 0.1) and medium composition (Modified Zarrouk at pH 9.5) and allowed to proliferate at a fixed light intensity (35 or $45 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and temperature (33.4°C). During each batch, the liquid phase was circulating in the illuminated and stirred culture chamber ($V_{cc} = 51\text{mL}$) and

in the non-illuminated liquid loop ($V_{LL} = 9\text{mL}$) at an average flow rate of $1\text{mL}\cdot\text{min}^{-1}$ allowing to assume a perfect mixing of the full liquid volume (60mL). The cyanobacterium was grown in a batch regime at conditions maintaining exponential cell proliferation during 1 week, then emptying the culture chamber and using the biomass remaining in the circulation loop ($V_{LL} = 9\text{mL}$) to start the next batch. The manual replacement of the Zarrouk medium reservoir (90 mL) by the crew for 4 times, allowed for 4 successive batches to be run. This experiment allowed sufficiently large sample volumes (15 mL), to be taken at the end of each batch using RNAlater® to stabilize and protect cellular DNA, RNA and proteins of the sample for the further biomolecular analysis after the return of the experiment on Earth. The accumulative total radiation dose could be measured by means of dosimeters (luminescent detectors, type TLD-OSLD) and analysed also after the recovery of the experiment. Automatic measurements of temperature, gas pressure, optical density, fluorometry were online acquired during the experiment: every second for pressure and temperature, twice by day for optical measurements. The biomass concentration was directly correlated to the optical density. The fluorescence was recorded to calculate quantum yield. The gas pressure increase is the result of the oxygen production by *Limnospira indica* PCC 8005, so that online gas pressure measurement permits to calculate the instantaneous oxygen production rate r_{O_2} , and the total (or cumulated) oxygen produced (assuming no gas accumulation in the liquid). Cogne et al (2003^c) have shown that the oxygen production measured by this way is consistent with the stoichiometry of the growth.

$$r_{O_2}(t) = \frac{V_{GC}}{R.T} \frac{dP(t)}{dt} \quad \text{and} \quad O_2(t) = \frac{P_{cumul}(t).V_{GC}}{R.T} \quad \text{where} \quad P_{cumul}(t) = \int_0^t P(t).dt$$

In batch, the oxygen produced is linked to the biomass produced, by the $Y_{O_2/X}$ yield:

$$r_{O_2}(t) = Y_{O_2/X}.r_x(t) \quad \text{and} \quad O_2(t) = Y_{O_2/X} (X(t) - X(t_0))$$

The ISS flight experiment (reactors A1, A4, B1, B4) was reproduced on ground at the same time with the same hardware (i.e. ground reactors GM1, GM2, GM3, GM4). Each of the reactors was run following the same timeline and the same incubation conditions. During the experiment, 4 batches were run and for each batch the timeline was decomposed in several stages: batch incubation (Batch1: 14d; Batch 2: 6d; Batch 3: 8d; Batch 4: 6d); automated sampling (30') followed by manual reservoir replacement by the crew (2h).

3 Photobioreactor model

3.1 Model structure and assumptions

Cogne et al. (2003^b) have proposed, developed and implemented the principle of a dynamic model for a similar membrane photobioreactor. The photosynthetic growth of microalgae under light limiting conditions has been modelled associating four main sub-models (Figure 4), namely a radiative-transfer model, a biological model, a liquid phase mass balance model and a gas phase mass balance model. The four sub-models are coupled and interdependent from each other. Biological phenomena (biomass growth, oxygen production rate) are rate-limited by the light availability inside the culture. Therefore, a radiative-transfer model is required to express light attenuation which itself is function of the biomass concentration. Similarly, gas and liquid phases are linked by gas/liquid equilibrium and gas transfer rate through the membrane. The magnetic stirrer is supposed to provide an efficient agitation in the liquid phase ensuring perfectly mixed conditions in the liquid phase of the reactor within the time scale of the accumulation of substrates and products and of characteristic time of biomass growth. The location of the stirrer at the membrane wall permits to minimize the

thickness of the liquid boundary layer, reducing the mass transfer resistance between the liquid bulk and the membrane. The nutrients (N, S, P sources) were in excess, but below inhibition levels. Temperature and gas pressure are controlled, respectively by the Biolab thermal control unit and by the pressure relief valve of the reactor; pH is uncontrolled and increases progressively due to CO₂ fixation for the biomass growth. Consequently as pH affects the ionic equilibrium of the solution and can affect the growth, a pH prediction model is also included in the liquid sub-model.

The state variables are calculated by solving the model Ordinary Differential Equations (ODE) and Algebraic Equations simultaneously using Matlab® R2015a ODE15s solver.

3.2 Radiative transfer (light) model

Considering the culture is heterogeneous in terms of light availability, it is treated as a system with uniformly distributed parameters along its depth (z). To determine the attenuation of light inside a photosynthetic culture, a radiative-transfer model designed for rectangular reactors lightened on one side, was adopted. This model will determine the value of irradiance, G, for any depth, z of the culture, following the two-flux model analytical solution of the dimensionless Eq. 1, (Cornet and Dussap, 2009) that has already been proved efficient in several studies (Cogne et al. 2005, Ifrim et al. 2014, Pruvost et al., 2016):

$$\frac{G_z}{q_0} = 2 \left(\frac{n+2}{n+1} \right) \frac{(1+\alpha)e^{\delta(L-z)} - (1-\alpha)e^{-\delta(L-z)}}{(1+\alpha)^2 e^{\delta L} - (1-\alpha)^2 e^{-\delta L}} \quad (\text{Eq 1})$$

n is the degree of collimation for the radiation field: n = 0 for isotropic intensities and n = ∞ for collimated intensity;

$$\delta = \frac{(n+2)}{(n+1)X\sqrt{E_a(E_a+2bE_S)}} \text{ is the two-flux extinction coefficient;}$$

$\alpha = \sqrt{\frac{E_a}{(E_a + 2bE_s)}}$ is the linear scattering modulus.

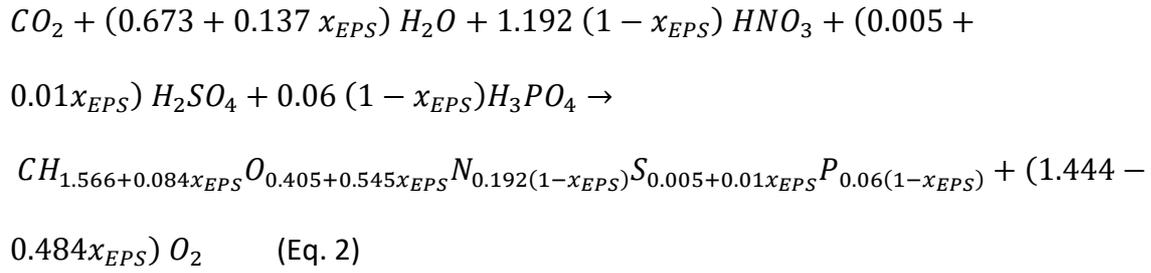
E_a and E_s are the mass absorption and the mass scattering coefficients and b is the backward scattering fraction (dimensionless). q_0 represents the hemispherical incident light flux or photons flux density (PFD). X represents the biomass concentration inside the photobioreactor and L is the depth of the photobioreactor. The optical and radiative properties of *Limnospira indica* PCC 8005 (*Arthrospira platensis*) E_a , E_s and b are determined experimentally by spectrophotometric tools using an integrating sphere. For the current study, collimated intensity is assumed, so that Eq.1 is used considering $n = \infty$.

The light availability is defined by the lightened liquid volume fraction: $\gamma = \min\left(1, \frac{L_0}{L}\right)$, where L_0 is the depth for which light intensity is equal to the light compensation point; γ represents the illuminated fraction of the reactor and depends on the incident light intensity and on the biomass concentration (Figure 5a).

3.3 Biological model

The biological model is composed of the growth kinetic model, accounting for the photosynthetic activity of the cells, and for the stoichiometry of the photosynthetic growth leading to the relationships between the compounds (nutrients and biomass) consumption and production rates.

Stoichiometry of the photosynthetic growth is summarized into a single mass and elemental (C,H,O,N,S,P) balanced equation (Eq. 2) where x_{EPS} is the molar fraction of exopolysaccharide in the total biomass (Dauchet et al., 2016):



This overall equation was obtained from a structured approach of the metabolism of *Limnospira indica* PCC 8005 including anabolic reactions rates coupled to the rate of chemical energy carriers (J_{ATP}) and of reduced power cofactors (J_{cov}) driven by photosynthesis (Z scheme) (Cornet et al. 1998). It was demonstrated (Dauchet et al. 2016) that the energy transducing process can be described by the ratio (J_{ATP})/(J_{cov}), which is termed the $P/2e^-$ ratio, and thus can be handled with the theory of the linear energy converter in terms of Linear Thermodynamics of Irreversible Processes (LTIP) as:

$$\frac{P}{2e^-} = \frac{(1+0.91x)}{0.91(0.91+x)}$$

x is obtained as the solution of the following second order equation:

$$0.91(1 + 1.82x + x) - (0.91 + x)(1 + 0.91x)(1 + \bar{\beta}) = 0$$

$$\bar{\beta} = \frac{1}{L} \int \int \int_L \frac{Gz}{K+Gz} dz$$

x_{EPS} is calculated from $P/2e^-$: $x_{EPS} = 1.33 (P/2e^- - 1.23)$.

The mass fraction of exopolysaccharide that is predicted using the LTIP approach (Figure 5b) is consistent with experimental observations and metabolic analysis of *Limnospira indica* PCC 8005 growth for different light incident fluxes conditions (Cogne et al. 2003^a). For $P/2e^-$ values greater than 1.5, the light energy absorbed by the microorganisms is above their metabolic capacities and can lead to physiological limitations. Therefore the working

conditions for this micro PBR were fixed at a light incident flux q_0 of 7.6 or 9.8 W.m⁻², which guaranties never to be in such light inhibiting conditions, even at start-up with a low biomass concentration (Figure 5b). In these conditions the mass fraction of exopolysaccharide in the total biomass was always maintained below 35%, reducing the problematic of cell aggregation and biofilm formation (Cornet et al 1998, 2009).

The respiration of *Limnospira indica* PCC 8005 in the dark was assumed to be negligible for a perfectly mixed illuminated bioreactor with a low residence time of the liquid medium in the dark zone considering light inhibition of respiration in the illuminated zone. The kinetic of growth is equal to the average photosynthetic growth rate associated to Eq. 2, $\langle r_x \rangle$, calculated for the full liquid volume of the reactor by integrating the local light flux G_z along the culture depth (Cornet et al. 1998; Cornet and Dussap, 2009):

$$\langle r_x \rangle = (1 - f_d) \rho_M \Phi K E_a X \frac{1}{L} \int_0^L \frac{G_z}{K + G_z} dz$$

f_d is the design dark fraction of the reactor, ρ_M the maximum energetic yield for photon conversion, Φ the mass quantum yield for the Z-scheme of photosynthesis, K the half saturation constant for photosynthesis. The kinetic rate of a compound (i) involved in the mass balanced growth equation Eq. 2 is calculated:

$$\langle r_i \rangle = Y_{i/x} \langle r_x \rangle$$

$Y_{i/x}$ is the mass yield g(i). gX⁻¹ calculated accordingly to the stoichiometric coefficients of Eq. 2.

The carbon source for the growth in Eq. 2 is CO₂, but in practice both dissolved CO₂ and HCO₃⁻ support photosynthesis. On ground, in a pH-controlled reactor, the carbon source for cyanobacteria growing at pH 9 +/- 1 remains mainly in the bicarbonate HCO₃⁻ form.

Therefore classical models neglect the issue of carbon limitation; carbon is either considered as Total Inorganic Carbon (TIC) or HCO₃⁻. For the current study, pH was not controlled leading to pH increase above 10 in the culture medium and to a potential C limitation. A Monod limiting term lim_c was introduced in the biomass growth kinetic equation for the carbon source considered (HCO₃⁻): $lim_c = HCO_3^- / (K_C + HCO_3^-)$, with K_C the half saturation constant for HCO₃⁻. Because of intracellular bicarbonate accumulation mechanism (Giordano et al. 2005) and the influence of both light limitation and pH, it was difficult to get reliable values for K_C from literature data. Most of values for cyanobacteria are ranging between 10⁻² – 10⁻⁴ mol C.L⁻¹. For the current study a value of 5 10⁻³ mol HCO₃⁻.L⁻¹ was used.

3.4 Liquid model and pH model

The evolution of compounds concentration in the liquid phase for the micro PBR operated in batch was expressed in term of mass balance equation for a perfectly mixed reactor:

$$\frac{d(i)}{dt} = \langle r_i \rangle - r_{GL(i)}$$

$r_{GL(i)}$ is the gas-liquid transfer rate for the compound (i) through the membrane expressed in same unit as $\langle r_i \rangle$. Considering the PTFE hydrophobic membrane, this term was relevant only for molecular CO₂ and O₂ and was equal to zero for other compounds.

The CO₂ required for the growth (fixed by RubisCo) was obtained via dehydration of the bicarbonate inside the cell (HCO₃⁻ <-> CO₂aq + OH⁻), a reaction that releases hydroxyl ions

(OH⁻) causing an increase in pH for the pH-uncontrolled micro PBR. pH affects both the growth and the relative distribution of ionic species. A model predicting pH evolution as a function of net CO₂ uptake was used. The model was first described by Achard et al. (1994) and used by Cogne et al (2003^a) and for the prediction of pH in a toric PBR with pH control by periodic gas CO₂ pulses (Ifrim et al, 2014). The pH model results in a multi-solute system of 10 distinct species: H₃PO₄, H₂PO₄⁻, HPO₄²⁻, PO₄³⁻, CO₂, HCO₃⁻, CO₃²⁻, H₂O, OH⁻ and H⁺, linked by chemical equilibrium relations. The chemical equilibrium relations were expressed as the one for water: $K_w = (a_{H^+} + a_{OH^-})/a_w$, where $a_i = \gamma_i c_i$ is the activity for a compound (i), γ_i being the molar activity coefficient based on concentration and c_i the compound concentration. Activities were calculated by the model of Pitzer (Edwards et al. 1978). Finally, the thermodynamic model for calculating the chemical equilibrium of species in the liquid phase was composed of 19 unknown (concentrations and activities) and solved using 19 relations (10 Pitzer relations for activities, 6 chemical equilibrium relations, 2 compounds species mass balances, 1 electroneutrality relation). The pH itself was calculated as $pH = -\log(H^+)$

3.5 Gas pressure model and gas/liquid equilibrium

The evolution of partial pressure of CO₂ and O₂ in the micro PBR gas compartment is expressed as:

$$\frac{dP_i(t)}{dt} = \frac{R T}{V_{GC} 10^{-3}} V_L r_{GL(i)}$$

$r_{GL(i)}$ is in units of mol(i).L⁻¹.h⁻¹; the volume is expressed in L and pressures in Pa. Total pressure was obtained as sum of partial pressures.

The gas transfer rate through the membrane was calculated as:

$$r_{GL(i)} = \frac{A k_i}{V_L} (H_i c_i - P_i)$$

With A the membrane surface (m^2), k_i the membrane permeability for (i) in $mol.Pa^{-1}.m^{-2}.h^{-1}$, H_i the Henry constant for (i) in $Pa.L.mol^{-1}$.

4 Results and discussion

4.1 Experimental results: Ground and ISS

Figure 6 presents the results for the 4 ISS reactors namely A1, B1, A4, B4. The pressure of the gas compartment was recorded online (Figure 6). The experiment was designed in order that all four reactors were operated in same conditions, so that the same results were expected. Due to experimental and technical issues, this was not always the case. For the first batch (culture start up) only the reactor B4 gave the expected biomass production and pressure profile with a pressure increase up to the relieve set point of 1.25 bar. Reactor A1 had a lower pressure increase than expected in batch 1, 2 and 3, but suddenly recovered the expected pressure profile at the end of batch 3 and for the entire batch 4. Reactor B1 had never start (no biomass growth and no pressure increase) and was stopped after 21 days. Reactor A4 showed no measurable biomass production for the start up first batch. The growth started at the end of batch 2 with a low-pressure increase profile in the last 2 batches (number 3 and 4), which looks like the pressure profile of reactor A1-batch1 but with a crushed shape. Inspection of the reactor after its return on Earth indicated a pressure leak from 1.15 bar. The biomass profiles of reactor A4 -batches3, 4- were similar to the A1-batch1. For reactor B4, the growth stopped after 290h, and biomass decay was then

observed. Medium renewal for the batch 2 did not permit to restore the culture. No gas production was recorded potentially due to a malfunctioning pressure sensor or of the pressure release valve.

The online monitoring data of the 4 ground reactors, namely GM1, GM2, GM3 and GM4 are presented in Figure 7. Only pressure results are presented; fluorescence measurements require about 20 min, so that on ground biomass sedimentation occurs leading to a non-reliable value for OD. Several issues when running occurred also for ground experiments. Batch 1 for GM1 and GM2 had to be stopped due to a liquid leak that required repair. The 2 reactors were restarted for batch2. After their restart, reactors GM1 and GM2 had a similar pressure profile (batches 2 and 3). GM3 showed a low gas production few days after its start-up. A stirring failure was identified when visually inspecting the reactor that has been confirmed after dismantling the reactor after the experiment. GM3 reactor was then ran with mixing of the liquid compartment only by the way of the external liquid loop ($1 \text{ mL} \cdot \text{min}^{-1}$), leading to heterogeneity of the liquid phase and gas transfer limitation in liquid boundary layer surrounding the membrane surface. GM3 reactor was kept in this degraded mode and is further used as a reference to characterize a reactor with stirring difficulties. GM4 reactor had presented no technical failures, but like for the B4 ISS reactor at the end of the batch 1, the biomass and oxygen production suddenly decreased, and could only be recovered in batch 4. As the growth decrease was not observed when restarting GM1 and GM2 (batch 2,3,4), the interruption of GM4 and B4 were interpreted as linked to the long batch duration (14 days for batch 1, versus 7 days for other batches), which has led to toxic high pH and/or carbon substrate limitation.

For each reactor, analysis of pressure and biomass (Figure 8) allowed to calculate the oxygen production rate and yield $Y_{O_2/X}$ (Table 1). Theoretically $Y_{O_2/X}$ is constant for a constant light flux and is predicted equal to $1.9 \text{ gO}_2\cdot\text{gX}^{-1}$ by the stoichiometric equation. For A1-batch4 and B4-batch1 the calculated oxygen production yield $Y_{O_2/X}$ was close to the theoretical one, taking into account the experimental gross errors in calculations due to the OD to dry mass conversions and the small volumes of the reactors. The values in the order of $0.5 \text{ gO}_2\cdot\text{gX}^{-1}$ for A1-batches 1-3 were too low when compared to stoichiometric theoretical value, suggesting either a pressure problem (such as gas leakage or pressure sensor malfunction) or a biomass overestimation. Considering the results of ISS A1 batch 1-3 until day 27 (~650h) are similar to the results obtained for on-ground GM3 reactor that has been identified as a reactor that has encountered a stirrer issue, it is concluded that the same difficulty has occurred on the ISS reactor. A normal stirring has been recovered for ISS A1 batch 4 indicating that the stirrer has recovered a normal functioning, showing the reversibility of the malfunction as it has been often observed on ground for this case of dysfunction.

The experiment was designed as a 4 reactors - 4 batches/reactors for both ISS and ground. Except the initial biomass concentration, which could not be strictly controlled, impacting the early growth phase (lag phase and adaptation phase), all reactors were operated in same conditions (temperature, light, reactor design). So ideally, the 16 batches were planned to be 16 replications that can be compared between ISS and ground. In a worst case, knowing that at least for the ISS it was not possible to correct a technical issue, it was expected that relevant results could be obtained for some batches. Over the 32 batches, 3 gave the expected oxygen production rate of $0.33 \text{ mmol O}_2\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ and 7 a production rate of $0.23 \pm$

0.03 mmol O₂.L⁻¹.h⁻¹ (Table 1). For 6 batches the oxygen production rate calculated was 0.1 +/- 0.03 mmol O₂.L⁻¹.h⁻¹. 16 batches have failed (either technical or inoculation defaults) and no pressure build-up could be used. The cumulative pressure was a good way to compare the different batches (Figure 9) and to discriminate reactors with low oxygen production.

For batches with 0.1 +/- 0.03 mmol O₂.L⁻¹.h⁻¹ (A1-b1-b2-b3, GM1-b4, GM3-b2-b3), the calculated growth rate is lower by a factor of 3 than the normal growth rate with the applied light energy transfer conditions. It has been observed on-ground (GM1-b4, GM3-b2-b3) that such a functioning was characteristic of a dysfunction of the magnetic stirrer leading to degraded O₂ transfer from the liquid phase to the gas phase leading to a high O₂ gas partial pressure into the liquid phase. A low production rate of 0.1 mmol O₂.L⁻¹.h⁻¹ is characteristic of limiting O₂ transfer between liquid and gas coming from a stirring issue. This behaviour has been observed both on ground and on ISS, leading to higher oxygen dissolved concentrations into the liquid phase, therefore inhibiting biomass growth. Moreover, the sudden re-increase in O₂ production rate in ISS reactor A1-b3-b4 confirms this explanation and was interpreted as an unblocking of the stirrer.

One ISS batch (A1-b4) can be compared to the ground batches GM4-b1 and GM4-b4 for the maximal production rates obtained of 0.33 mmol O₂.L⁻¹.h⁻¹. Even considering that the number of replications is not sufficient to draw firm conclusions, it can be claimed that beyond technical issues it is possible to achieve similar productivities and rates of production biomass and oxygen on ground and in microgravity.

The last group of batches with an intermediate calculated productivity of 0.23 +/- 0.03 mmol O₂.L⁻¹.h⁻¹ lower than the nominal productivity (B4-b1, GM1-b2-b3, GM2-b2-b3-b4, GM3-b1) was mainly observed on ground experiments. These ground reactors have often shown some

difficulties with irregular adherence of the stirring barrel leading to a similar interpretation as previously. It was also often observed in previous ground experiments that this intermediate productivity could be explained by the presence of a gas bubble in the liquid compartment, reducing the liquid volume and increasing the gas volume. If it still difficult to conclude whether this group of batches with intermediate productivity would have to be related to a stirrer dysfunction (reduced liquid gas transfer rate) or to a bubble appearance in the liquid. It might also be explained by a pressure problem (such as gas leakage or pressure sensor malfunction), the reason being most probably identical for all reactors.

4.2 Model versus experiments

One of the objectives of the experiments was to validate and to use the theoretical model in microgravity conditions. For multiple experiments together, it was easier to use the cumulative pressure than the pressure profiles for comparing experimental data and model data. First the model must be validated with the ground experiments. As the current model assumes perfectly mixed and perfectly tight and isolated reactor, only the batches showing reasonable oxygen production profiles without indications of severe technical issues were used (Figure 9a). Reactors GM4-b1 and GM3-b1 are taken as reference experiments. As can be seen in Figure10, the model fits quite satisfactorily the experimental results for GM4-batch1, without any fitting or adjustment of the model parameters. This confirms the predictability of the model, which was already applied for various ground photobioreactors (Cogne et al., 2003^b, Cornet et al., 2009, Ifrim et al., 2014, Dauchet et al., 2016). The decrease of the growth is linked to a carbon limitation (bicarbonate in the model) due to the high pH value reached after 250h of culture. It has been calculated that the growth stop near

300h happens for a pH of 11.5, what was consistent with Pandey et al. (2010) who has observed a 50% decrease in pigments at pH 10 and 11, and no growth at pH 12 in flask cultures. Decrease of pigments content associated to metabolic and cell damage for pH above 11-11.5 was probably the reason why even after medium replacement for the batch 2, the culture could not be rescued.

The experimental results of GM3-b1 do not fit satisfactorily with the model without any parameter adjustment (Figure 11). The calculated O₂ productivity is lower than for GM4-b1 (Table 1). This behaviour could be explained either by a non-perfectly mixed liquid phase (GM3 had indeed issues with the magnetic stirrer) and/or by the presence of a gas bubble into the liquid, leading to an increased gas buffer volume and consequently to cushion the pressure increase. Knowing that the model parameters were not adapted for a non-perfectly mixed liquid volume, the model was further tuned (Figure 11) by considering a 5 mL gas bubble into the liquid phase resulting into a good fit of the experimental cumulative pressure with the model. This confirms that the presence of gas bubbles in the liquid compartment can lead to small changes in the gas / liquid volume ratio, which in turn can have a significant effect onto the gas pressure evolution.

Reactor B4-b1 and A1-b4 are the only 2 ISS reactors with a behaviour suggesting no technical problems related to mixing or to pressure measurement. A1-b4 showed exactly the same trend as GM4-b1. The theoretical model represents quite satisfactorily the behaviour of the last batch of this reactor. B4-b1 showed the same trend as the GM3-b11 reactor and again, the tuned model can be used to represent the ISS reactor (Figure 12). The pressure profile was better predicted than for ground experiment because environmental parameters (pressure, humidity, composition) on-board ISS and of Biolab facility were better controlled

than for the ground experiments. Again, the model failed to correctly represent the experimental results when pH has reached high values. After a threshold pH predicted at 11.3, the growth rate presents a sharp decrease. For ISS reactor, the limiting effect of bicarbonate availability at high pH was a bit lower than on ground, so that the culture was maintained only in light-limited conditions until the pH threshold was observed. This is explained by the fact the carbon limitation happens a bit later due to the higher CO₂ fraction in the gas flushing the reactor (ground air is 0.04% while ISS air is 0.4%). A better fit is obtained by reducing the half saturation constant for bicarbonate in the model. The mechanistic interpretation shows that gas-liquid transfer of CO₂ through the membrane involves a non-negligible transfer resistance of molecular CO₂ from gas to liquid (at pH higher than 9.5). Considering that for ISS reactors pCO₂ is 10 times higher than on ground conditions, the appearance of the gas transfer resistance limitation effect is therefore significantly hindered. The model of carbon uptake considers that bicarbonate is the main carbon source. However at the higher pH values (higher than 11) molecular CO₂ that is the chemical species undertaken by RUBISCO complex must be considered as the main carbon source. Therefore higher pCO₂ on ISS has a tendency to reduce the limitation in ISS conditions. Niederwieser et al. (2018^a) has discussed the influence of increased CO₂ partial pressure on algae growth in spacecraft conditions and they have given similar conclusions for the growth rates of algae.

5 Conclusion

The Arthrospira-B experiment was successfully run; relevant results have been obtained from online measurement of the oxygen production rate by *Limnospira Indica* PCC8005 in a

micro Photobioreactor operated in microgravity. Considering the 51 green algae space experiments reviewed by Niederwieser et al. (2018^b), Arthrospira-B is the first successful dynamic culture experiment in space, allowing direct measurement and calculation of reliable growth and oxygen production rates. However, three main issues for the ISS experiment have to be reported. 1) The remotely activated automated inoculation and reactor start-up failed for one reactor in space, but the procedure was successful for the 3 other space reactors, and all ground reactors. 2) There were some technical difficulties with the stirrer and gas pressure measurement, leading to a non-perfectly mixed liquid phase, for which the current model was not completely applicable. It was still interesting to note that the continuous circulation of the liquid in the liquid loop was sufficient to maintain a suitable liquid phase mixing for operating the PBR in ground and microgravity conditions with about 1/3 of the maximum oxygen productivity. The lower oxygen productivity in non-perfectly mixed liquid phase may be due to presence of gas bubbles into the liquid phase and limiting gas / liquid transfer across the liquid boundary layer at the surface of the membrane. It has also been observed that when a perfectly mixed behaviour was recovered, the oxygen production profile was recovered too. 3) The space bioreactor compatible with Biolab facility on ISS could not account for a pH sensor or regulation, so that pH of the process was uncontrolled, leading to a steep pH increase when bicarbonate was consumed. A complete model of pH reconstruction using element and electrolytes balances was derived and used to reconstruct post-flight the pH profiles of the cultures. The model predicted a max pH threshold value of 11.3-11.4 for biomass and oxygen production both on ISS and on ground, to avoid an unrecoverable biomass death. Except for reactors showing stirring difficulties, oxygen productivity and therefore biomass growth were comparable on ground and in microgravity experiments. The model also fits both on ground and microgravity experiments

except when high pH conditions (above 11) were reached. In microgravity the growth in such bicarbonate-pH limiting condition was less affected than in ground conditions, maybe due to higher CO₂ fraction in the gas used to flush the IEC on ISS.

The experiment had been planned with 16 batches in order to obtain enough data for comparison of the growth and oxygen production on-board ISS and on ground. But the technical issues encountered have reduced the number of results and a definitive conclusion on the influence or not of the microgravity on the growth of *Limnospira indica* PCC8005 remains difficult to assess. Nevertheless, it was observed that for on-board ISS and on ground reactors, the oxygen production rates measurements were comparable.

Consequently, the terrestrial bioreactor growth model is completely applicable for representing 0-g conditions, provided the modifications of physical environmental variables have been correctly assessed by a mechanistic approach in the model. The present model can still be improved if in the future for predicting the growth under degraded conditions such as high pH or imperfectly mixed reactor.

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2x Sample reservoirs

Medium supply

Rad sensors

gas chamber & pressure sensor

Reactor programming & commanding

Data logging & communication

Power converter from Biolab line 5V to 12V

OMU cuvette :
cell density (absorbance)
photosynthetic activity (fluorescence)

liquid pumping

temperature sensor

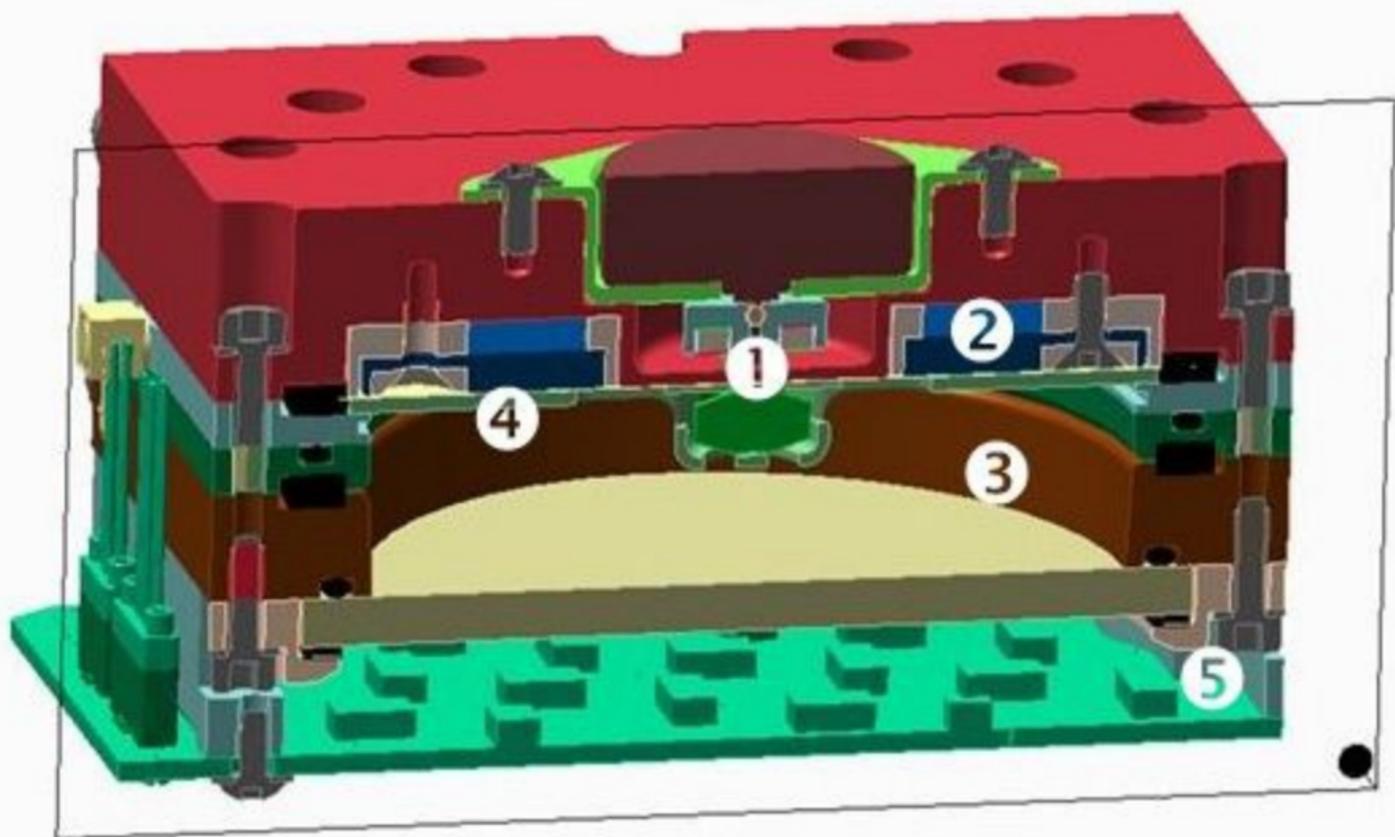
Liquid culture chamber
gas membrane
mixing (magnetic stirrer)

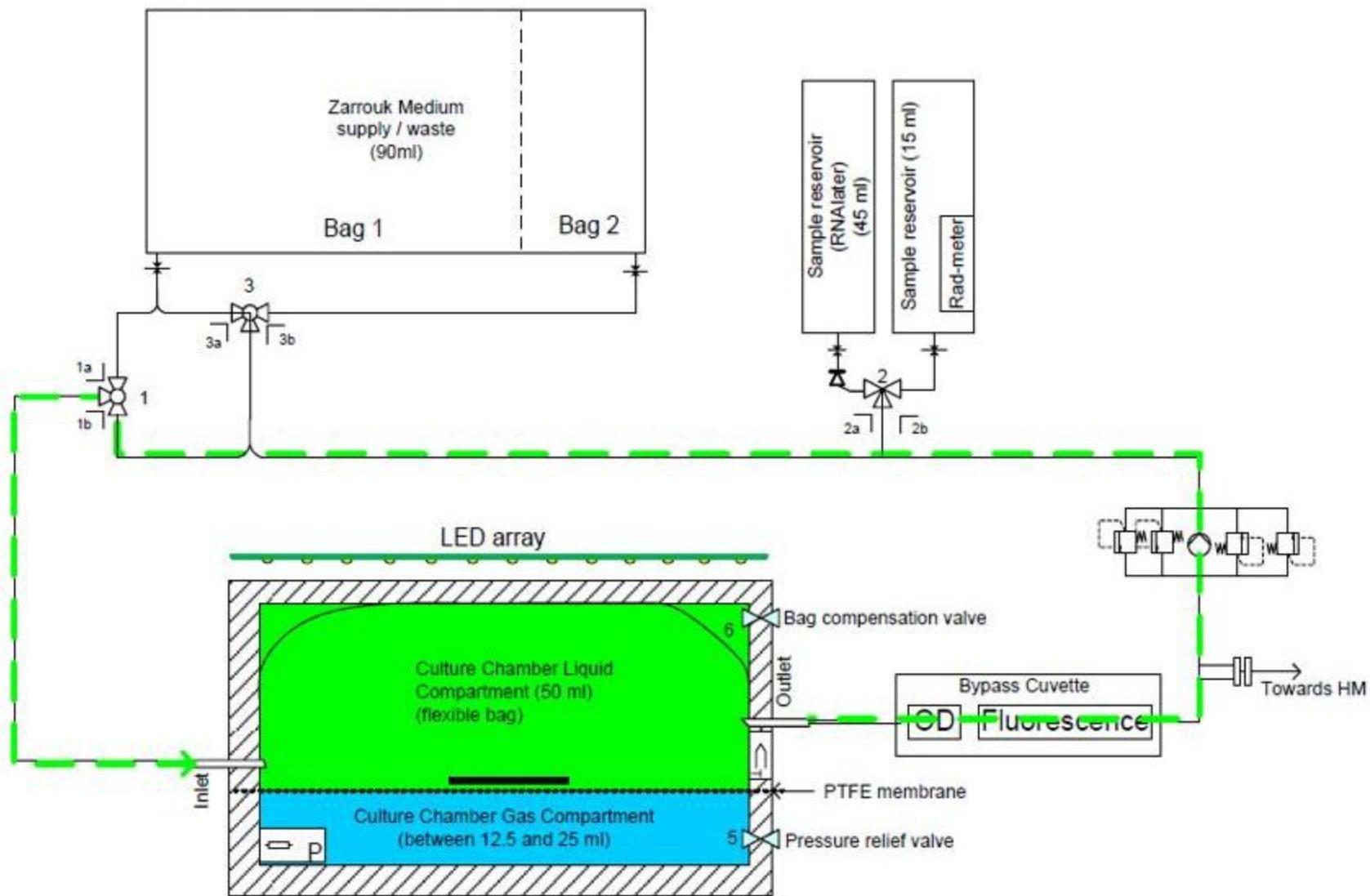
Hermetically sealed box

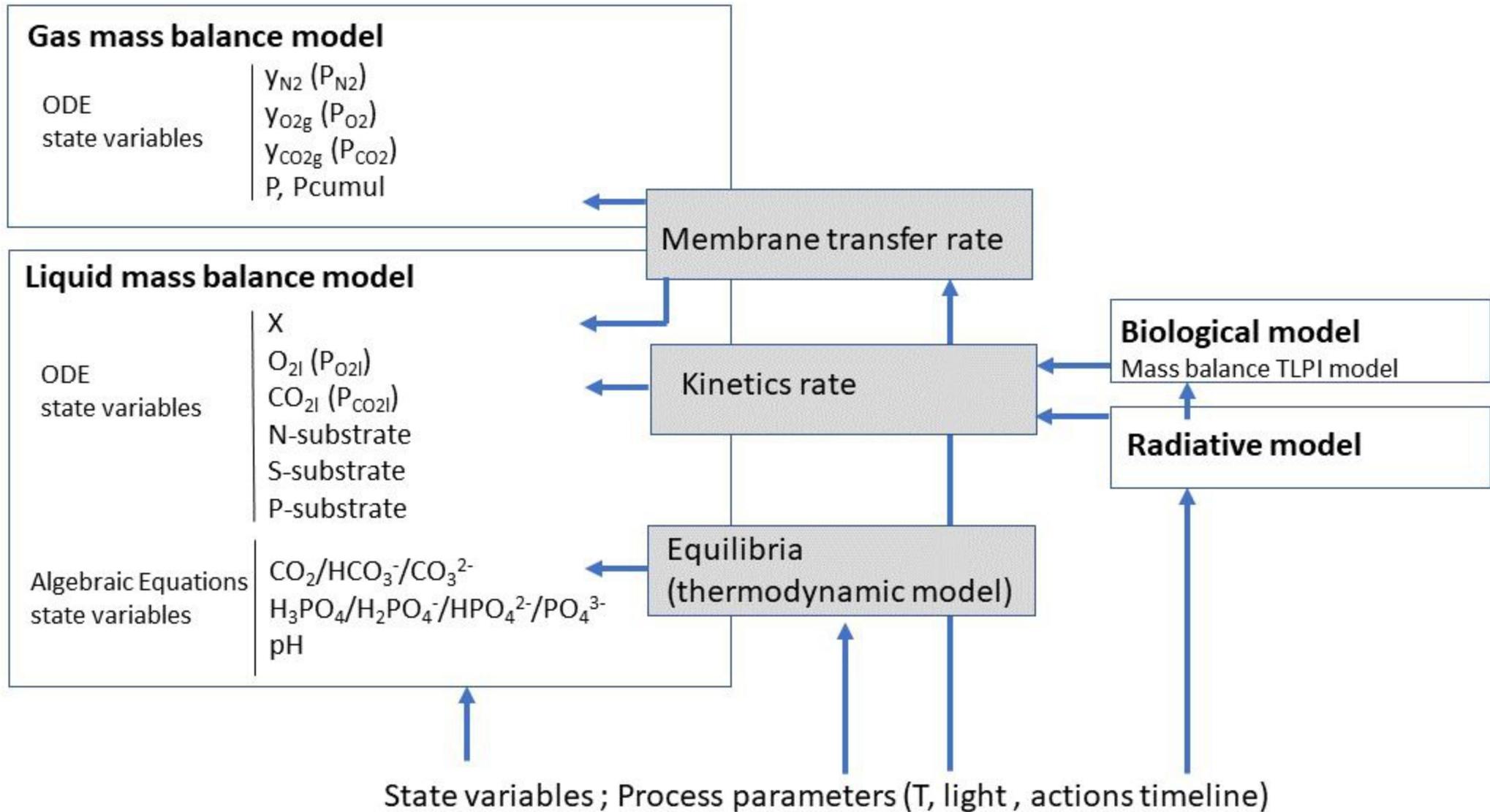
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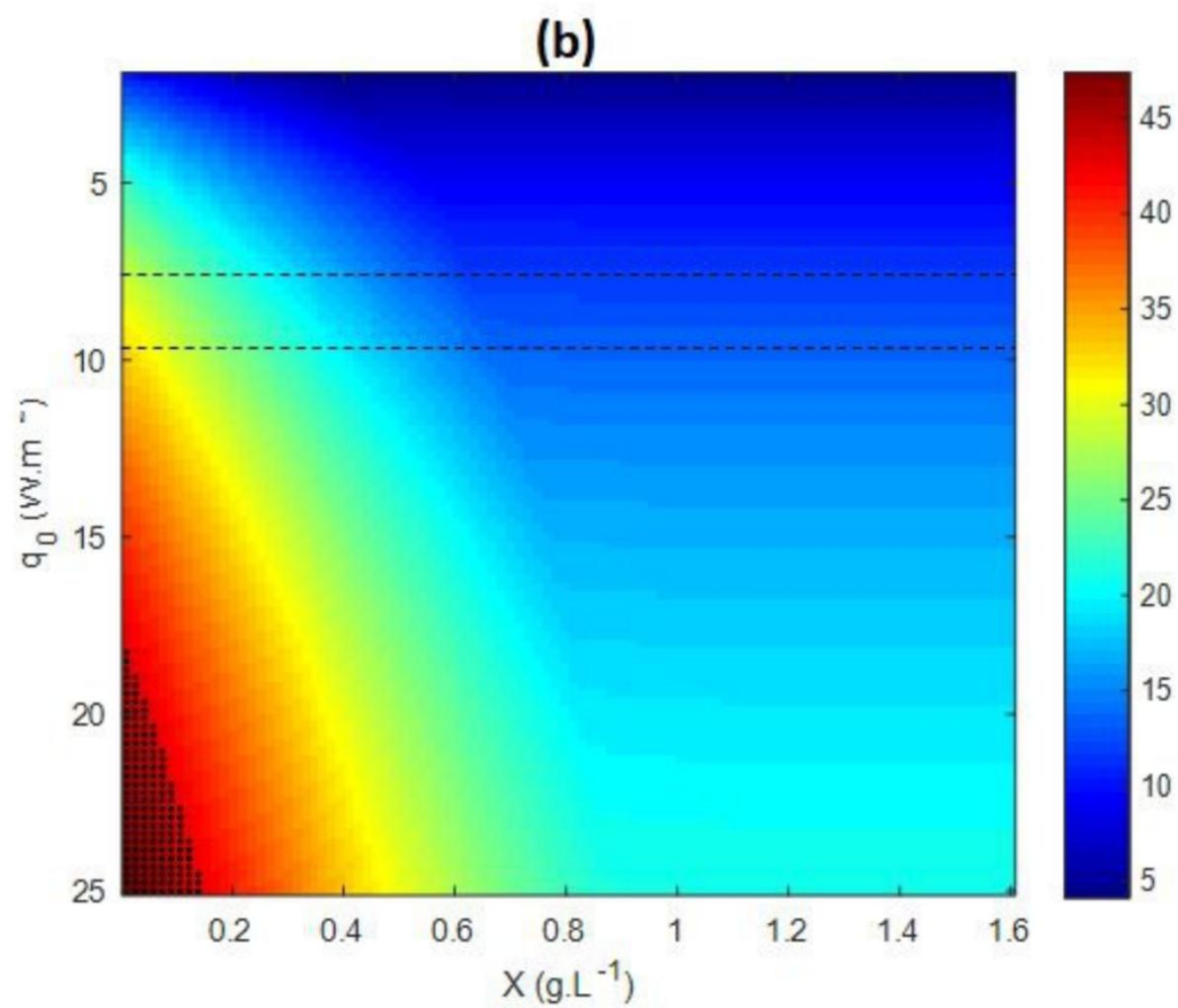
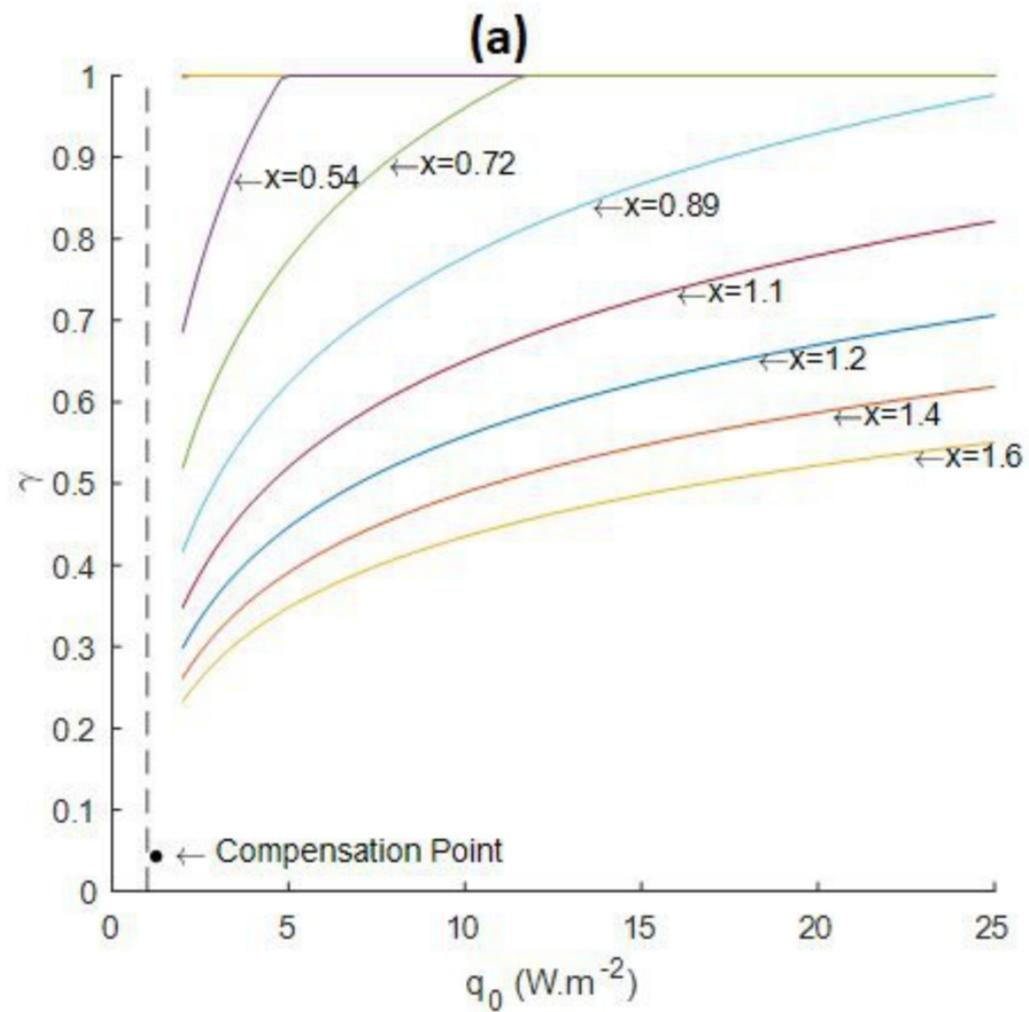
Illumination (white LED array)

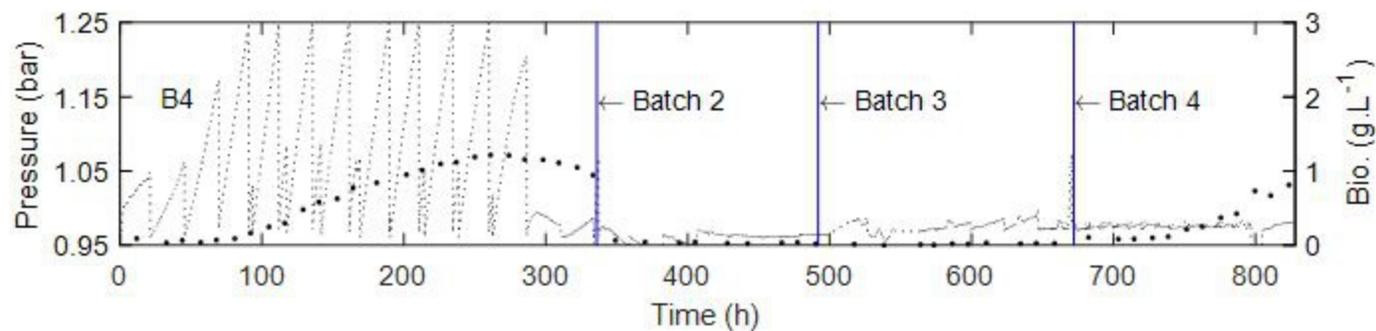
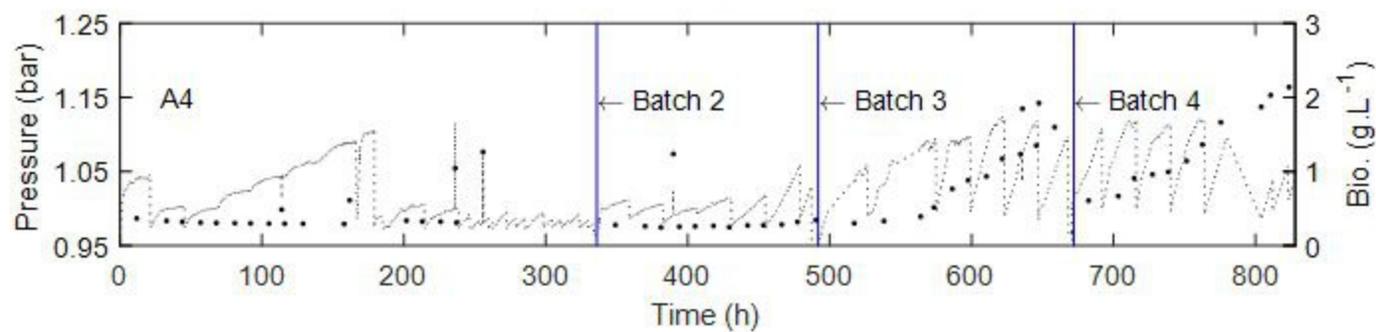
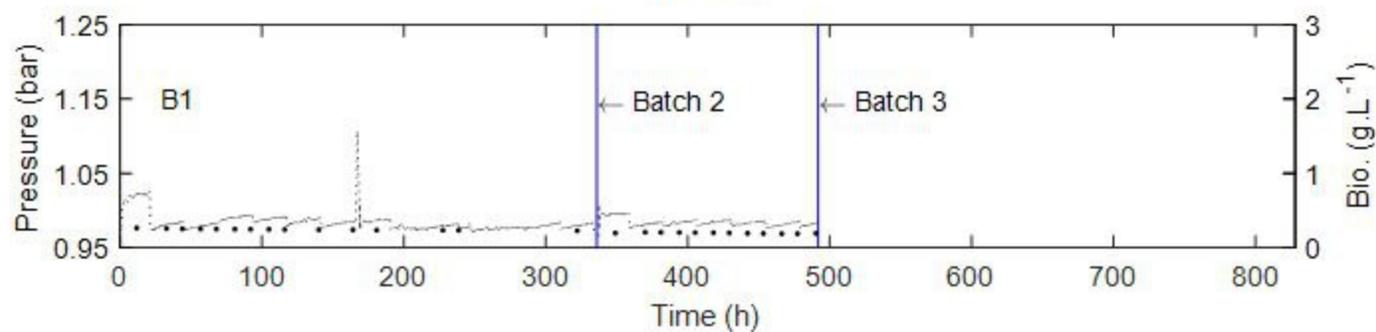
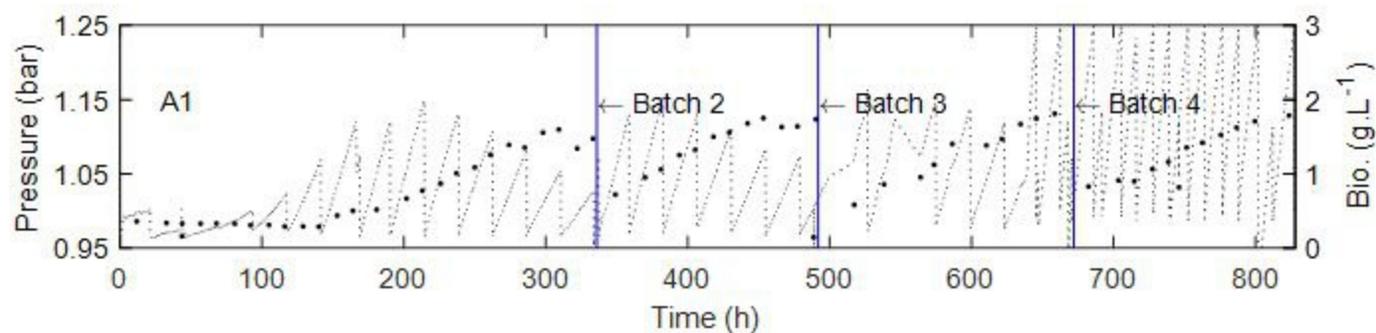
External source of energy and heat required.

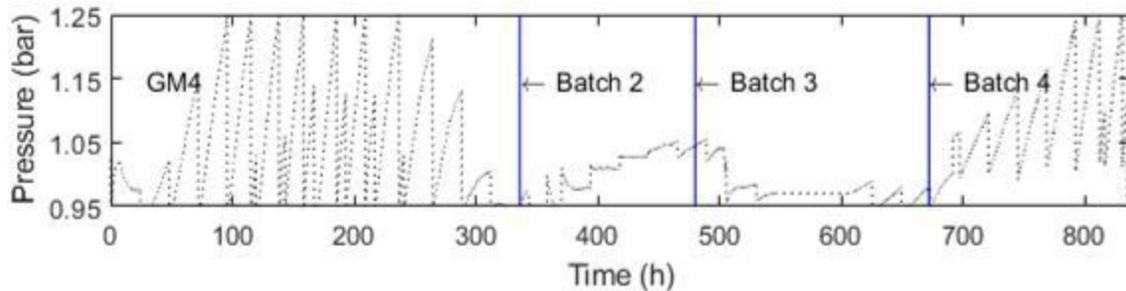
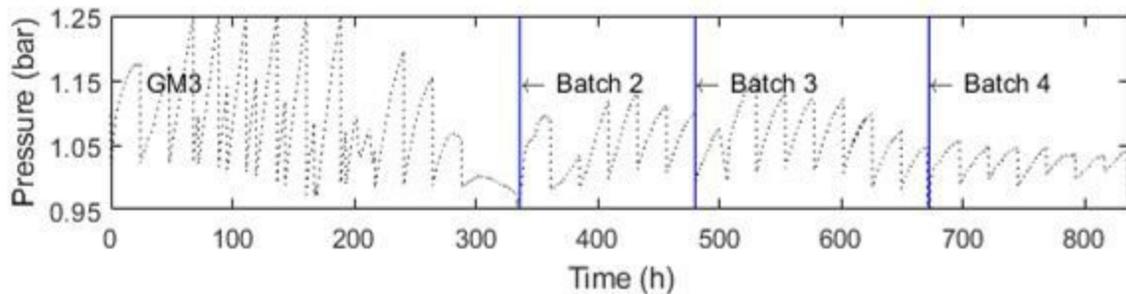
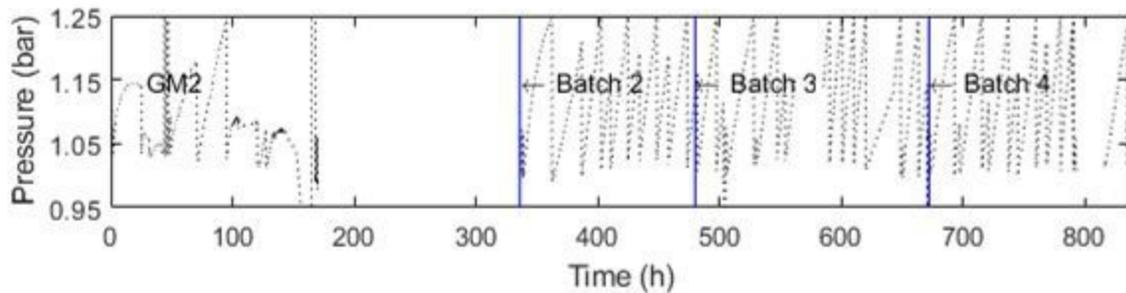
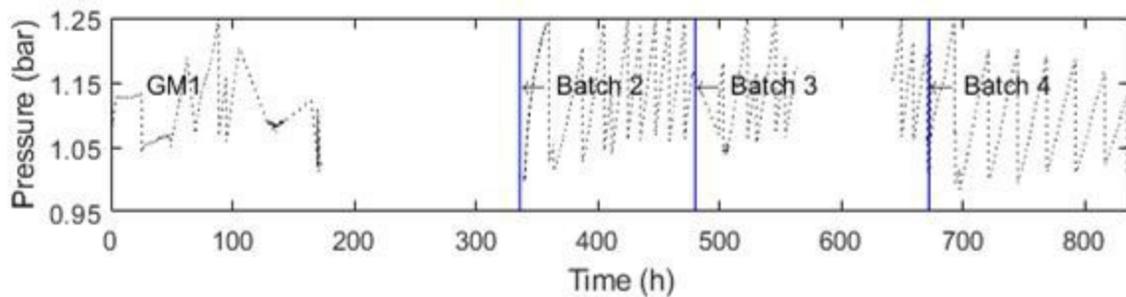


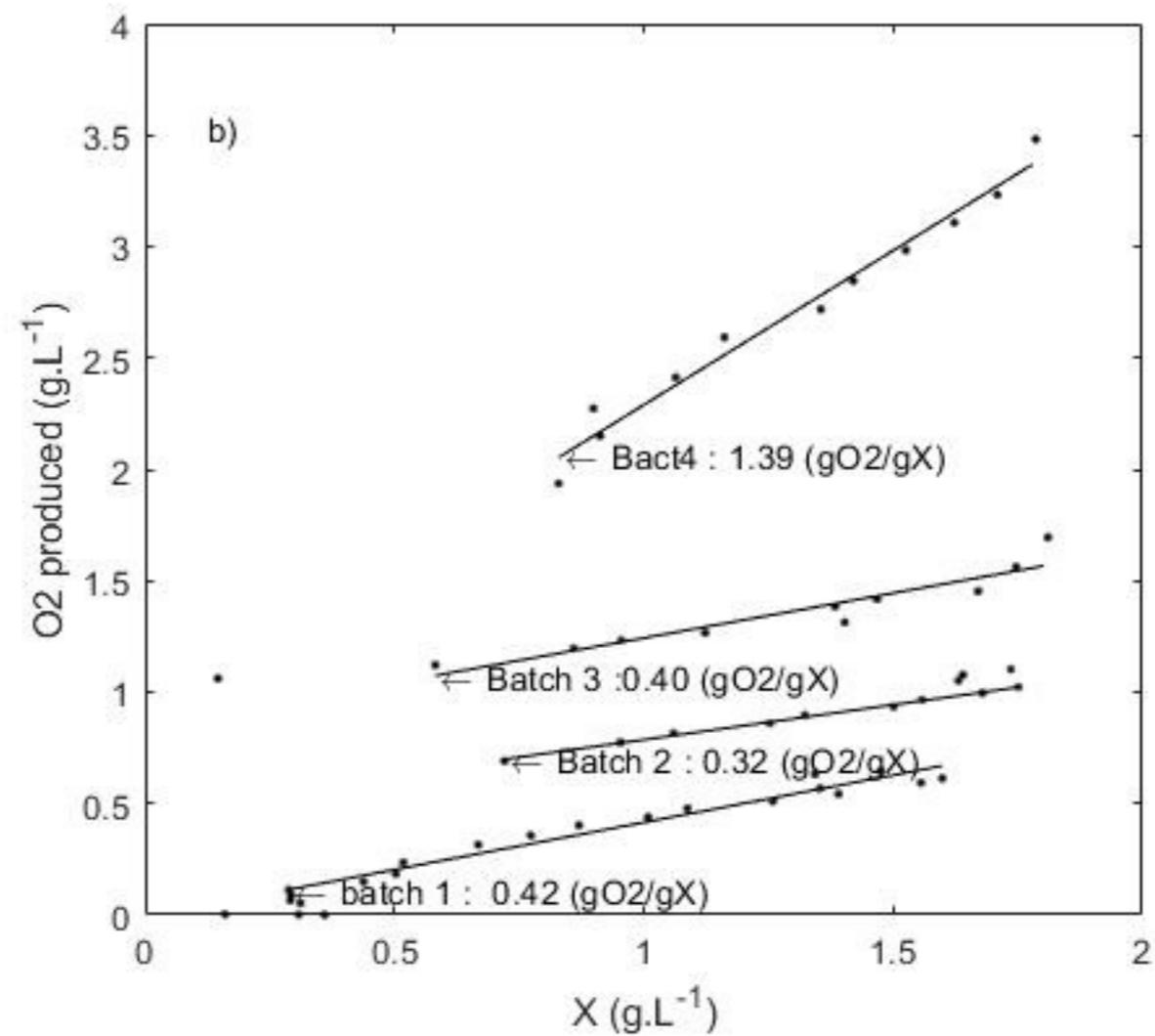
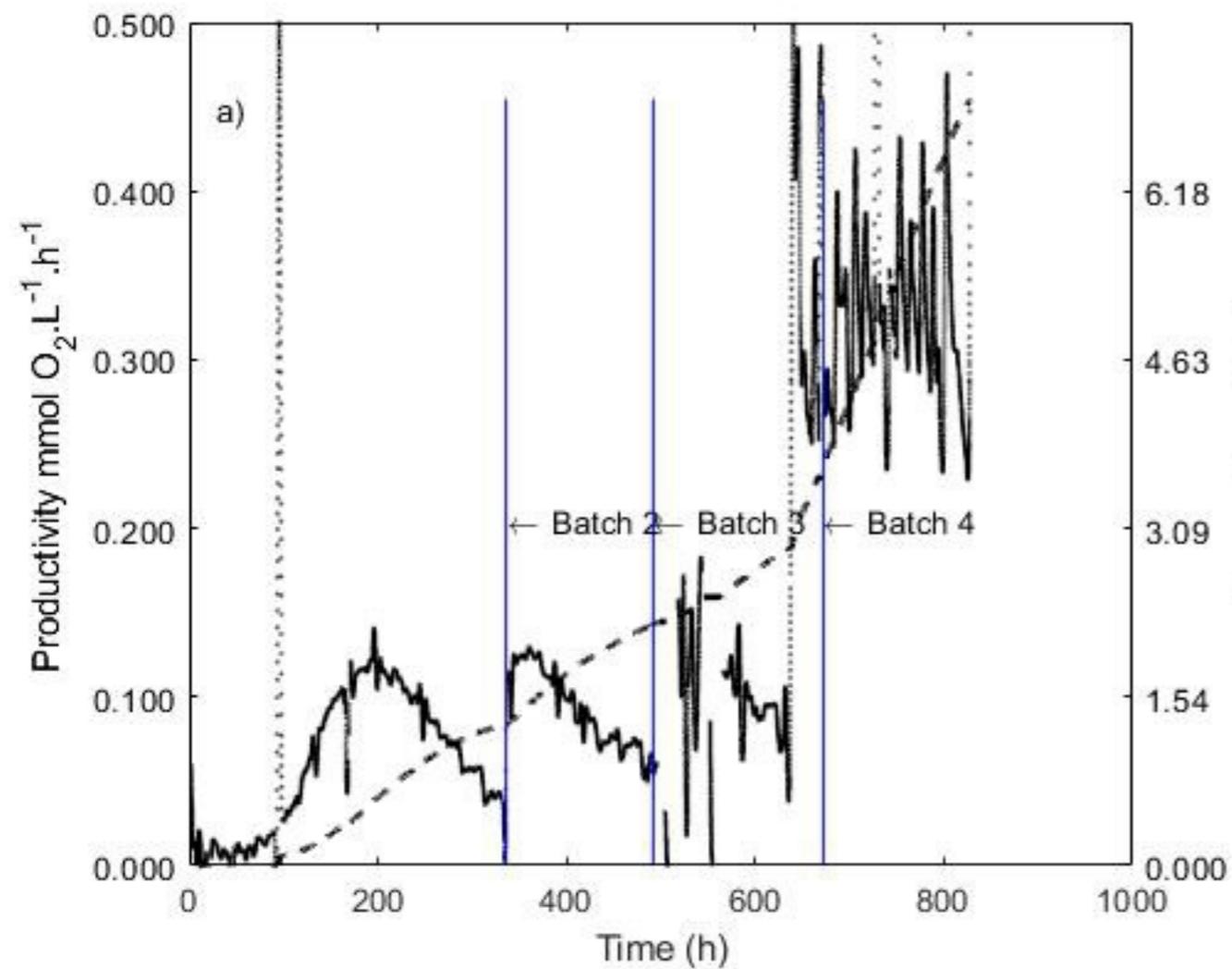


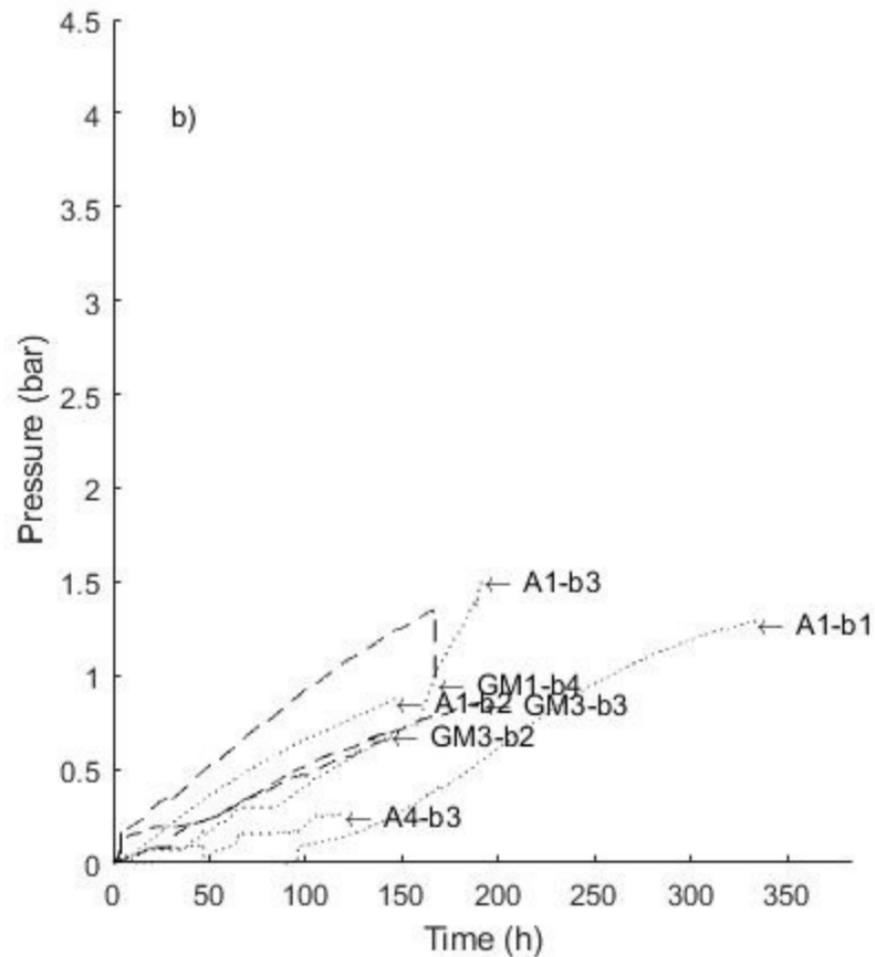
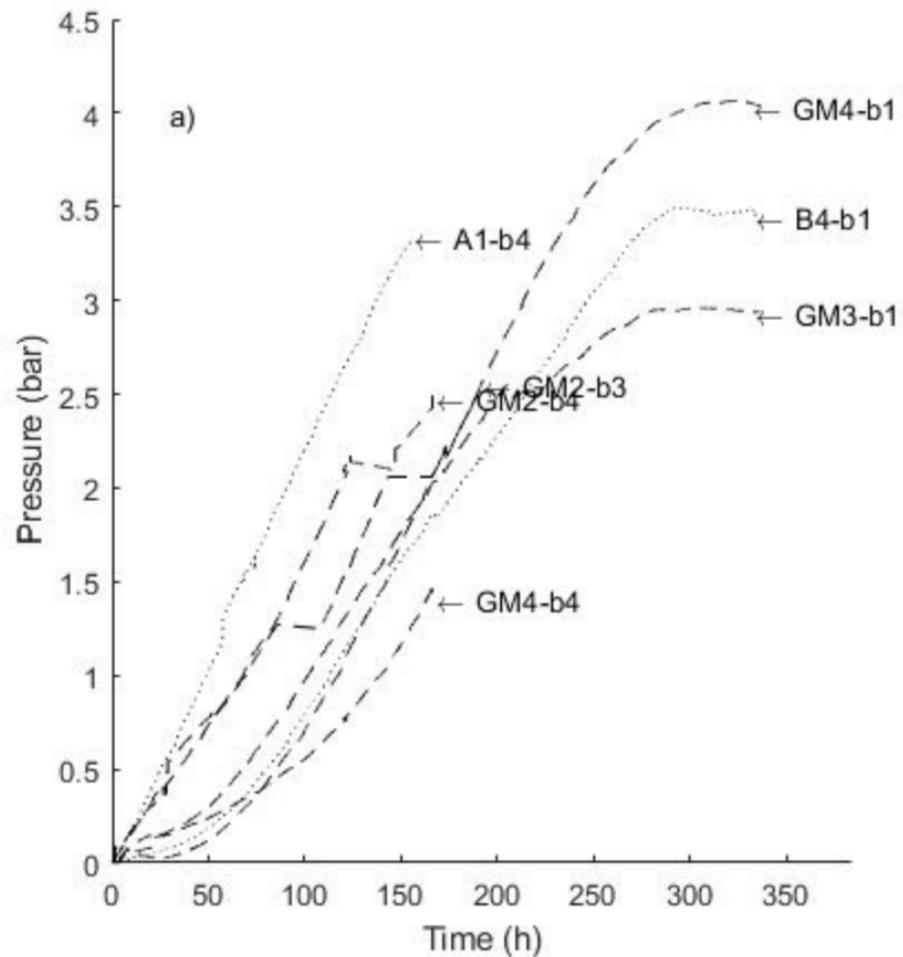


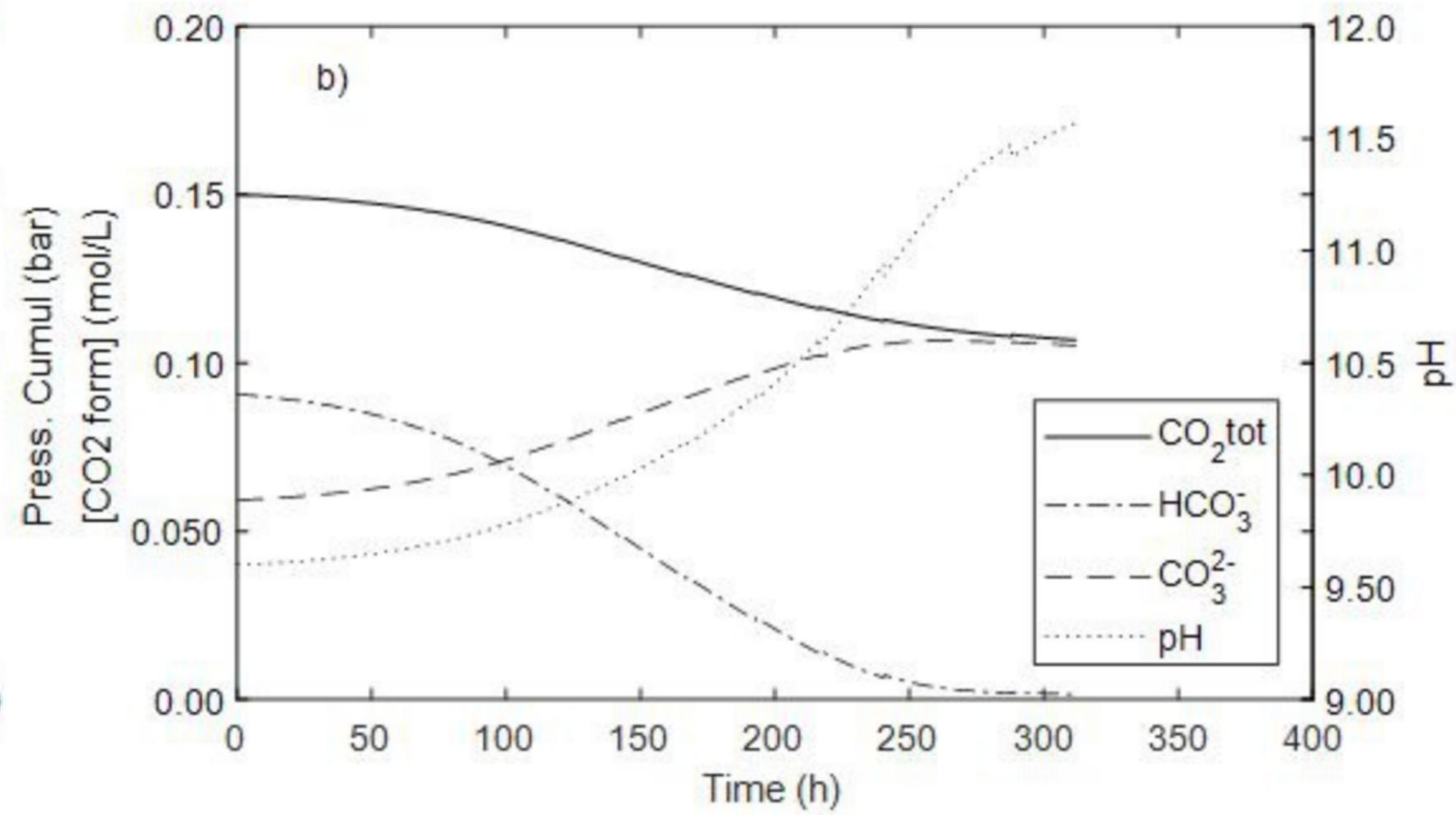
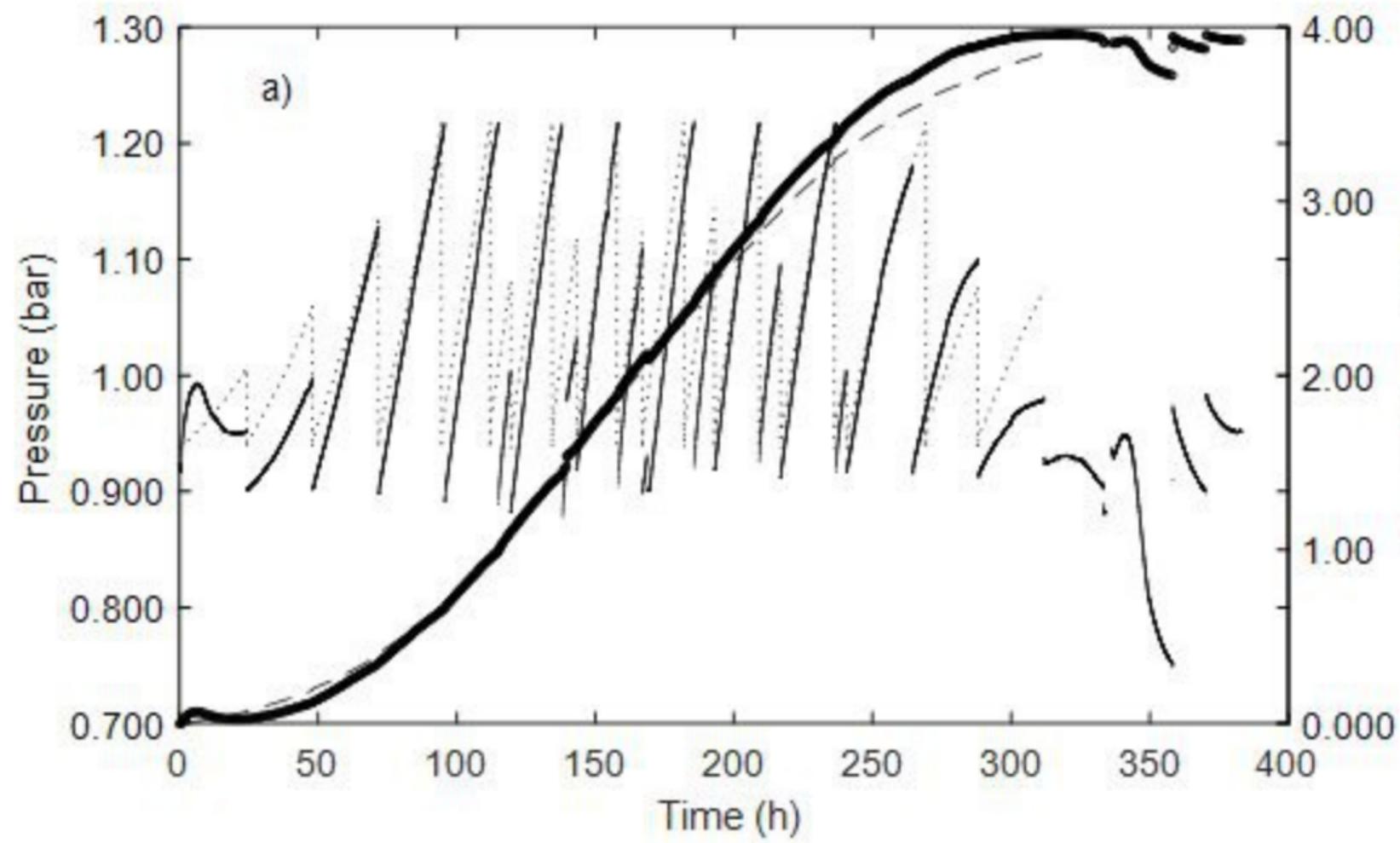


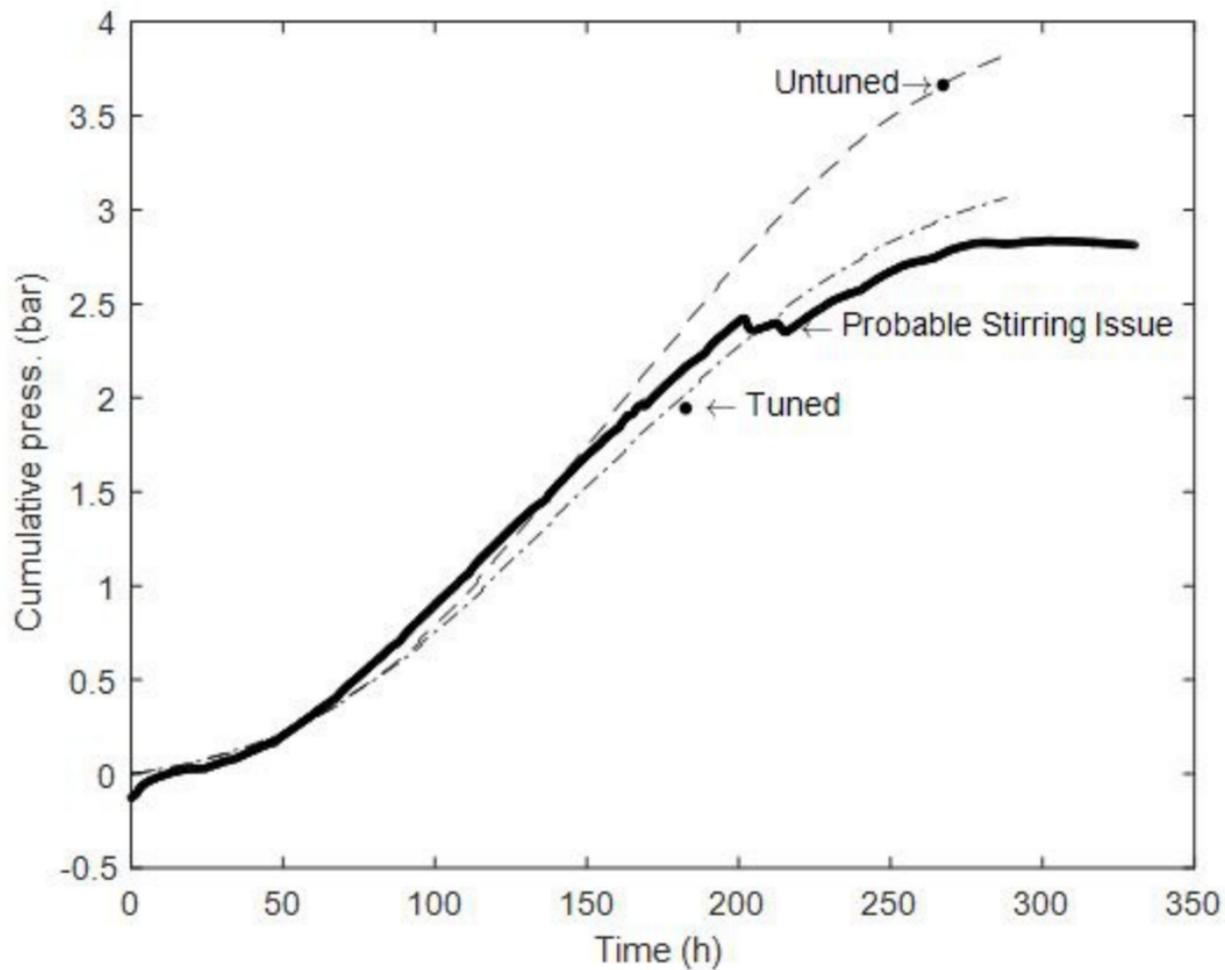


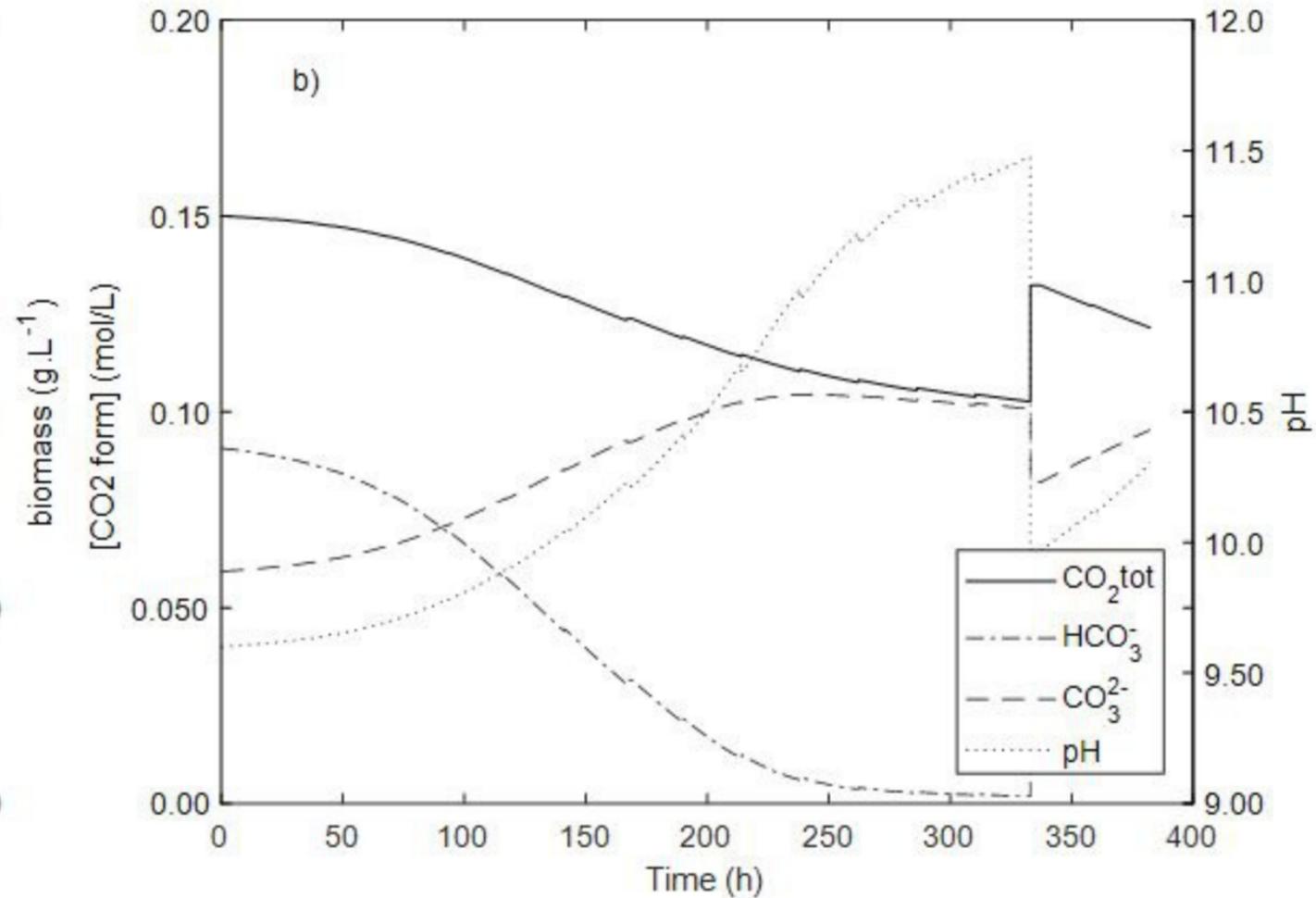
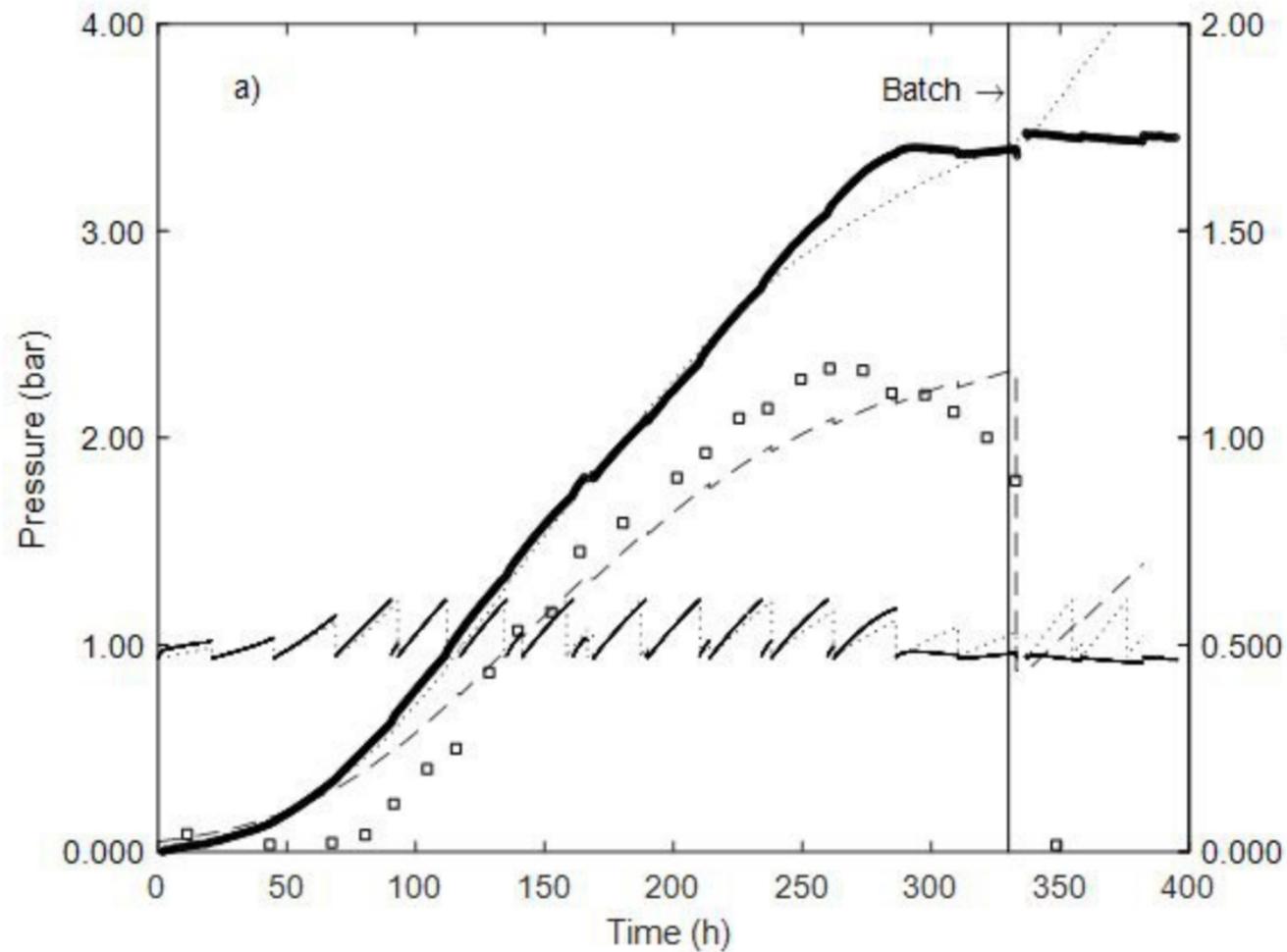












	Batch 1	Batch 2	Batch 3	Batch 4
A1	0.42 g.g ⁻¹ 0.10 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.32 g.g ⁻¹ 0.10 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.40 g.g ⁻¹ 0.10 mmolO ₂ .L ⁻¹ .h ⁻¹ c)	1.39 g.g ⁻¹ 0.35 mmolO ₂ .L ⁻¹ .h ⁻¹ b)
A4	nd	nd	0.20 g.g ⁻¹ 0.07 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.65 g.g ⁻¹ 0.19 mmolO ₂ .L ⁻¹ .h ⁻¹ c)
B1	nd	nd	nd	nd
B4	1.20 g.g ⁻¹ 0.24 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	nd	nd	nd
GM1	nd	0.25 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.20 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.12 mmolO ₂ .L ⁻¹ .h ⁻¹ b)
GM2	nd	0.26 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.22 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.23 mmolO ₂ .L ⁻¹ .h ⁻¹ b)
GM3	0.25 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.09 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	0.08 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	nd
GM4	0.33 mmolO ₂ .L ⁻¹ .h ⁻¹ b)	nd	nd	0.31 mmolO ₂ .L ⁻¹ .h ⁻¹ a)

Table 1: Yield O₂/biomass (g.g⁻¹) (calculated by linear regression from cumulative pressure/biomass concentration) and oxygen rate production (a) maximum if steady-state value not reached, b) averaged maximum from R_{O₂} calculation, c) Cumulative Pressure slope). Calculations were done considering the same theoretical volumes for the reactors (Liquid 60 mL and gas 24mL).